Genetic structure, survival mechanisms and spread
of downy mildew in Western Australian and
Australian vineyards.

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Declaration

I declare this thesis is my own account of my research and contains as its main content work, which has not previously been submitted for a degree at any tertiary education institution. This thesis was supported by an Australian Government Research Training Program Scholarship and Wine Australia PhD top up grant (GWR Ph1301).

Andrew S Taylor
“In the face of overwhelming odds I’m left with only one option, I’m going to have to science the shit out of this”

Mark Watney

*Ares III* mission to Mars

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Dedicated to

Norm and Doreen,

Kevin and Lilian.
Vale Trevor Wicks

1945-2016

I first met Trevor Wicks within months of completing undergraduate studies at university and starting employment at the Department of Agriculture in Western Australia in 2004. Trevor was asked to provide mentorship for a potato project in Sri Lanka. It was in part this mentorship that encouraged me to undertake this PhD and one which would continue until his passing.

Despite being based on the other side of the country Trevor taught me more about plant pathology than any other in the field. It is a rare quality to be able to both conduct rigorous scientific experiments and hold the attention of a large contingent of growers, often looking for the quick fix solution to what is regularly a complex biological issue. Trevor had this
quality and it would be something I would consistently quiz him about in order to better myself.

His love of travel was well known and this meant he had contacts and friends seemingly everywhere. It was through these contacts this PhD could end up as “complete” as it has become with assistance from overseas friends and colleagues being vital after his illness became apparent in the early months of 2016.

There will be no other horticultural plant pathologist like Trevor in Australia. His expertise covered an extensive range of commodities from vegetable to tree crops over many decades of dedicated service. I hope the work presented in this thesis would have made him proud.

He will be missed.
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“There is no I in team plant pathology”

An often used cliché in competitive team sports it is probably more fitting to someone who has undertaken a PhD journey in plant pathology, particularly in the case of a global pathogen and one that doesn’t like growing on agar plates. Without the assistance of more people than I can count completing this would not have been possible.

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Abstract

Western Australia (WA) was one of few grape growing regions of the world free from grapevine downy mildew, caused by the oomycete *Plasmopara viticola*, until two separate detections occurred in 1997 and 1998. The pathogen is now established in all growing regions of the state and growers are required to actively manage the disease due to potential economic loss. The introduction of *P. viticola* into WA raised a number of questions about the source of the incursion(s), its population structure, ability to survive in a climate not considered optimal for its development and the subsequent economic impact it is having on the WA viticulture industry.

Using a number of different detection methods 51 microsatellite markers have been identified for *P. viticola*. These markers were identified from isolates of European and American populations and had not been tested on Australian isolates. Using three isolates collected from geographically separated locations in Australia all 51 microsatellites were tested for their capacity to detect polymorphism within the Australian populations and the sequences lodged in GenBank. A total of 18 microsatellites were identified showing polymorphism.

A total of 413 samples from Australia (286), North America (69), France (32), Brazil (8) and Uruguay (18) were collected as part of a global survey. Both fresh leaf samples and DNA trapped on FTA cards were collected and the populations analysed using 16 of the 18 microsatellites previously identified. The populations were analysed for genetic diversity, mode of reproduction and cryptic *P. viticola* species present. It was determined the Australian and South American populations more closely relate to the French samples than those of North America, the origin of *P. viticola*. The only cryptic species discovered outside of North America was *P. viticola* clade *aestivalis*. The WA population had the lowest genetic diversity
of all populations, likely a result of the more recent introduction of the pathogen and suggests the 1997 and 1998 incursions are linked. Genetic analysis of the WA populations indicate they have a clonal mode of reproduction whereas the American and French populations are sexually reproducing. This is the first record of clonal reproduction within *P. viticola* and highlights a greater plasticity within the mode of reproduction than previously identified.

With relatively mild winters in comparison to northern hemisphere grape production systems it was hypothesised *P. viticola* could overwinter as mycelium in dormant grape buds in WA. To test this hypothesis a number of field trials and vineyard observations were conducted over two seasons. It was discovered *P. viticola* can infect green buds, and has the capacity to produce sporangiophores from them, but there is no evidence of any ability to overwinter and produce shoots infected with *P. viticola* the following season. This finding contrasts long held assumptions about the overwintering ability of *P. viticola* in dormant grape material.

Oospores are considered important for both *P. viticola* survival and the source of primary infection. However, a survey conducted in the early 2000’s failed to detect oospores in the majority of WA vineyards. Vineyards in the Margaret River and Swan Valley regions of WA having experienced downy mildew infection during the 2014/15 season were assessed for oospores. All vineyards were found to have downy mildew oospores. To determine mating type ratios 11 isolates were co-inoculated on leaf discs and oospores were detected in three crosses including a cross between the same isolate. There is the possibility a single mating type of *P. viticola* exists in WA with the capacity for secondary homothallism or selfing.
Despite *P. viticola* being present in WA since 1997/98 an economic assessment of the impact of the disease on WA viticulture had not been undertaken. A bioeconomic model was developed linking weather with spread, infection of the disease and production costs and revenue to estimate the cost of *P. viticola* over time. It was found *P. viticola* costs the WA viticulture industry AUD $7.3 million per year with cumulative costs over a 30 year period to be AUD $140 million.
Publications arising from this thesis

Peer reviewed journals


Chapter 3: In preparation

Conference proceedings


Final reports

Taylor AS (2017) Attend and present PhD research findings at the 8th International workshop on Grapevine Downy and Powdery mildew in Oregon. Final report to the Australian Grape and Wine Authority (Wine Australia), Project Number: AGT 1612.

Industry journals

## Contents

Chapter 1 Literature Review ........................................................................................................ 1

Chapter 2 Evaluation of microsatellite primers developed for grapevine downy mildew, *Plasmopara viticola*, on Australian isolates. ................................................................................. 25

Chapter 3 Population genetic structure of *Plasmopara viticola* in Australian vineyards with emphasis on the Western Australian population. .................................................................................. 35

Chapter 4 Overwintering of grape downy mildew as mycelium in Western Australian vineyards. ................................................................................................................................. 60

Chapter 5 Detection of grape downy mildew oospores in Western Australian vineyards and mating type crosses ........................................................................................................... 87

Chapter 6 An economic assessment of the impact on the Western Australian viticulture industry from the incursion of grapevine downy mildew .......................................................... 102

Chapter 7 General Discussion ..................................................................................................... 116

Appendix 1 ................................................................................................................................. 125

Appendix 2 ................................................................................................................................. 126

Appendix 3 ................................................................................................................................. 145

References .................................................................................................................................. 148
Chapter 1 Literature Review

Taxonomy

Grapevine downy mildew caused by the obligate biotroph *Plasmopara viticola* (Berk. & Curt.) Berlese & de Toni, is a member of the *Oomycota* class of plant pathogens, a group representing a number of economically significant pathogens of commercial agriculture and native ecosystems (Kamoun et al. 2015). Oomycetes are eukaryotes, resembling true fungi but are phylogenetically related to diatoms and brown algae in the kingdom *Stramenopila* (Kamoun et al. 2015; Luis et al. 2013; Voglmayr et al. 2004). Within the *Peronosporaceae* family *P. viticola* phylogenetically aligns with the downy mildews containing pyriform haustoria, along with genera of *Bremia*, *Basidiophora*, *Benua*, *Novotelnova*, *Paraperonospora*, *Plasmovema* and *Protobremia* (Luis et al. 2013; Thines 2014; Voglmayr et al. 2004). Whole genome-scale analysis of 37 oomycetes has closely aligned *P. viticola* amongst the *Phytophthora* Clade 1 species, including *P. infestans*, *P. parasitica* and *P. nicotianea* (McCarthy and Fitzpatrick 2017). A recent review of the phylogenetic relationship between the downy mildews and *Phytophthora* taxon suggests the current nomenclature within this group is likely to change (Bourret et al. 2018).

Originally, the cause of grapevine downy mildew was classified as *Botrytis cana* Lk. by Schweinitz in 1837 (Viennot-Bourgin 1981). In 1855 Berkeley renamed the fungus as a separate species, *B. viticola* Berk. & Curt (Viennot-Bourgin 1981). *B. viticola* was subsequently transferred to the genus *Peronospora* and classified as *Peronospora viticola* de Bary in 1863 (Viennot-Bourgin 1981). The name *Peronospora viticola* persisted until 1888 when it was transferred to the genus *Plasmopara* for the final classification of *P. viticola* (Berk. & Curt.) (Viennot-Bourgin 1981).
Viticulture in Western Australia

The commercial viticulture industry of Western Australia (WA) consists of both table and wine grape production with a gross total industry farm gate value of $79.6 million (ABS, 2012). Viticulture production extends from Carnarvon in the north to Albany in the south of the state with the wine growing regions being delineated into 9 separate geographical indicator (GI) regions (Figure 1.1). WA wine production is approximately 45 million litres annually, representing almost 5% by volume of total Australian production but represents 12% of total value through targeting fine wines (speciality and super-premium). The Margaret River region accounts for the largest wine grape production area (45%), followed by the Great Southern (26%) and the Geographe, Swan District and Pemberton regions (7%) (Wines of Western Australia 2014).
Figure 1.1. The grape growing regions of Western Australia minus Carnarvon and Geraldton in the north. The different colours designate a different geographical indicator region (Map kindly supplied by Wines of Western Australia).
**Spread and significance**

*P. viticola* originates from eastern North America where it is believed to have coevolved with genera of the Vitaceae family (Baldini et al. 2008; Rouxel et al. 2014; Schröder et al. 2011). Prior to the discovery of *P. viticola* clades (discussed below), several of these endemic North American *Vitis* species were considered to have developed a high level of resistance to the disease in comparison to the highly susceptible commercially produced European *Vitis vinifera* cultivars (Lafon and Clerjeau 1988). A number of oriental *Vitis* species also show resistance and or tolerance to *P. viticola* infection (Yu et al. 2012).

Downy mildew was first recorded in Europe in 1878 (Gessler et al. 2011). It is believed *P. viticola* was imported with grapevine rootstocks of wild American *Vitis* species as an effort to control the insect phylloxera (*Daktulosphaira viteifoliae*) which was causing significant issues for European viticulture at the time (Rossi et al. 2013; Rouxel et al. 2014). After its introduction to France (1878) spread was rapid in an eastward direction into major grape production regions of Europe and over the Mediterranean Sea to Algeria (1881) (Gessler et al. 2011; Rossi et al. 2013). There appears to be two distinct genetic and geographic populations of *P. viticola* in Europe, differentiated into Western and Eastern European vineyards (Fontaine et al. 2013). The rapid movement is most likely due to human mediated transfer of grapevine material throughout Europe (Gobbin et al. 2006). Globally, *P. viticola* is found in 98 countries (CABI 2017).

The first report of *P. viticola* in a commercial vineyard in Australia was in Rutherglen, Victoria in 1917, although it is possible a single infected vine, subsequently destroyed, occurred prior to this (de Castella and Brittlebank 1917). Following this detection downy mildew spread rapidly within grape growing regions of eastern Australia, being recorded in New South Wales (1918), South Australia (1921) and Queensland (1920-21).
(Emmett et al. 1992a; Laffer 1918; Osborn and Samuel 1922; Quinn 1924). The rapid spread of the disease across Eastern Australia was caused by a combination of wind and human aided dispersal (Osborn and Samuel 1922). The disease was first reported in New Zealand in the 1925/26 season (Brook 1992).

The first recorded detection in WA occurred in a remote Kalumburu mission in 1997 on 14 vines of unknown cultivar (Riley 1998). All cultivated vines in the mission (25) were destroyed and a survey of the East Kimberley found no further infections on native Vitaceae species (Riley 1998). The first outbreak of P. viticola in commercial grapevine regions of WA occurred in October 1998 (McKirdy et al. 1999). It is not known whether the 1997 and 1998 detection are related. However, given the detection of the disease in 45 of 70 vineyards surveyed across the state within two weeks of the 1998 detection (McKirdy et al. 1999), some considered it to be present in commercial production areas prior to that season. Analysis of weather conditions in WA, conducted in the 1980’s, indicated it was feasible for the disease to have been introduced and to have remained undetected (McLean et al. 1984). Despite the implementation of an exotic disease response plan, the sheer extent of the spread meant eradication was not feasible (McKirdy et al. 1999). One year on from the 1998 detection, downy mildew had been recorded in the major production regions of Margaret River (Fisher 1999) and Carnarvon (Colin Gordon pers. comm.). The disease has now been found in all growing regions of WA (Williams et al. 2007a).

**Molecular contributions**

The development of molecular markers for P. viticola has made a considerable difference to the understanding of the life cycle and epidemiology of downy mildew as well as its population structure across different grape growing regions (Rossi et al. 2013). Initially the development of Random Amplified Polymorphic DNA (RAPD’s) allowed the detection
of differences among genotypes but these were soon superseded with the development of microsatellite (Simple Sequence Repeats (SSR)) markers, having the capacity to withstand contaminant DNA in the sample and still detect differences among the P. viticola genotypes (Gessler et al. 2011).

Gobbin et al. (2003b) identified five SSR markers, BER, CES, GOB, ISA and REX to be used in P. viticola population studies using isolates from Switzerland and Italy. REX was found to be monomorphic and therefore of no use in differentiation between genotypes but could be useful on a larger scale analysis such as an introduction of P. viticola strains from abroad (Gobbin et al. 2003b). Using the other four SSR’s markers the population of P. viticola has been investigated in Europe (Gobbin et al. 2005; Gobbin et al. 2006), Italy (Gobbin et al. 2003a), Greek Islands (Rumbou and Gessler 2006), Australia (Hug 2005; Williams 2005) and even at a single vine scale in southern Switzerland (Matasci et al. 2010). Using these four microsatellites a high level of gene and genotypic variability exists in European populations, highlighting its success as an introduced pathogen (Gobbin et al. 2006). Studies investigating fungicide resistance in P. viticola populations have also included a selection of these microsatellites to determine if there are any association with allele combinations and the detection of resistance (Gisi et al. 2007; Matasci et al. 2008; Scherer and Gisi 2006).

In Australia the genetic diversity of isolates in WA is low, intermediate in NSW and high in VIC, based on the four microsatellites BER, CES, GOB and ISA (Hug 2005). There are some genetic similarities between isolates in eastern Australia (SA, NSW, QLD) but it appears WA isolates are genetically dissimilar to those in eastern Australia (Williams 2005). In both the Hug (2005) and Williams (2005) studies, only single samplings occurred for eastern Australia in comparison to multiple sampling occasions for locations in WA.
Furthermore, these studies were both conducted prior to 2005, only seven years after the major incursion in WA, so there is the potential genotypic recombination has increased the level of genetic differentiation in WA in the years since these studies. It remains unclear as to where the 1997 Kalumburu and 1998 Swan Valley incursions originated from.

Since the development of the initial set of SSR markers a number of new markers have been identified to aid in the characterisation of *P. viticola*. A second set of seven microsatellite markers were developed by Delmotte et al. (2006) designated Pv7, Pv13, Pv14, Pv16, Pv17, Pv31 and Pv39. Using both the original SSR set and five of those developed by Delmotte et al. (2006) the population genetic structure of *P. viticola* in the Western Cape province of South Africa was studied by Koopman et al. (2007). Yin et al. (2014) used six of the Delmotte et al. (2006) markers to determine the genetic structure of *P. viticola* in seven viticulture regions of China. A large scale sampling across mainland Europe of *P. viticola* isolates, investigating the introduction and spread, using a combination of the Gobbin et al. (2003b) and Delmotte et al. (2006) microsatellites, found little genetic diversity despite two distinct populations existing (Fontaine et al. 2013). The low diversity found in the Fontaine et al. (2013) study contrasts the findings of Gobbin et al. (2006), potentially as a result of the differences in the sampling scale and microsatellites used between the two studies.

With the use of microsatellite-enriched and direct shotgun pyrosequencing libraries Rouxel et al. (2012) identified 31 microsatellite markers having different loci to both those identified by Gobbin et al. (2003b) and Delmotte et al. (2006). These microsatellites have been used to characterise populations, variation in pathogenicity, fungicide resistance and host resistance studies in a number of growing regions (Delmas et al. 2017; Delmas et al. 2016; Kosev et al. 2015; Li et al. 2016). As each study used different microsatellites it is
difficult to interpret findings across them. A population analysis of *P. viticola* using any of the newly developed molecular markers has not been conducted with Australian isolates.

Improvements in molecular markers have led to a new understanding of *P. viticola* populations in its native USA. Using both nuclear and mitochondrial markers Schröder et al. (2011) determined isolates in the United States fit into three distinct phylogenetic clades. Further studies using genetic markers and large scale sampling across eight eastern North American states and parts of Canada, of both cultivated and native host species; have revealed five cryptic species of *P. viticola* (Rouxel et al. 2014; Rouxel et al. 2013). These five clades show a level of host specialisation and the ability to infect European *Vitis* species indicates a host range expansion from native to cultivated hosts (Rouxel et al. 2013). Of the clades described, both *P. viticola* clade *vinifera* and *P. viticola* clade *aestivalis* were found to infect *V. vinifera* hosts. It is unknown whether both or one of these clades is the causal organism for grape downy mildew in Australia. The discovery of *Vitis* specialised clades means some of the host susceptibility studies conducted in the past have less relevance due to the clade of the isolate used being unknown.

**Symptoms and susceptibility**

*P. viticola* is known to attack all green parts of the vine (Lafon and Clerjeau 1988) possessing functional stomata (Gessler et al. 2011). Leaf lesions appear first and are the most obvious, appearing as yellow, oily lesions on the upper surface of the leaf (Nicholas et al. 1994). These are commonly referred to as ‘oilspots’ (Fig. 1.2a). A chocolate halo can surround the oilspot but this fades as the oilspot ages (Emmett et al. 1992b; Nicholas et al. 1994) (Figure 1.2b). Depending on the age of the leaf the lesions may appear circular or angular, limited in their size and spread via the leaf veins (Lafon and Clerjeau 1988) (Figure 2c). This produces a mosaic pattern on the upper surface of the leaf. If conditions are
favourable the oilspots enlarge rapidly and may merge with other spots on a leaf to cover the majority of the leaf surface (Nicholas et al. 1994) (Figure 1.2d). During warm humid nights a dense, white, cottony growth appears on the under surface of the leaf where the oilspot occurs (Lafon and Clerjeau 1988) (Figure 1.2e). This is referred to as ‘down’, hence the disease’s common name. As the oilspot ages the centre of the lesion dries out, becoming necrotic and may fall away whilst the outer ring remains yellow and viable (Emmett et al. 1992b; Nicholas et al. 1994) (Figure 1.2f).

Younger leaves are more susceptible to infection than older leaves (Reuveni 1998). Early models of downy mildew spread use shoots 10cm long (modified Eichhorn-Lorenz growth stage 12) as the initial guide for infection as it was presumed stomata on shoots younger than this are not functional (Kennelly et al. 2007). Kennelly et al. (2007) found shoots, and the subsequent leaves, were susceptible to infection at modified Eichhorn-Lorenz (E-L) stage 4 (budburst, first leaf tissue visible) (Appendix 1, (Coombe 1995)). The development of age related resistance, known as ontogenic resistance, in grapevine leaves occurs through the production of compounds acting as defence mechanisms against infection (Reuveni 1998; Steimetz et al. 2012). Although all V. vinifera cultivars are susceptible to downy mildew a number have been assessed for their level of susceptibility and differences or tolerances are apparent (Boso et al. 2011; Boso et al. 2014; Boso and Kassemeyer 2008). Kortekamp and Zyprian (1999) found leaf hairs provided a basic natural barrier to infection and it is possible this might also provide slight susceptibility differences among cultivars of V. vinifera.

Infection on young shoots and tendrils appear as oily brown lesions, potentially extending along the shoot and into the leaf stalk (Nicholas et al. 1994) (Figure 1.3a, b, c). Tips of infected shoots thicken and curl in what is commonly known as a “shepherds crook”
Lafon and Clerjeau 1988). The nodes are more susceptible to infection than the internodes of
the shoot (Emmett et al. 1992b) (Fig. 1.3d, e, f). If infected early in the season shoots and
tendrils turn brown, dry up and drop or are broken from the vine (Lafon and Clerjeau 1988).

Flower inflorescences are highly susceptible to infection and appear oily brown when
infection occurs (Emmett et al. 1992b) (Fig. 1.4 a). During warm (>13°C), humid conditions
these are then covered in white sporangia (Emmett et al. 1992b) (Fig. 1.4b). Early season
infection on young clusters may cause the entire inflorescence to wither and die or only
sections of the inflorescence may be affected (Emmett et al. 1992b) (Fig. 1.4c, d, f). Berries
are susceptible to infection until they reach pea-size (7 mm diameter, modified E-L31)
(Emmett et al. 1992b; Nicholas et al. 1994) (Fig. 1.4e). Previously infected berries at this
point harden, develop a purple hue, before shrivelling and falling from the bunch (Lafon and
Clerjeau 1988; Nicholas et al. 1994) (Fig. 1.5). Although mature berries are resistant the
pedicels and bunch stems remain susceptible throughout the season (Emmett et al. 1992b;
Nicholas et al. 1994).
Figure 1.2. A) “oilspot” lesions on a leaf B) young “oilspot” surrounded by brown halo C) on mature leaves the lesions are constricted to the leaf vein margins creating a mosaic appearance D) oilspots may merge, eventually covering the whole leaf surface E) under warm, humid night time conditions sporangia or “down” is produced on the underside of leaves F) In old oilspots or those killed with fungicide applications the centre begins to turn brown, dry out and fall away.
Figure 1.3. A) infections appear as oily brown lesions on current season stems B) leaf infection has moved systemically through the petiole C) sporulating stem infection D) infection of a bunch extending to the node E) node infection F) node infection in lignified wood.
Figure 1.4. A) oily brown infected inflorescence on the left and uninfected healthy inflorescences on the right B) infected inflorescence covered in sporangia or white “down” C) the entire inflorescence can be infected during the season or D) a portion of the flower clusters can be infected E) berries greater than 7mm in diameter become resistant to infection F) when an entire inflorescence is infected the bunch stem dries up and hardens, these easily break from the vine.
Figure 1.5. Berries are susceptible to infection until approximately 7mm; the berries infected prior to this stage develop a purple hue and begin to shrivel.
Lifecycle

The lifecycle of *P. viticola* on *V. vinifera* is dimorphic, consisting of both a sexual (primary infection) and asexual (secondary infection) stage (Rossi et al. 2008b) (Fig. 1.6). The primary and secondary infections overlap for part of its lifecycle (Rossi et al. 2008b). The disease can be polycyclic within a season in areas where weather conditions favour development of disease. Temperate growing regions experiencing frequent summer rainfall favour the downy mildew lifecycle whilst relatively dry growing regions, or those experiencing a Mediterranean climate, are unfavourable (Emmett et al. 1992b).

![Diagrammatic representation of downy mildew of grapevines caused by *P. viticola* from Agrios (1978).](image-url)
Primary infection

In temperate regions *P. viticola* overwinters as oospores in the decaying leaf litter and berries on, or slightly below, the soil surface where maturation and germination occurs (Gessler et al. 2011; Gobbin et al. 2005; Lafon and Clerjeau 1988; Rossi et al. 2008a). The oospores themselves are 20-120µm in diameter, have two membranes which are covered by the wrinkled wall of the oogonium (Lafon and Clerjeau 1988).

*P. viticola* is a heterothallic organism, requiring two distinct mating types (P1 and P2) (Wong et al. 2001) to merge where the antheridium and oogonium of the two mating types fuse to produce oospores within the leaf (Burrano 2000; Lafon and Clerjeau 1988). The merging of oilspots and development of oospores can occur throughout the season but peaks during autumn when weather conditions are no longer conducive for asexual reproduction and is characterised by the presence of mosaic leaf lesions (Burrano 2000; Emmett et al. 1992b). Mating type characterisation has been carried out for *P. viticola* populations in North America and Europe (Scherer and Gisi 2006; Wong et al. 2001), but not yet for Australian populations. The presence of oospores in WA is rare, occurring in only one of 16 vineyards visited in surveys conducted between 2001 to 2003 (Killigrew et al. 2005). It is not known whether this is a result of a bottleneck situation (founder effect) caused by the recent introduction of the disease or the difficulties in viewing oospores in grapevine leaf material. Despite downy mildew occurring in New Zealand since 1925/26 the presence of oospores was only detected there in 2012 (Wood et al. 2012).

Germination of oospores occurs when a germ tube is produced at which a pyriform macrosporangium develops (Gregory 1912; Lafon and Clerjeau 1988; Vercesi et al. 1999). The germ tubes are between 29-56µm long whilst the macrosporangium is 27x45µm (Vercesi et al. 1999). The macrosporangia contain between 30-56 zoospores (Lafon and Clerjeau...
1988). The macrosporangia or the individual zoospores they contain, or a combination of both are spread from the soil layer to the underside of leaves via rainsplash or wind (Gessler et al. 2011). Zoospores infect the leaf via the stomata and this can occur at a range of temperatures (Williams et al. 2007a). It is assumed the infection process is then the same as for secondary infection (Gessler et al. 2011). Williams et al. (2007c) has shown in the absence of favourable climatic conditions and the availability of free water downy mildew sporangia have the capacity to directly germinate. The infectivity and subsequent effect this has in causing a downy mildew epidemic is unknown (Williams et al. 2007c).

It is considered a rule of thumb for oospore driven primary infection to occur at least 10mm of rainfall, temperature ≥10°C over a 24hr period is required (Nicholas et al. 1994; Rossi and Caffi 2012). However, Rossi and Caffi (2012) found if germinated oospores are present in the leaf litter any rainfall event can transfer inoculum to the grapevine leaves. Whilst infection severity was unrelated to the amount or intensity of rainfall the probability of infection was higher in the lower canopy (Rossi and Caffi 2012). Increased rain intensity did lead to greater coverage splashed by drops (Rossi and Caffi 2012).

After colonisation of the stomata via primary infection *P. viticola* develops an extensive network of mycelium in the intracellular spaces of the mesophyll tissue (Kortekamp 2005). The mycelium produces many haustoria, capable of penetrating the cell walls of the mesophyll (Burruano 2000). After penetration, enzymes are released that degrade polymers into simple sugars, amino acids and fatty acids which are metabolised by *P. viticola* (Luis et al. 2013). Usually, four to five days after infection typical oilspot lesions appear on the adaxial surface of the leaf (Burruano 2000) but this can be longer, up 25 days, depending on temperature and relative humidity (Rosa et al. 1993).
Maturation and germination of oospores

Oospore development begins with the formation of male and female gametangia, appearing as swollen hyphal tips, and pair through facilitation of an adhesive secreted by the antheridium (Judelson 2009). Meiosis occurs simultaneously within each gametangia whilst a fertilization tube develops and a single haploid nucleus is transferred from the antheridium to the oogonium (Judelson 2009). A single diploid nucleus establishes within the oogonia from up to 10 nuclei that originally form (Judelson 2009). After fertilization a plug is formed to isolate the oogonial cytoplasm from the rest of the thallus and maturation involves the establishment of a thick multilayered wall (Judelson 2009).

The oospore remains dormant and will not germinate, even under favourable conditions, because of undetermined internal physiological dormancy, only broken with age (Rossi and Caffi 2007). Externally, water availability in the leaf litter and temperature are key factors in breaking dormancy and germination (Rossi and Caffi 2007). Water can be supplied to the leaf litter via rainfall, dew or absorption from atmospheric humidity and therefore oospore maturation can occur without the specific requirement of rainfall (Rossi and Caffi 2007). The process of oospore germination occurs from a minimum of 10°C to 28°C, with optimum of between 20-22°C (Burruano et al. 2006). Optimal germination occurs if the outer wall is ruptured, possibly after a light freeze and sufficient humidity (Gessler et al. 2011).

Historically, downy mildew epidemics were believed to be caused by a limited number of successful oospore germination and infection events, followed by widespread asexual multiplication and spread via secondary infections (Gobbin et al. 2005; Rossi et al. 2008a). However, using a floating leaf disk test Hill (1998) showed oospore infection events occurred from bud burst to midseason or later in cooler regions receiving summer rainfall. Through the use of SSR markers Gobbin et al. (2005) was able to show new genotypes were
continuously added to the disease epidemic and only approximately 25% of those samples underwent asexual infections.

It was believed the germination of oospores occurred rapidly with the production of germ tube and terminal macrosporangia (Gregory 1912). However, Jermini et al. (2003) found the variability in the time taken to produce macrosporangia to be between 0 to 22 days, with the number of macrosporangia produced per oospore containing leaf being large. For any vineyard the population of oospores will consist of different age-physiological cohorts, germinating at different rates with their development influenced by climatic conditions and possible leaf effects (Jermini et al. 2003). This could create variable oospore germination not only in the year following formation and maturation but may extend up to the following 3 years (Emmett et al. 1992b). A study on the long term viability of oospores in Italian vineyards found oospores were capable of germination for up to 65 months (~5.4 years) (Caffi et al. 2011).

Secondary infection

Under favourable conditions; relative humidity of 95-100%, minimum temperature 13°C and at least 4 hours of darkness the oomycete sporulates by producing tree-like sporangiophores and sporangia which emerge through the stoma on the abaxial side of the leaf (Blaeser and Weltzien 1978; Gessler et al. 2011; Lafon and Clerjeau 1988). Several sporangiophores may emerge from an individual stoma but only one matures while the others degenerate (Burruano 2000). The sporangia are detached from the sporangiophore via the dissolution of a cross wall callose (Lafon and Clerjeau 1988) and spread via wind or rain. The optimal temperature for sporulation is between 18-22°C (Lafon and Clerjeau 1988), however in Italy sporulation has been observed between 10.6 and 32.9°C (Caffi et al. 2013).
Once sporulation occurs the lesion can continue sporulating for several days. Caffi et al. (2013) found lesions sporulated from between 2 and 10 days with sporangia produced at a high rate for the first 4 days then at a lower rate until sporulation ceased. Longevity and productivity of foliar lesions has been linked to how often they have been induced to sporulate; under conditions promoting sporulation every 1-2 days lesion productivity fell 90% over a 10 day period, whereas under sub-optimal, non-sporulating conditions lesions maintained their production potential for 22-24 days (Kennelly et al. 2007). The nutrient status of the leaf tissue on which the lesion occurs may also play a role in the duration of the lesion sporulation but this has yet to be explored (Caffi et al. 2013).

Despite the high productivity and longevity of individual lesions in producing sporangia the success rate of secondary infections is low (Gobbin et al. 2005). The total number of sporangia produced per 1mm² over the lifetime of a lesion can range from 382 to 2.7 million, but sporangia production from individual lesions is influenced greatly by humidity, the number of dark moist hours and the maximum temperature during the sporulation period (Caffi et al. 2013). As a result, long range sporangia migration via secondary infection may have been overestimated in the past (Gobbin et al. 2005).

**Overwintering as mycelium**

The ability of *P. viticola* to overwinter as mycelium has been noted as far back as 1904 (Barrett 1939). It is believed in regions experiencing mild winters, such as California, the disease can survive as mycelium in buds and persistent leaves (Barrett 1939; Lafon and Clerjeau 1988). However, as noted by Killigrew (2006) these statements are not supported by references or experimental data. Microscopic analysis of grapevine wood was found to contain mycelium of *P. viticola* in Georgia (Chrelashvili 1993) but there is no indication as to whether it was capable of spore production the following season.
If *P. viticola* were to possess the ability to overwinter in dormant grapevine buds, this would not make it unique. Powdery mildew of grapevines caused by the biotroph *Erysiphe necator* can overwinter in grapevine buds and produce shoots in the following season covered in inoculum (Rumbolz and Gubler 2005; Ypema and Gubler 2000). Known as ‘flag shoots’ these are the main source of primary infection in most vineyards in Australia (Emmett et al. 2006).

Killigrew (2006) showed through both epi-fluorescence microscopy and detection of DNA that *P. viticola* can successfully colonise grapevine buds and tendrils. However, bud inoculation experiments in this study were only conducted over a 5-6 week period. Infections were not tracked over the grapevines’ entire dormancy period, and the ability of the disease to reinfect in subsequent spring periods was not investigated. Although cuttings of infected wood were collected from previous season’s shoots and buds it was noted that the detection of *P. viticola* in compound buds >12 months old was difficult due to characteristic hyphae of *P. viticola* being absent (Killigrew 2006). Both grapevine leaves and berries are susceptible to infection when young but as they age develop ontogenic resistance to downy mildew (Kennelly et al. 2005; Steimetz et al. 2012).

Lesion productivity and sporangia survival is influenced by humidity, temperature and the phenological stage of the vine (Kennelly et al. 2007) and it therefore remains unclear as to whether the survival of *P. viticola* as mycelium plays a significant role in any disease epidemic (Hall 1989).

The development of microsatellite markers for *P. viticola* has allowed a greater understanding of the population structure of the pathogen. Microsatellite data from the Greek islands indicated three genotypes were present over two consecutive epidemics and it was proposed this was due to the ability of the disease to overwinter asexually (Rumbou and
Gessler 2006). It is possible asexual overwintering may also occur in South African populations of *P. viticola* (Koopman et al. 2007). The low genetic diversity seen in populations in WA (Hug 2005) and absence of oospores in many vineyards in WA (Killigrew et al. 2005) supports the hypothesis of asexual survival of *P. viticola*. Both of these studies were conducted within six years of the downy mildew incursion in WA and it is possible the population structure of the disease has changed since. If genotypes from natural infections were matched to those of systemically infected grapevine buds or mycelium surviving over a >12 month period then this would indicate the ability of the disease asexually overwinter in grapevine material.

**Economic impact**

*P. viticola* has a direct and indirect economic cost to viticulture production. Infection causing a loss of foliage and inflorescence can range from 50-100% if conditions are favourable for the disease (Emmett et al. 1992b). Infection also results in a reduction in photosynthetic capacity of the area surrounding the lesion (Caffi et al. 2010a). This leads to a reduction in the carbohydrate reserves of the vine, leading to a loss of vigour and yield in subsequent seasons (Emmett et al. 1992b). Prior to the introduction of chemical control measures significant yield losses were sporadically recorded in France, Germany and Italy with some losses up to 70% (Gessler et al. 2011). Several vineyard-level economic analyses have been performed to ascertain the impact of downy mildew in the various climatic regions of Australia. For instance, Scholefield and Morison (2010) found profit reduction ranged from AUD 218 in the region to $814/ha/annum region. Cumulative annual losses and costs of control have been estimated in low rainfall years to be over $13 million and greater than $47 million in wet seasons for Australian producers (Magarey et al. 1991).
While these microeconomic studies are important from a regional perspective, they are difficult to use when providing policy advice at the level of the state. To date, only one aggregate analysis is presented in the literature where annual costs of downy mildew in Australia are estimated to be AUD $32-64 million (Magarey and Butler 1998). This broad estimate uses a static approach to cost estimation, and does not explore the intertemporal effects of the disease. Intertemporal being the impact of the current choices made and how these affect the options available in the future. Hence, it is not known if the economic significance of downy mildew is increasing or decreasing over time.

Generic economic models estimating the consequences of pest and diseases over time have been developed in recent years (Cook et al. 2011). These models are derived from the reaction diffusion models originally developed by Fisher (1937) which have been shown to provide a reasonable approximation of the spread of a diverse range of organisms (Cook et al. 2011; Cook et al. 2012; Dwyer 1992; Holmes 1993; McCann et al. 2000; Okubo and Levin 2001). However, to date these models have not been applied to downy mildew.
**Purpose of this thesis**

It has been 20 years since grape downy mildew was first detected in commercial viticulture in WA and there have been significant developments in the understanding of the disease since. Using molecular tools, economic analysis and information on the disease lifecycle this thesis aimed to answer some remaining gaps in the knowledge of the disease in a Mediterranean climate such as WA. More specifically the thesis aimed to:

- Characterise the evolutionary history of *P. viticola* in WA and Australia including its probable source of origin or origins? How many potential introductions have occurred in WA? The current genotypic structure across Australia and how this compares to known populations overseas? Whether a single or multiple cryptic species exist in Australia? This will provide insight into the introduction of important agricultural pathogens and the potential by which they survive the WA climate.

- To determine the survival of *P. viticola* between growing seasons in WA, be it via the production of oospores or as mycelium overwintering in dormant grapevine buds? Whether *P. viticola* infection in buds can produce downy mildew “flag shoots” the following season? If green grapevine buds are susceptible to *P. viticola* infection and subsequent sporulation? Do both mating types of *P. viticola* exist within WA? And has the presence of *P. viticola* oospores increased within grape growing regions of WA since its initial detection in the late 1990’s? Understanding how the pathogen survives overwinter in WA is important as commercial management decisions are based around this aspect of its lifecycle.

- What has the economic cost of the *P. viticola* incursion into WA been on grape growers and the community? The economic impact of this incursion event can be used to model and assess the potential impact of other important agricultural pathogens of biosecurity risk to WA.
Chapter 2


2. Abstract

Grapevine downy mildew, caused by *Plasmopara viticola*, is a serious economic disease for Australian viticulture. Using different detection techniques 51 new and revised microsatellite primers have been developed for *P. viticola* based on American and European isolates. Thus far, only a subset of the developed microsatellites have been tested for polymorphisms among Australian *P. viticola* populations. This study tests all 51 microsatellites, under the same PCR conditions, for polymorphism on Australian isolates from geographically distinct regions. Of the 51 microsatellites, 18 showed polymorphism between the isolates tested and or *P. viticola* GenBank sequences. A selection of these microsatellites will be used for a larger population analysis study on Australian and international isolates.

2. Introduction

Grapevine downy mildew, caused by the oomycete *Plasmopara viticola*, is considered one of the most destructive diseases of grapevines, especially in regions experiencing warm and humid growing seasons (Wilcox et al. 2015). The disease spread worldwide with the movement of grapevine material and is now recorded in some 98 countries, with a range of climatic growing conditions (CABI 2017). It was first recorded in Australia in 1917 and is known to cause significant losses during sporadic wet growing seasons (de Castella and Brittlebank 1917; McLean et al. 1984). Despite infrequent crop failures significant input costs
are spent annually on fungicides to prevent potential epidemics from occurring (Scholefield and Morison 2010).

In recent years a total of 51 new and revised microsatellite or simple sequence repeat (SSR) primers for *P. viticola* have been developed for use in population analysis (Delmotte et al. 2006; Gobbin et al. 2003b; Matasci et al. 2010; Rouxel et al. 2012). The primer regions in each study were detected independently using different microsatellite detection techniques and with different optimal PCR conditions. European and American populations were the focus of primer development (Delmotte et al. 2006; Gobbin et al. 2003b; Rouxel et al. 2012).

To date, a comparison of all 51 primers under the same PCR conditions has yet to be made. In Australia the genetic diversity of the downy mildew population has only been tested using 4 of the primers developed by Gobbin et al. (2003) on a limited number of growing regions and isolates (Hug 2005; Williams 2005). Using three isolates from three geographically distinct viticulture regions in Australia, Western Australia (WA), Victoria (VIC) and Queensland (QLD) this study aims to test all 51 primers generated by two research groups under the same conditions. The purpose was to evaluate a set of primers that were (1) polymorphic using Australian isolates, (2) reliably amplified using standard reagents, and (3) amplified using the same temperature profiles for PCR thus enabling multiplexing, where multiple microsatellites are amplified in the same PCR reaction.

2. Materials and Methods

*Samples*

Infected leaves containing oilspots were collected from vineyards in WA, VIC and QLD and sent via post to WA for analysis. Individual leaves were placed in individual ziplock bags to prevent any contamination of potential genotypes. Only leaves with distinct
individual oilspots were considered for assessment. Individual sporangia were bulked up on detached Chardonnay leaves in the laboratory prior to DNA extraction.

**DNA Extraction and PCR amplification**

DNA was extracted from a one centimetre squared section of a sporulating oilspot. Extracts were performed as described by the Powerplant® Pro DNA Isolating Kit protocol (Mo Bio Laboratories, California, USA). A total of 60-80µl of elution buffer was used at the final step.

PCR amplifications were carried out with a final volume of 25µl containing 5µl 5x colourless buffer (Promega, Wisconsin, USA), 2.5µl MgCl₂ solution (25mM), 1µl bovine serum albumin (10mg/mL Fisher Biotec, Western Australia), 1.5µl dNTP (10mM Promega, Wisconsin, USA), 0.5µl of unlabelled forward and reverse primer (10µM), and 0.125 U of GoTaq® Hot Start polymerase (5u/µl, Promega, Wisconsin, USA) and 2ul of DNA. PCR reactions were performed using a BioRad T100 Thermocycler with the following conditions: 94°C for 4 min, 35 cycles at 94°C for 30s, 55°C for 30s, 72°C for 35s with final extension of 72°C for 5 min.

PCR products were viewed on a 1% agarose gel to which Sybr safe DNA gel stain (4µl) was added. 1µl of loading dye was added to 5µl of product and compared against a 100bp ladder (Axygen Bioscience, California, USA) and a negative control. Gels were run for 1.5hrs at 75V. Those primers with poor amplification in one or all isolates or showed no polymorphism between isolates were discarded from further analysis.

Initially, a subset of microsatellites were chosen for PCR amplification with serial dilution of DNA to determine optimal concentration. Undiluted DNA was compared with 1 in 10 and 1 in 100 dilutions on the same 1% agarose gel, as described above. A DNA dilution
of 1 in 10 was found to provide the optimal band strength for the initial test microsatellites and was subsequently used in all PCR amplifications.

**Sequencing**

The PCR product was cleaned by passing through a Sephadex G-50 spin column (Sigma Aldrich, Missouri, USA) as described previously (Sakalidis 2011). A sequencing reaction was performed for the forward and reverse of each primer. Sequencing reactions of 10µl consisted of 1µl of sequence buffer, 2µl of BigDye, 0.5µl of primer, and 2.5µl of the PCR cleaned product. The sequencing reaction were performed using a GeneAmp PCR system 2700 (Applied Biosystems, California, USA) with the following conditions: 96°C for 2 min, 25 cycles at 96°C for 10s, 50°C for 5s, 60°C for 4min.

The sequencing product was cleaned via Sephadex G-50 spin columns as described earlier except only 10 µl of product was passed through the column. The cleaned product was then sent to Australian Genome Research Facility (AGRF, [http://www.agrf.org.au/](http://www.agrf.org.au/)) for sequencing.

Sequence similarities of the Australian isolates were compared with *P. viticola* microsatellite sequences lodged in GenBank using the Geneious v9 software [http://www.geneious.com/](http://www.geneious.com/) (Kearse et al. 2012). No microsatellite sequences for the primers designed by Gobbin et al. (2003) and Matasci et al. (2010) were available on GenBank.

2. Results

Of the 51 microsatellites tested 12 (Pv7, Pv76, Pv91, Pv93, Pv96, Pv102, Pv126, Pv127, Pv134, Pv136, Pv138 and REX) presented weak or no bands for one or all three isolates when viewed on the agarose gel. They were subsequently removed from further analysis (Table 1).
Visually, there appeared no difference between the microsatellites ISA, BER, CES and GOB and the revised versions of the same primers, when compared on the agarose gel. Strong bands were observed for all microsatellites and all three Australian isolates. Distinct size differences between the isolates were observed for microsatellites ISA, GOB and CES. The BER microsatellite appeared monomorphic between the Australian isolates.

Based on sequence data a number of microsatellites showed polymorphism within the different Australian isolates and those of the GenBank sequence including Pv14, Pv65, Pv74, Pv104, Pv133, Pv137, Pv140, Pv142, Pv143, Pv146, Pv147, Pv148 (Table 1). Of the remainder microsatellites Pv13, Pv39, Pv61, Pv103 differ to the sequence available on GenBank but were monomorphic among the Australian isolates. Primers Pv16, Pv17, Pv31, Pv67, Pv83, Pv87, Pv88, Pv100, Pv101, Pv124, Pv135, Pv139, Pv141, Pv144, Pv145 were not polymorphic among the Australian isolates and the GenBank sequence (Table 1).
Table 2.1. Assessment of 51 microsatellite primers developed for grapevine downy mildew on Australian isolates.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Source</th>
<th>GenBank accession</th>
<th>Size*</th>
<th>Sequenced</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>BER</td>
<td>F:5’AAATGCAATGGCTTCTCACCTCG, R:5’CTCTGCCTGAAAAGGCCTGC</td>
<td>Gobbin et al. 2003</td>
<td></td>
<td>179-185</td>
<td>y</td>
<td>Monomorphic(^1)</td>
</tr>
<tr>
<td>CES</td>
<td>F:5’CTTGCAGTGAAGGCTG, R:5’GCTCTACCTCACAATCTCAG</td>
<td>Gobbin et al. 2003</td>
<td></td>
<td>143-186</td>
<td>y</td>
<td>Polymorphic(^2)</td>
</tr>
<tr>
<td>GOB</td>
<td>F:5’CTTGGAAAGTATTACATGCTACC, R:5’TTGAGAACACGACAGCTTA</td>
<td>Gobbin et al. 2003</td>
<td></td>
<td>210-434</td>
<td>y</td>
<td>Polymorphic(^2)</td>
</tr>
<tr>
<td>ISA</td>
<td>F:5’ATTAGCGGAGTGGACGTG, R:5’GAGAAGTCTCGCAGAATGTA</td>
<td>Gobbin et al. 2003</td>
<td></td>
<td>118-144</td>
<td>y</td>
<td>Polymorphic(^2)</td>
</tr>
<tr>
<td>REX</td>
<td>F:5’CGTGTGCGATAGCCAAACTT, R:5’TTGCATTCGAGATCATGTA</td>
<td>Gobbin et al. 2003</td>
<td></td>
<td>164</td>
<td></td>
<td>Weak or no bands on gel</td>
</tr>
<tr>
<td>BER NEW</td>
<td>F:5’CAAGCAATGGCTACGTCTC, R:5’GGCTACCTCTGACTGCTC</td>
<td>Matasci et al. 2010</td>
<td></td>
<td>179-185</td>
<td>y</td>
<td>Monomorphic(^1)</td>
</tr>
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<td>CES NEW</td>
<td>F:5’CTTGTGCTGAGCTG, R:5’CATCACATGTTGTAAGTCTG</td>
<td>Matasci et al. 2010</td>
<td></td>
<td>143-186</td>
<td>y</td>
<td>Polymorphic(^2)</td>
</tr>
<tr>
<td>GOB NEW</td>
<td>F:5’CTTGGAAAGTATTACATGCTACC, R:5’ATGGAGTCTACATTGACATC</td>
<td>Matasci et al. 2010</td>
<td></td>
<td>210-434</td>
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<td>ISA NEW</td>
<td>F:5’GGGATGGGGTACAGCTAC, R:5’GAGATTCGCAACAGCATGCA</td>
<td>Matasci et al. 2010</td>
<td></td>
<td>118-144</td>
<td>y</td>
<td>Polymorphic(^2)</td>
</tr>
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<td>Pv7</td>
<td>F:5’CTTTCCAGAAGGGAGGTTA, R:5’GGCTACCTCTGCATCTCTGAGAAA</td>
<td>Delmotte et al. 2006</td>
<td>DQ217575</td>
<td>289-297</td>
<td>y</td>
<td>Weak or no bands on gel</td>
</tr>
<tr>
<td>Pv13</td>
<td>F:5’CGATGATAGGTGCCACATT, R:5’CGGTGACATTGCACCTCG</td>
<td>Delmotte et al. 2006</td>
<td>DQ217576</td>
<td>214-220</td>
<td>y</td>
<td>Not polymorphic(^3)</td>
</tr>
<tr>
<td>Pv14</td>
<td>F:5’CAGAATAATATCTGCCGCTAG, R:5’AATGGCATATCTGACGACCG</td>
<td>Delmotte et al. 2006</td>
<td>DQ217577</td>
<td>120-128</td>
<td>y</td>
<td>Polymorphic(^4)</td>
</tr>
<tr>
<td>Pv16</td>
<td>F:5’TAAATTAGTGGTGCGCTCA, R:5’TCGGAGTCTTCAGGATGCTG</td>
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<tr>
<td>Pv17</td>
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<td>DQ217579</td>
<td>160-172</td>
<td>y</td>
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<td>Pv31</td>
<td>F:5’TCCCTACGATGAAAGCTTCC, R:5’TTTTTCTAAGGCCTGGTG</td>
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<td>DQ217580</td>
<td>241-247</td>
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<td>Monomorphic(^5)</td>
</tr>
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<td>Pv39</td>
<td>F:5’ACGCAATGGGCCTGACGTG, R:5’CAACAGAGGAGGTTGCTGAGTG</td>
<td>Rouxel et al. 2012</td>
<td>DQ217581</td>
<td>174-176</td>
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<td>Pv61</td>
<td>F:5’TCTTCTAATGGATAGGCGAC, R:5’GGTACCTCCGGAGCTGAATA</td>
<td>Rouxel et al. 2012</td>
<td>JQ219983</td>
<td>181-187</td>
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<td>Not Polymorphic(^3)</td>
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<td>Pv65</td>
<td>F:5’CTTTGTGCCCAGCAGCTGATG, R:5’CGCTTTGGCTAGTCCATTA</td>
<td>Rouxel et al. 2012</td>
<td>JQ219972</td>
<td>196-202</td>
<td>y</td>
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<td>Name</td>
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</tr>
<tr>
<td>Pv74</td>
<td>F’S:GCAACGTGTCGAAGCCTTGA, R’S:GCATTATGAGGACCTCAGG</td>
<td>Rouxel et al. 2012</td>
<td>JQ219984</td>
<td>176-182</td>
<td>y</td>
<td>Polymorphic$^4$</td>
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<td>Pv76</td>
<td>F’S:CTGTTGCTGATGCAGCTGAC, R’S:GGCGGTGACTAAGTCTGTG</td>
<td>Rouxel et al. 2012</td>
<td>JQ219974</td>
<td>136-140</td>
<td></td>
<td>Weak or no bands on gel</td>
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<tr>
<td>Pv78</td>
<td>F’S:TCGAGCATGTTTCTCATCAT, R’S:ACCGGTACTTTTTCGCCCTC</td>
<td>Rouxel et al. 2012</td>
<td>JQ219985</td>
<td>238-242</td>
<td>y</td>
<td>Monomorphic$^5$</td>
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<tr>
<td>Pv87</td>
<td>F’S:CTGTCATTTCAACACAGG, R’S:CTATAAAAGCAGCTGAGCA</td>
<td>Rouxel et al. 2012</td>
<td>JQ219986</td>
<td>152-154</td>
<td>y</td>
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<td>Pv91</td>
<td>F’S:ACACGCTTTCGCGAAGATAA, R’S:TGAAAGTTACGTGTCGCC</td>
<td>Rouxel et al. 2012</td>
<td>JQ219975</td>
<td>142-146</td>
<td></td>
<td>Weak or no bands on gel</td>
</tr>
<tr>
<td>Pv93</td>
<td>F’S:TTCACACCGGACTAGCGGTAT, R’S:GTACCTGTCAGCCCTCTC</td>
<td>Rouxel et al. 2012</td>
<td>JQ219976</td>
<td>147-151</td>
<td></td>
<td>Weak or no bands on gel</td>
</tr>
<tr>
<td>Pv96</td>
<td>F’S:TAGCTTCCATTTTCGCGGTG, R’S:ATACCTGTAAAGCAGGAA</td>
<td>Rouxel et al. 2012</td>
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<td>Pv100</td>
<td>F’S:TGATAAGATACCGCAGACGG, R’S:TTGTATTGAAGCAGTGAACGC</td>
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<td>JQ219978</td>
<td>231</td>
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<td>Pv101</td>
<td>F’S:ACACCGCGCAGAAGATTTA, R’S:GGGATATACGTGCAAAATCTC</td>
<td>Rouxel et al. 2012</td>
<td>JQ219979</td>
<td>263-266</td>
<td>y</td>
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</tr>
<tr>
<td>Pv102</td>
<td>F’S:GATGCCTTTGTGCAATGTCT, R’S:AAAGGATCTAATGCACGTGCG</td>
<td>Rouxel et al. 2012</td>
<td>JQ219980</td>
<td>273</td>
<td></td>
<td>Weak or no bands on gel</td>
</tr>
<tr>
<td>Pv104</td>
<td>F’S:CTACGCTCAGGAGTGACACA, R’S:GACATTGGCGACCTAAAGAT</td>
<td>Rouxel et al. 2012</td>
<td>JQ219982</td>
<td>321-324</td>
<td>y</td>
<td>Polymorphic$^4$</td>
</tr>
<tr>
<td>Pv124</td>
<td>F’S:ACGACAGAGGCGGATTCTGC, R’S:GACCTGAGGCCTTGGAC</td>
<td>Rouxel et al. 2012</td>
<td>JQ219988</td>
<td>139-142</td>
<td>y</td>
<td>Monomorphic$^5$</td>
</tr>
<tr>
<td>Pv126</td>
<td>F’S:GCTCTCTGAGAGGTACGTGCG, R’S:GCCCTTCTAGGTTCAGGCC</td>
<td>Rouxel et al. 2012</td>
<td>JQ219989</td>
<td>182-206</td>
<td></td>
<td>Weak or no bands on gel</td>
</tr>
<tr>
<td>Pv127</td>
<td>F’S:GTGAACAGGAGGAGATTGCCAGAAGAC, R’S:GAACTGCTCAGCCATAGGATTGT</td>
<td>Rouxel et al. 2012</td>
<td>JQ219990</td>
<td>213-223</td>
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<td>Weak or no bands on gel</td>
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<tr>
<td>Pv133</td>
<td>F’S:ACGCACACAGGAGATTCTGC, R’S:GCACCTGCTCTTACCTTC</td>
<td>Rouxel et al. 2012</td>
<td>JQ219991</td>
<td>178-181</td>
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<td>Source</td>
<td>GenBank accession</td>
<td>Size*</td>
<td>Sequenced</td>
<td>Comment</td>
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<tr>
<td>Pv134</td>
<td>F:5’CATGCTCACGTAGACCTCCA, R:5’AATGCAGAGCTCCCATACAAGG</td>
<td>Rouxel et al. 2012</td>
<td>JQ219992</td>
<td>220-226</td>
<td></td>
<td>Weak or no bands on gel</td>
</tr>
<tr>
<td>Pv135</td>
<td>F:5’GGTGCTCTGCTTCGACACTT, R:5’CGCCACACAAGTCAACCTTCC</td>
<td>Rouxel et al. 2012</td>
<td>JQ219993</td>
<td>217-220</td>
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<td>Monomorphic</td>
</tr>
<tr>
<td>Pv136</td>
<td>F:5’GGTGCTCGTGGAAACAGAGC, R:5’ATCGCTCTGCCAGAATGAC</td>
<td>Rouxel et al. 2012</td>
<td>JQ219994</td>
<td>161-164</td>
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<td>Weak or no bands on gel</td>
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<tr>
<td>Pv137</td>
<td>F:5’AAGTGCGACACATCAAGG, R:5’TGGCAATAAATTTATGCAGT</td>
<td>Rouxel et al. 2012</td>
<td>JQ219995</td>
<td>243-256</td>
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</tr>
<tr>
<td>Pv138</td>
<td>F:5’CGACGACATGCTCAGTACG, R:5’GAATTTGCGAAGGAGAT</td>
<td>Rouxel et al. 2012</td>
<td>JQ219996</td>
<td>225-235</td>
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<td>Pv139</td>
<td>F:5’GACCGCGCAATGGACTCTA, R:5’CGCGCAAATATTGAAAGCTG</td>
<td>Rouxel et al. 2012</td>
<td>JQ219997</td>
<td>126-133</td>
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<tr>
<td>Pv140</td>
<td>F:5’GCTTGAAGAAATGGGAAAGCG, R:5’CCCAGAAGGCTTATGACG</td>
<td>Rouxel et al. 2012</td>
<td>JQ219998</td>
<td>172-201</td>
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<td>Pv141</td>
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<td>Rouxel et al. 2012</td>
<td>JQ219999</td>
<td>190-192</td>
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<tr>
<td>Pv142</td>
<td>F:5’TTATGCGACACCAATCTCTG, R:5’AGGCGCAAATACGAGAGG</td>
<td>Rouxel et al. 2012</td>
<td>JQ220000</td>
<td>209-219</td>
<td>y</td>
<td>Polymorphic</td>
</tr>
<tr>
<td>Pv143</td>
<td>F:5’CTGAAATAAAGCAACACGCA, R:5’TGGCGCAAAATTGACG</td>
<td>Rouxel et al. 2012</td>
<td>JQ220002</td>
<td>121-135</td>
<td>y</td>
<td>Polymorphic</td>
</tr>
<tr>
<td>Pv144</td>
<td>F:5’ACGAAGAAATGCACCTAACG, R:5’GTCTGCGTTTATGCAGTA</td>
<td>Rouxel et al. 2012</td>
<td>JQ220003</td>
<td>161-192</td>
<td>y</td>
<td>Monomorphic</td>
</tr>
<tr>
<td>Pv145</td>
<td>F:5’GACTTGAAGGAAGCCATCCA, R:5’CTCTCTGAAACCTGGT</td>
<td>Rouxel et al. 2012</td>
<td>JQ220004</td>
<td>204</td>
<td>y</td>
<td>Monomorphic</td>
</tr>
<tr>
<td>Pv146</td>
<td>F:5’CTGCAGACCTGAGAAAAGCG, R:5’ACGTCGCCACCCAGG</td>
<td>Rouxel et al. 2012</td>
<td>JQ220005</td>
<td>242-245</td>
<td>y</td>
<td>Polymorphic</td>
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<tr>
<td>Pv147</td>
<td>F:5’TGCATACGAGGTCCAGAGG, R:5’TTCTAAGCTCAGAAGAAGCGT</td>
<td>Rouxel et al. 2012</td>
<td>JQ220006</td>
<td>189-219</td>
<td>y</td>
<td>Polymorphic</td>
</tr>
<tr>
<td>Pv148</td>
<td>F:5’CGAATGATGTTTGGCGTTTATT, R:5’GAACGCTGAGAGGACGT</td>
<td>Rouxel et al. 2012</td>
<td>JQ220001</td>
<td>134-137</td>
<td>y</td>
<td>Polymorphic</td>
</tr>
</tbody>
</table>

*Size ranges from the associated published source. Monomorphic based on visual examination of gel. Polymorphic based on visual examination of gel. Australian isolates identical to each other but do differ from sequence on GenBank. Polymorphic among Australian isolates based on sequence data. Australian sequences identical to each other and the sequence on GenBank.
2. Discussion

Despite the use of only three Australian isolates in this study, 18 of the 51 microsatellites developed for *P. viticola* have exhibited polymorphism, either visually on agarose gels or within sequence datum. The three isolates were chosen due to the large geographical distances between them and the expectation of variability within those populations, based on details of the first detection of *P. viticola*, being 1917 for Victoria (de Castella and Brittlebank 1917), early 1920’s for Queensland (Emmett et al. 1992a) and 1998 for Western Australia (McKirdy et al. 1999). It would be expected those microsatellites exhibiting polymorphism amongst these three isolates could be used for an expanded number of isolates collected from grape production regions across Australia.

Limited comparisons can be made from the two previous genetic studies on Australian isolates of grape downy mildew by Hug (2005) and Williams (2005) as they only tested the four primers (ISA, BER, CES and GOB) developed by Gobbin et al. (2003b). Comparisons are further complicated as there is no sequence data available for these primers on GenBank. However, the BER primer was considered monomorphic amongst isolates tested from WA, SA, NSW and VIC by Hug (2005) but polymorphism was found amongst isolates from WA in the Williams (2005) study. There were no differences between the number and allele sizes between isolates for the BER primer from NSW, QLD and SA (Williams 2005). Based on visual assessment of the agarose gel this study has found no distinct differences between the isolates from VIC, QLD and WA.

Of the microsatellites tested, Pv13, Pv17, Pv65, Pv74, Pv103, Pv137, Pv140, Pv142, Pv143, Pv146, Pv147, Pv148, BER New, CES New, GOB New, ISA New will be used for a population study on a larger number of isolates from across Australia (Chapter 3). Although some of these microsatellites did not exhibit polymorphism amongst the tested isolates (Pv13,
Pv17, Pv103, BER New) they were chosen based on the published size range of the fragment so the number of primers could be maximised in the fewest multiplex reactions required. As northern hemisphere isolates are to be included in the larger study these four primers will provide comparison between American and European isolates and those from Australia. Polymorphism has been found within and between isolates from Europe and America for Pv13, Pv17, Pv103 and BER NEW (Delmotte et al. 2006; Gobbin et al. 2006; Rouxel et al. 2012).
Chapter 3

Population genetic structure of *Plasmopara viticola* in Australian vineyards with emphasis on the Western Australian population.

3. Abstract

Downy mildew of grape, caused by *Plasmopara viticola*, has been a major pathogen affecting vineyards in Australia for over a century. However, the disease was not observed in commercial production areas of Western Australia until 1998. The source of the *P. viticola* population in Western Australia remains unknown while the genetic diversity of the pathogen in other Australian states has rarely been investigated in contrast to populations in the northern hemisphere. DNA was extracted from 381 *P. viticola* samples of *Vitis vinifera* and alternate hosts, collected via fresh and herbarium leaves from populations within Australia and FTA cards from North America, Brazil and Uruguay. 32 DNA samples were provided from a French population. The populations were analysed and compared using 16 microsatellite markers. Representative samples from within Australia, Brazil and Uruguay were also tested to determine which cryptic *P. viticola* species are present. Our findings suggest the Australian and South American populations of *P. viticola* are more closely related to the European population than the North American, the reported source of origin of the pathogen. The Western Australian population had similarities to the South Australian population and the tight clustering of samples suggests a single introduction into Western Australia. *P. viticola* clade *aestivalis* was the only cryptic species detected in Australian and South American populations. Analysis of the Western Australian population suggests it is reproducing clonally, but further research is required to determine the mechanism as to how this is occurring.
3. Introduction

Grapevine downy mildew caused by the oomycete *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni is a global pathogen recorded in over 90 countries worldwide (CABI 2017; Emmett et al. 1992b). Endemic to eastern north America, it is believed *P. viticola* was imported to Europe with native American *Vitis* species used as rootstock to prevent infection from the insect grape phylloxera; which at the time was causing significant losses for European viticulture (Fontaine et al. 2013; Gessler et al. 2011; Gobbin et al. 2006; Rossi et al. 2013; Rouxel et al. 2014; Viennot-Bourgin 1981). After its introduction to France in 1878 it spread rapidly eastward into major grape production regions of Europe and over the Mediterranean Sea to Algeria by 1881 (Fontaine et al. 2013; Galet 1977; Gessler et al. 2011; Rossi et al. 2013). The rapid movement was believed to have been aided by human mediated transfer of grapevine material throughout Europe (Fontaine et al. 2013; Gobbin et al. 2006). Further human aided global spread followed with detections in Brazil in 1893, South Africa in 1907, and New Zealand in 1926 (Koopman et al. 2007; Viennot-Bourgin 1981; Woodfin 1926).

The first Australian report of *P. viticola* was its occurrence in several commercial vineyards in Rutherglen, Victoria in 1917 (de Castella & Brittlebank, 1917). Following this detection, downy mildew spread rapidly within grape growing regions of eastern Australia, being recorded in New South Wales in 1918, South Australia and Queensland in 1920-21 and Tasmania in 1959 (Emmett et al. 1992a; Laffer 1918; Osborn and Samuel 1922; Quinn 1924). The rapid spread of the disease across eastern Australia was a combination of wind and human aided dispersal despite climatic conditions being considered less conducive for disease development than they are in Europe (Osborn & Samuel, 1922).
It was thought natural isolation from other grape growing regions (McLean et al. 1984), an absence of suitable spring and summer rainfall (Emmett et al. 1992b), and restrictions in movement of host material and machinery from other states and territories (McKirdy et al. 1999), P. viticola would be prevented from establishing in the state of Western Australia. However, in 1997 a detection occurred in Kalumburu in the north of the state on a small number of vines which were subsequently eradicated (Riley, 1998). This is a very remote area, accessible only by unsealed roads and separated by desert and approximately 2,200 km from commercial viticulture in the southwest of Western Australia. In October 1998 a further detection occurred in the commercial production area of the Swan Valley, in south west Western Australia, and it was soon determined that eradication was not possible (McKirdy et al. 1999). P. viticola has since been found in all growing regions of Western Australia (Williams et al. 2007a). The source of P. viticola in Western Australia remains unknown, as well as whether the 1997 and 1998 detections were related.

Recently, a number of cryptic species or clades, within the P. viticola species complex have been identified with specificity to different Vitis species and related hosts (Rouxel et al., 2014; Rouxel et al., 2013). The cryptic species were delineated through a combination of genetic analysis using multiple genes, sporangia morphology and virulence exhibited in cross-inoculated experiments of different host plants (Rouxel et al., 2013). As the type specimen of P. viticola is yet to be examined and greater morphological analysis is required a provisional nomenclature system of clades is used in place of forma specialis (Rouxel et al., 2014). A survey of eastern North America highlighted the presence and distribution of all P. viticola cryptic species throughout its centre of origin on native and introduced Vitis hosts (Rouxel et al., 2014), several of which are known to occur in Australia.
The development of *P. viticola* specific microsatellite markers has enabled a greater understanding of the genetic diversity in populations and subsequent spread dynamics of the pathogen in a number of different production systems (Delmotte et al. 2006; Gobbin et al. 2005; Gobbin et al. 2003b; Gobbin et al. 2006; Koopman et al. 2007; Kosev et al. 2015; Li et al. 2016; Rouxel et al. 2012). To date, the genetic diversity in the Australian grape downy mildew population has only been evaluated in a small number of growing regions with a limited number of isolates (Hug, 2005; Williams, 2005), using only four microsatellite markers developed by Gobbin et al. (2003b).

This chapter focuses on populations in Western Australia to determine the likely origin and population structure relative to Australian and global populations. Over 400 isolates were sampled, including herbarium specimens, to test several hypotheses. Isolates were genotyped using microsatellites. A series of questions were asked to characterize the evolutionary history of downy mildew in Western Australia: Did populations in Western Australia emerge from Australian populations? If not, were they introduced from Europe or North America? Were there multiple introductions into WA? What cryptic species are present in Australia? Is the population in Western Australia randomly mating? The answers to these questions will provide insights into how a grape pathogen established itself in Western Australia, a viticulture production area previously considered climatically unfavourable for establishment, and provide insights to avoid further introductions of pathogens in agricultural and natural ecosystems.

3. Materials and Methods

**Sampling**

*P. viticola* sample
A total of 413 samples (Appendix 2, Table A2.1) were collected as part of this study from Australia, United States of America, France, Brazil, and Uruguay as highlighted in Fig. 3.1. Samples were collected predominantly from *V. vinifera* but also included alternate hosts (Table 3.1).

Figure 3.1. Global sampling of *P. viticola* isolates indicating the number of samples from each state within each country. Circles indicate the location of isolate collection. (Map produced by P. Gardiner DPIRD).
Table 3.1. Summary of global sampling of *P. viticola* isolates indicating number and year of sampling, host species, sample type and collector.

<table>
<thead>
<tr>
<th>Country</th>
<th>State</th>
<th>Year*</th>
<th># samples</th>
<th>Vitis species</th>
<th>Sample type</th>
<th>Collector(s)</th>
</tr>
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<tr>
<td></td>
<td>QLD</td>
<td>2015, 2017</td>
<td>29</td>
<td>V. vinifera, V. labrusca</td>
<td>Fresh</td>
<td>Stephen Tancred</td>
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<td></td>
<td>NSW</td>
<td>1976, 2015, 2016</td>
<td>43</td>
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<td>Fresh, Herbarium</td>
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</tr>
<tr>
<td></td>
<td>SA</td>
<td>2013, 2015, 2016, 2017</td>
<td>65</td>
<td>V. vinifera</td>
<td>Fresh</td>
<td>Various</td>
</tr>
<tr>
<td></td>
<td>TAS</td>
<td>2015, 2017</td>
<td>8</td>
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<td>Fresh</td>
<td>Dennis Patten</td>
</tr>
<tr>
<td></td>
<td>VIC</td>
<td>2015, 2016</td>
<td>31</td>
<td>V. vinifera</td>
<td>Fresh</td>
<td>Various</td>
</tr>
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<td></td>
<td></td>
<td>28</td>
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<td>Andrew Taylor</td>
</tr>
<tr>
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<tr>
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<td></td>
<td>Virginia</td>
<td>2016</td>
<td>8</td>
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<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
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<td>DNA</td>
<td>Delmas et al. (2017)</td>
</tr>
</tbody>
</table>

*Year is considered the year of harvest to differentiate southern hemisphere production systems which run over calendar years.*
**Collection strategy**

*Australia:* The presence of *P. viticola* in Australian grape growing regions is sporadic with incidence and severity being restricted by the timely application of fungicides and climatic conditions (Wicks and Hall 1990; Wicks et al. 1991), making structured sampling regimes problematic. Therefore, in order to obtain a meaningful number of samples for analysis, vineyard owners and consultants across Australia were sent sample packs from 2014 to 2016 and asked to return infected leaves if present. Fresh samples were also collected opportunistically by the authors. GPS coordinates of the samples were also requested for data analysis purposes. A 1 cm² section was removed from individual oil spot lesions for DNA extraction. Herbarium specimens of *P. viticola* from leaves of dried host plants from the Western Australian Herbarium and Agricultural Scientific Collections Trust in New South Wales were also included in the study.

*North and South America:* *P. viticola* DNA from infected leaves from a range of *Vitis* host plants were collected during 2016 and 2017 by transfer to Whatman FTA micro cards (GE Healthcare Life Sciences, United Kingdom). Leaves with individual active oil spots were collected and kept overnight in humid zip-lock bags for sporulation. Using the direct leaf press method outlined in Whatman FTA Protocol BD05 each individual oil spot was transferred to an individual card. GPS coordinates and host cultivar were recorded on the card.

*France:* DNA samples from the Bordeaux region in France were kindly supplied by Delmas and co-authors (2017).
**DNA extraction**

Extractions from fresh leaf tissue were performed according to the Powerplant Pro DNA Isolation Kit protocol (Mo Bio Laboratories, California, USA). A total of 60-80µl DNA was eluted at the final step. For herbarium samples, the same protocol was used except the samples were ground using a micro-pestle with the addition of silica beads at the lysis stage. The homogenate was maintained at 55°C overnight prior to further steps. Only 50µl of the elution solution was used and this was heated to 37°C prior to use.

DNA extractions from FTA cards followed the protocols described by Owor et al. (2007) and Ndunguru et al. (2005) for viral DNA. Initially 3 leaf discs were punched from the cards and washed with 300 µl of Tris–HCl buffer (10mM Tris–HCl pH 8) for 5 minutes before removing the buffer. The discs were then sequentially washed for 5 minutes with 300 µl each of 70% ethanol and two washes with the FTA Purification Reagent (GE Healthcare Life Sciences, United Kingdom). After drying for two hours in a new 1.5mL eppendorf, 20 µl of Tris–HCl buffer (10mM Tris–HCl pH 8) was added and the solution was maintained overnight at 4°C.

**DNA sequencing and analysis**

For determination of cryptic *P. viticola* species, representative samples from each state in Australia and from the international sources were selected for sequencing of the partial ITS region with primers designed by Rouxel et al. (2013). PCR amplifications were carried out with a final volume of 25µl containing 5µl 5x colourless buffer (Promega, Wisconsin, USA), 2.5µl MgCl₂ solution (25mM), 1µl bovine serum antibody, 1.5µl dNTP (10mM Promega, Wisconsin, USA), 0.5µl of unlabelled forward and reverse primer (10µM), 0.125 U of GoTaq Hot Start polymerase (5u/µl, Promega, Wisconsin, USA), and 2ul of 1:10 diluted DNA. PCR reactions were performed using a BioRad T100 Thermocycler with the following conditions:
95°C for 4 min, 40 cycles at 95°C for 40s, 58°C for 45s, 72°C for 90s with final extension of 72°C for 10 min.

The PCR and sequencing products were cleaned using Sephadex G-50 spin columns (Sigma Aldrich, Missouri, USA) and sent to the Australian Genome Research Facility (AGRF, http://www.agrf.org.au/) for sequencing as described in Chapter 2. Representative sequences of the five known clades within the *P. viticola* species complex were used as a backbone to identify isolates recovered in the current study. Sequences were aligned using MAFFT algorithm plugin in the Geneious v9 software http://www.geneious.com/ (Kearse et al. 2012). Bayesian estimation of phylogeny was conducted using MrBayes plugin in Geneious using GTR+G substitution model over 1 000 000 generations and trees were saved each 1000 generations. Burnin was set at 200 000 generations well after likelihood values had converged to stationary, leaving 800 trees from which the consensus trees and posterior probabilities were calculated.

**Microsatellite multiplexing**

A total of 16 microsatellite primers were selected to be used for population analysis based on the evaluation of Australian *P. viticola* isolates in Chapter 2 (Appendix 2, Table A2.2). The 16 microsatellites were combined into 3 multiplex reactions based on previously published allele sizes (Delmotte et al. 2006; Gobbin et al. 2003b; Rouxel et al. 2012) using Multiplex Manager v1.2 (Holleley and Geerts, 2009). Each forward primer was assigned one of four fluorescent dyes (VIC, 6FAM, PET, NED, Applied Biosystems, California, USA (Table S1) and pig tails “GTTT” were added to the 5’ end of the reverse primer to reduce stutter peaks (Brownstein et al. 1996). Multiplex PCRs were performed with the Qiagen Multiplex PCR kit using the microsatellite cycling protocol, with a BioRad T100 Thermocycler, and an annealing temperature of 57°C for 35 cycles. Initially 25 µL reactions
were conducted but these were later reduced to 10 µL. The PCR products were diluted 1:100 in molecular grade ultrapure H$_2$O and 2 µL of the diluted product was added to 15 µL of HiDi™ Formamide (Applied Biosystems, California, USA) containing GeneScan™ 600 LIZ size standard v2.0 (Applied Biosystems, California, USA), with a ratio of 15 µL of standard to 1500 µL HiDi™Formamide. Samples were run using the GeneMapper50_POP7 module and G5 filter set on an ABI PRISM 3730XL automated DNA sequencer (Perkin-Elmer Applied Biosystems, Foster City USA). DNA from a single WA isolate was included in all submitted plates as a reference isolate to ensure PCR reproducibility. Allele sizes were scored using GeneMarker software (SoftGenetics, State College, Pennsylvania).

**Microsatellite data analysis**

Microsatellite allele sizes for each isolate were entered into GenAlEx 6.5 data format (Peakall and Smouse 2012). The formatted data was uploaded into the R package *poppr* 2.5.0. for population analysis (Kamvar et al. 2015; Kamvar et al. 2014). Raw data was initially analysed to investigate whether asexual reproduction was occurring and therefore its influence on the determination of mating structure within populations. Clonality was established in a number of populations (Appendix 2, Table A2.3) and as a result the data was clone corrected to remove duplicated genotypes as were loci with 5% missing data. A sample hierarchy of country and state from where the isolate was collected (country/state) was used for clone correction. Clone corrected data prevents bias within the statistical analysis by collapsing the samples into one observation per multilocus genotype and is recommended for populations having both sexual and asexual modes of reproduction (Grünwald et al. 2017). A genotype accumulation curve was created to ensure the number of loci used were sufficient to capture most of the genetic diversity within each of the populations. For comparison

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1 Brian J Knaus, Horticulture Crop Research Unit, USDA-ARS, Corvallis collaborated on the R code and population statistical analysis for this chapter.
purposes, American states were subsequently pooled into a single population named North America and the Brazil and Uruguay samples were pooled to form a South America population.

Population diversities, richness, and evenness were calculated in *poppr* by calculating the Shannon-Wiener index (Shannon 2001), Stoddart and Taylor’s index (Stoddart and Taylor 1988), Simpson’s index (Simpson 1949), Nei’s gene diversity (Nei 1978), evenness and the expected number of multilocus genotypes, with a minimum of 10 samples Simpson (1949). In populations where all unique multilocus genotypes are equally distributed, Stoddart and Taylor’s index will equal the number of isolates, expected multilocus genotypes will equal to 10 and Simpson’s index, Nei’s diversity and evenness will equal 1. For inference of mode of reproduction, clonal or sexual, within all populations the index of association and the standardised index of association were calculated. An index of association value of zero indicates linkage equilibrium and is used to infer a sexually recombining population. If it is significantly greater than zero, linkage disequilibrium exists and the population is considered clonal. Statistical significance for linkage disequilibrium between the microsatellite loci of all populations was calculated using 999 permutations. Using the R package *pegas*, all loci for each population were tested to determine whether they were at Hardy-Weinberg equilibrium (HWE). Both the $\chi^2$ and the exact test, based on Monte Carlo permutations (999), were calculated with the null hypothesis that all alleles are randomly joining to create genotypes and meet HWE assumptions.

To determine the extent of genetic similarity between populations of different countries and states within countries an analysis of molecular variance (AMOVA) was conducted (Excoffier et al. 1992). A total of 1000 permutations were performed to determine whether significant differences occurred at each level of the hierarchy. A minimum spanning
network (MSN) was developed in the poppr package to differentiate individual genotypes and populations using Bruvo’s distance (Bruvo et al. 2004). A discriminant analysis of principal components (DAPC) (Jombart et al. 2010) was performed to determine clustering between the populations used in this study. As no known reference population was included in the study, the DAPC used sequential K-means clustering to determine the optimal number of groups based on comparisons of the Bayesian Information Criterion (BIC) for different group numbers.

3. Results

**DNA sequencing**

Sequences were successfully obtained from the partial ITS region from 19 samples collected during this study from both fresh leaves and FTA cards. Three of the five cryptic species as outlined by Rouxel et al. (2014) were detected from the 19 samples sequenced (Fig. 3.2). All samples from each state of Australia, Brazil and Uruguay, from both *V. vinifera* and *V. labrusca* host species, aligned with the *P. viticola* clade *aestivalis*. A single isolate from a *V. riparia* host in New York, USA aligned with *P. viticola* clade *riparia*. A *P. viticola* isolate from a *P. tricuspisdata* host plant in Oregon, USA aligned closely with the *P. viticola* clade *quinquefolia* sequence acquired from GenBank from the Rouxel et al. (2014) study. Sequence data and collection information from the 19 samples has been deposited in GenBank (Appendix 2, Table A2.4).

**Microsatellite and clone correction**

Of the 16 microsatellites, the loci BER and PV74 had >5% missing values and were removed from further analysis. Despite appearing to indicate polymorphism, the loci GOB and CES, with compound repeats, made accurately determining allele calls difficult and were also
removed from further analysis. Genotype accumulation curves were used to determine how
the number of loci used in a study affects the number of genotypes observed. Ideally they
show a point of levelling where the addition of more loci does not result in more genotypes,
indicating a sufficient number of loci were used in the study. Genotypic accumulation curves
conducted on all populations indicated the 12 remaining loci were sufficient to describe the
amount of genetic diversity in each population (Appendix 2, Fig A2.1). The loci PV103 and
PV146 were polymorphic across the entire datum set but had a number of populations where
only a single allele was present in the population. Clone correction of the data reduced the
total number of samples from 413 to 321 with the Western Australian samples reduced the
greatest from 110 to 27 (Table 3.1 and 3.2).
Figure 3.2. Bayesian phylogeny based on ITS1 sequence data generated in MrBayes using the GTR + G substitution model showing relationship *P. viticola* cryptic species. The posterior probability is shown at the nodes. *Plasmopara euphrasiae* is included as an outgroup taxon. WA=Western Australia, SA=South Australia, QLD=Queensland, VIC=Victoria, NSW=New South Wales, TAS=Tasmania, BRA=Brazil, URY=Uruguay, VI=Virginia, OR=Oregon, NY=New York. Samples KF652198, JF897779, JF897782, JF897781, and JF897780 were used as ‘known’ samples reported from Rouxel et al. (2013) and Rouxel et al. (2014).

**Genotypic diversity, AMOVA, Index of Association and Hardy Weinberg**

Of the total 321 clone corrected isolates there were 318 distinct multilocus genotypes between all populations in the study indicating a high level of genetic diversity in all populations (Table 3.2). With only 8 isolates the TAS population was relatively small and could not be compared meaningfully with other populations within the study. All remaining populations except WA and NSW had expected multilocus genotypes equal to the sample
size of 10 and Stoddard and Taylor values equal to the number of isolates, indicating unique genotypes within all populations (Table 3.2). On a global scale the North American population had the highest MLG diversity as indicated by the Shannon (4.23) and Simpson (0.99) indices. The SA population had the second highest genotypic diversity, and was highest amongst all Australian states, based on the same indices. Nei’s gene diversity ranged from 0.62 for the North American population to 0.26 for the WA population. The analysis of molecular variance (AMOVA) showed there was significant differentiation between Australian states (d.f = 9, SSD = 5.75, \( P = 0 \)) (Appendix 2, Table A2.5).

Despite the diversity indices indicating all populations in this study being genetically diverse the index of association analysis provided evidence of linkage disequilibrium within the WA, QLD, NSW and South American populations (Table 3.2). The associations of the loci within these populations were all significantly different from 0 (\( P < 0.01 \)) (Table 3.2), rejecting the hypothesis of linkage equilibrium and indicating random mating or sexual reproduction is not occurring within these populations. Within all populations there were a number of loci with significant departures from HWE (Appendix 2, Fig. A2.2). For the populations of WA, QLD, North America and South America there were more loci deviating from HWE (7 loci) than those in HWE. The HWE results for WA, QLD suggested these populations were not randomly mating. A greater proportion of loci were within HWE than in departure for the France, SA and VIC populations and therefore met the assumptions of random mating.

**Minimum spanning network (MSN)**

The WA multilocus genotypes cluster tightly together within the MSN, indicating a close genetic relationship despite the diversity within the population. This clustering supports a scenario of a single introduction into WA, potentially from either SA or France with
subsequent clonal divergence in WA. The close proximity of a number of multilocus
genotypes from SA and France suggested these populations were most closely related to the
WA population. The range of multilocus genotypes within the eastern Australian populations,
South America and France are too diverse to identify any unique groupings. The multilocus
genotypes from North America appeared to be the least genetically linked to all other
populations analysed in this study, forming a large distinct grouping of multilocus genotypes
(Fig.3.3).

**Discriminant Analysis of Principal Components (DAPC)**

A DAPC analysis provided further support for the clustering observed in the MSN.
First, we performed K-means clustering using 40 principal component axes to determine how
many groups to use and their membership. After four groups there did not appear to be an
appreciable decrease in our optimality criterion (BIC) by adding more groups (Appendix 2,
Fig. 2.3). We performed DAPC using 40 principal component axes and four discriminant
analysis axes with the sample divided into two through four groups and plotted the results in
ggplot2 (Wickham 2009) (Fig. 3.4). For K = 2 the North American population is a distinct
cluster from the remaining populations in this study (Fig. 3.4). At K = 3 the Western
Australian and South Australian populations are predominantly differentiated from the
remaining populations (Fig. 3.4). This analysis supports a likely scenario of one introduction
into WA, most likely for other regions in Australia with potential origin from France.
Table 3.2. Genetic diversity statistics within all populations examined in this study.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>MLG</th>
<th>eMLG</th>
<th>SE</th>
<th>H</th>
<th>G</th>
<th>lambda</th>
<th>E.5</th>
<th>Hexp</th>
<th>Ia</th>
<th>rbarD</th>
<th>p.rD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AU_Western Australia</td>
<td>27</td>
<td>26</td>
<td>9.87</td>
<td>0.33</td>
<td>3.24</td>
<td>25.14</td>
<td>0.96</td>
<td>0.98</td>
<td>0.26</td>
<td>1.00</td>
<td>0.14</td>
<td>0.01</td>
</tr>
<tr>
<td>AU_South Australia</td>
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<td>63</td>
<td>10</td>
<td>1.38E-06</td>
<td>4.14</td>
<td>63</td>
<td>0.98</td>
<td>1</td>
<td>0.44</td>
<td>0.2</td>
<td>0.02</td>
<td>0.12</td>
</tr>
<tr>
<td>AU_Queensland</td>
<td>26</td>
<td>26</td>
<td>10</td>
<td>1.09E-06</td>
<td>3.26</td>
<td>26</td>
<td>0.96</td>
<td>1</td>
<td>0.45</td>
<td>1.41</td>
<td>0.15</td>
<td>0.01</td>
</tr>
<tr>
<td>AU_New South Wales</td>
<td>39</td>
<td>38</td>
<td>9.94</td>
<td>0.24</td>
<td>3.63</td>
<td>37.10</td>
<td>0.97</td>
<td>0.99</td>
<td>0.39</td>
<td>0.69</td>
<td>0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>AU_Victoria</td>
<td>31</td>
<td>31</td>
<td>10</td>
<td>1.78E-06</td>
<td>3.43</td>
<td>31</td>
<td>0.97</td>
<td>1</td>
<td>0.42</td>
<td>-0.02</td>
<td>-0.00</td>
<td>0.7</td>
</tr>
<tr>
<td>AU_Tasmania</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>2.08</td>
<td>8</td>
<td>0.88</td>
<td>1</td>
<td>0.39</td>
<td>0.92</td>
<td>0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>N_America</td>
<td>69</td>
<td>69</td>
<td>10</td>
<td>5.43E-06</td>
<td>4.23</td>
<td>69</td>
<td>0.99</td>
<td>1</td>
<td>0.62</td>
<td>0.11</td>
<td>0.01</td>
<td>0.14</td>
</tr>
<tr>
<td>S_America</td>
<td>26</td>
<td>26</td>
<td>10</td>
<td>1.09E-06</td>
<td>3.26</td>
<td>26</td>
<td>0.96</td>
<td>1</td>
<td>0.49</td>
<td>0.86</td>
<td>0.09</td>
<td>0.01</td>
</tr>
<tr>
<td>FR_Bordeaux</td>
<td>32</td>
<td>32</td>
<td>10</td>
<td>0</td>
<td>3.47</td>
<td>32</td>
<td>0.97</td>
<td>1</td>
<td>0.51</td>
<td>0.15</td>
<td>0.02</td>
<td>0.11</td>
</tr>
<tr>
<td>Total</td>
<td>321</td>
<td>318</td>
<td>9.99</td>
<td>0.05</td>
<td>5.76</td>
<td>315.11</td>
<td>1.0</td>
<td>0.99</td>
<td>0.58</td>
<td>1.74</td>
<td>0.16</td>
<td>0.01</td>
</tr>
</tbody>
</table>

N = number of isolates in the population, MLG = number of multilocus genotypes, eMLG = expected number of multilocus genotypes at the smallest samples size >10, SE = Standard error based on the eMLG, H = Shannon-Wiener index of MLG diversity, G = Stoddard and Taylor index of MLG diversity, Lambda = Simpson’s index, Hexp = Nei’s unbiased gene diversity, Ia = Index of association, rbarD = Standardized index of association, p.rD = p value (rbarD) = significance of the standardized index of association (p < 0.01).
Figure 3.3. Minimum spanning network (MSN) of all isolates of *P. viticola*, clone corrected, in this study based on Bruvo’s distance. Each node represents a multilocus genotype with nodes of the same colour indicating the same population with country/state hierarchy. The greater the genetic distance the thinner and lighter the colour of the line. The larger the node size the greater the number of samples with the same microsatellite profile.
Figure 3.4. DAPC of the *P. viticola* isolates in this study for up to 4 populations. Samples of the same colour represent the same genetic cluster (K). Bars of mixed colours are admixed individuals/samples.
3. Discussion

Viticulture in Australia has been historically linked to Europe with multiple introductions of grapevine planting material occurring during the 18th and 19th centuries (Beeston 2001; Kerridge and Antcliff 1999), after which *P. viticola* was first identified. Our findings indicate the populations of *P. viticola* within Australia and Europe are also genetically linked. Analysis of the microsatellite markers from all populations in this study except those from North America could not be clearly differentiated, suggesting the spread of the pathogen to Australia and South America most likely occurred from European production systems, where *V. vinifera* species were historically cultivated, rather than North America populations. It is unclear whether the introduction of *P. viticola* into Australia occurred directly from Europe or from a secondary population such as South Africa or Brazil, as vine material is known to have been collected from there on passages to Australia (Beeston 2001; Kerridge and Antcliff 1999). The number of Brazilian samples in this study was too small to make clear inference of its relationship to the Australian population. With only a single population of *P. viticola* from Europe included we were unable to determine whether the populations in Australia cluster more closely with the western or eastern European cluster of *P. viticola* as identified by Fontaine et al. (2013).

The North American population exhibits the highest level of genetic diversity amongst the populations of *P. viticola* in this study. This is not surprising given *P. viticola* is believed to have originated in north eastern America and five cryptic species of the pathogen are found to exist there (Rouxel et al. 2014). *P. viticola* was first described in North America in 1834 compared to 1878 for France (Wilcox et al. 2015). A genetic bottleneck or introductions from a single source population was proposed for the weak structure of the *P. viticola* population in Europe (Fontaine et al. 2013). This study has found the population
genetic diversity amongst the French samples is similar to the South American populations and a number of the states in Australia. The WA population has the lowest genetic diversity of all the populations studied and is likely a further example of a demographic bottleneck, given the pathogen was only detected in 1997/98 (McKirdy et al. 1999; Riley 1998), and quarantine requirements still restrict any potential for further introductions of new genotypes. In an earlier study using microsatellite primers Hug (2005) found WA had low genetic diversity when comparing *P. viticola* populations from different states of Australia.

Whilst the European and Australian populations of *P. viticola* had a lower genetic diversity than the North American population the diversity differs enough to warrant the need to test newly bred resistant cultivars of *V. vinifera* in multiple locations. A number of countries, including France and Australia, have breeding programs introducing and releasing *V. vinifera* cultivars with known resistance to *P. viticola* (Dry et al. 2017; Venuti et al. 2013; Weidemann-Merdinoglu et al. 2017). Breakdown of host resistance has already been observed from a *P. viticola* isolate of European origin (Peressotti et al. 2010).

An aim of this research was to determine the most likely source of the WA population of *P. viticola*. The AMOVA analysis suggest there are significant differences between the populations within different states of Australia, although it does appear from the DAPC and MSN data there are similarities between the WA population and SA when up to four population groups are assigned. The lower genetic diversity within the WA population also suggests the 1997 and 1998 samples of *P. viticola* in WA are related, despite the detections being several thousand of kilometres in distance apart. The inclusion of herbarium samples highlights no major population changes have occurred within the WA population since the 1997 incursion. It would be expected that if both the 1997 and 1998 incursions were from different sources this would highlight two distinct populations in the microsatellite analysis.
Trace back of the host material of the 1997 detection in Northern WA did not define a conclusive source but there was a possibility vines were introduced from the Northern Territory (NT) (Riley 1998). Until isolates of *P. viticola* from the NT are analysed we cannot conclusively determine whether the source of the WA population of *P. viticola* is from SA or the NT or whether there is a relationship between all three populations. However, the potential of a natural pathway does exist in northern Australia as several native *Vitaceae* species, including *Ampelocissus* and *Cissus* species, are reported to be susceptible to *P. viticola* infection (Emmett et al. 1992b). Within WA, the movement of the pathogen from Kalumburu and the Swan Valley or vice versa is difficult to ascertain if planting material is not involved, given the geographical distances between these areas would make any form of natural spread unlikely.

Sequence analysis on representative samples taken from all states in Australia and those from populations in Brazil and Uruguay revealed all isolates to be *P. viticola* clade *aestivalis*. This clade is likely to have originated from colonisation of a *V. aestivalis* host and undergone host range expansion to *V. labrusca* and the European grapevine species *V. vinifera* (Rouzel et al. 2013). The representative samples in this study came from both *V. labrusca* and *V. vinifera* hosts. The *P. viticola* clade *aestivalis* is the predominate clade infecting *V. vinifera* cultivars in North America (Rouzel et al. 2014) and therefore it is not surprising this cryptic species is the most widely spread in global *P. viticola* populations. Representative isolates of *P. viticola* clade *riparia* and *P. viticola* clade *quinquefolia* in this study were isolated from *V. riparia* and *P. tricuspidata* hosts, respectively, confirming the findings of Rouzel et al. (2013) and Rouzel et al. (2014). The discovery of cryptic species within *P. viticola* may represent new biosecurity threats to the native *Vitaceae* species present within Australia, some of which have conservation status due to restricted distribution (Herbarium 1998).
The grape downy mildew lifecycle is dimorphic, consisting of both sexual and asexual reproductive stages (Rossi et al. 2008b), so it is not unusual to observe clones within population studies. After clone correcting the data the index of association analysis for the WA, NSW, QLD and South America populations appear to be reproducing either asexually or via selfing. This is interesting as the isolates for several of these populations, particularly WA, were collected over several seasons. Studies in Greece and South Africa have also highlighted the presence of the same genotype over successive seasons (Koopman et al. 2007; Rumbou and Gessler 2006). It has been proposed *P. viticola* can survive in an asexual form within grapevine material in areas experiencing mild winters, including Greece, South Africa and Australia (Killigrew 2006; Koopman et al. 2007; Rumbou and Gessler 2006). It has long been thought the pathogen may be able to overwinter within dormant buds similar to grape powdery mildew, *Erysiphe necator* (Killigrew 2006; Rumbou and Gessler 2006), but a recent investigation suggests this is not the case in WA (Chapter 4). The sheer distances between isolates collected within mature vineyards in WA, over 250km, indicate natural spread between regions is unlikely to occur in a single season.

Findings in this study raise questions about the current understanding of the mating system and plasticity within *P. viticola*. European and American populations of *P. viticola* are strictly heterothallic, with two distinct mating types being required for the formation of oospores (Scherer and Gisi 2006; Wong et al. 2001). This study indicates random mating is still occurring within French and North American populations and occurs where optimal climatic conditions are prevalent. The prevailing Mediterranean climate experienced in WA is only favourable to the development of *P. viticola* sporadically, leading to regular extinction and recolonization events (Hug 2005; McLean et al. 1984; Weltzien 1981). Similar conditions occur within growing regions of Greece and South Africa (Koopman et al. 2007; Rumbou and Gessler 2006). Recent surveys of the majority of the viticulture regions within
WA have detected *P. viticola* oospores (Chapter 5). The presence of oospores in a clonal population may be evidence of *P. viticola* secondary homothallism or selfing as a mechanism to survive periods of unsuitable climatic conditions. This would not be unusual for an oomycete pathogen in WA, with *Phytophthora cinnamomi* reported to survive the dry hot summers by producing selfed oospores in a number of hosts, despite the presence of both mating types (Crone et al. 2013). The breakdown of mating type regulation may result as an evolutionary advantage for heterothallic oomycetes to on occasion form oospores for resisting harsh environments (Judelson 2009). Scherer and Gisi (2006) described the observation of oospore formation without crossing of isolates and suggested both mating types may be present within the same lesion. Rather, the presence of both mating types in the same lesion could be evidence of the ability of *P. viticola* to self. The formation of selfed oospores would explain the rapid and long distance spread of the pathogen and its ability to survive after extinction events in WA. Further investigations are warranted in order to understand the survival mechanism and reproduction of *P. viticola* under sub-optimal environments.

The successful use of the FTA cards in amplifying and storage of *P. viticola* DNA during this study will aid in the understanding of the population structure of the pathogen globally. Less is known about populations of *P. viticola* than other economically damaging oomycete pathogens such as *Phytophthora infestans*. This lack of knowledge is in some part due to *P. viticola* being a biotroph, requiring plant material to be collected, stored appropriately and in some instances mailed to different research labs in order to obtain results. This creates issues in regards to perishable samples and quarantine regulations with the movement of plant material. The FTA cards, with viability reported to last over a decade (Smith and Burgoyne 2004), remove some of the problems encountered in working with *P. viticola* populations.
3. Conclusion

Fontaine et al. (2013) discussed the requirement to understand the incursion pathway of *P. viticola* into new world viticulture. This study has highlighted the movement of the pathogen to two new world viticulture producers in Australia and South America and in doing so has increased the knowledge surrounding the population structure of *P. viticola* globally. The addition of South Africa to *P. viticola* population studies is required to completely understand any relationship with Australian populations given the historical movement of grape material between the two countries. The discovery of clonal or selfing populations of *P. viticola* existing with the presence of oospores presents potential capacity for long distance movement of the pathogen previously not considered possible.
Chapter 4

Overwintering of grape downy mildew as mycelium in Western Australian vineyards.

4. Abstract

With mild winters and few of oospores in Western Australian vineyards it has been proposed grape downy mildew can overwinter as mycelium in dormant buds, producing flag shoots similar to grapevine powdery mildew the following season. The ability of *P. viticola* to infect grapevine buds was investigated using naturally and artificially inoculated grapevine cuttings over two seasons. Immature green buds were capable of being infected by *P. viticola* and under humid conditions sporulation can occur. Infected buds were often killed by *P. viticola* infection, confirmed by the presence of *P. viticola* DNA in the dead tissue. Where the buds were not killed healthy shoots developed, despite *P. viticola* lesions being present. It is unclear how the host plant defence mechanisms prevent *P. viticola* from infecting dormant shoot tissue in these instances. There was no evidence of *P. viticola* flag shoots in WA vineyards.

4. Introduction

Western Australia (WA) was one of the few grape growing regions of the world free from grape downy mildew, *Plasmopara viticola*, until its discovery in commercial vineyards during the 1998/99 season (McKirdy et al., 1999). Despite the rapid spread of the disease to all viticulture regions in the state in subsequent years (Williams et al., 2007), vineyard surveys revealed a lack of oospores in grape growing regions (Killigrew et al., 2005). Oospores are both the survival structures of the pathogen and the source of primary infections...
throughout the growing season (Gobbin et al., 2005, Wilcox et al., 2015, Rossi et al., 2013). With few oospores detected in WA it was considered the primary source of inoculum was mycelia in canes and buds infected during the previous season (Underdown et al., 2008, Killigrew et al., 2005). It was further proposed shoots systemically infected with *P. viticola*, downy mildew “flag shoots”, may arise from infected buds in the following season but conclusive evidence of this is yet to be seen (Killigrew, 2006). Flag shoots being shoots heavily infected with fungal growth they stand out like white flags in the vine (Wilcox et al. 2015).

As reported in Galet (1977), the potential ability of *P. viticola* to overwinter as mycelium in dormant buds was noted as early as 1889 by Cuboni. Regions with mild winters, such as California and WA, are considered favourable for the pathogen to survive as mycelium in buds and persistent leaves (Barrett, 1939, Lafon and Clerjeau, 1988, Emmett et al., 1992, Rumbou and Gessler, 2006). On the basis of these observations, presumably from California, Agrios (1978) diagrammatically represented the survival of mycelium in dormant wood as a source of primary infection. This disease cycle was later reprinted in the Compendium of Grape Diseases first edition (Pearson and Goheen, 1988). A number of studies have microscopically detected the presence of mycelium in overwintering canes and buds, Istvanffi 1904 in Galet (1977), Barrett (1939), Chrelashvili (1993), Killigrew (2006), but there is no follow up evidence the mycelium present is viable and capable of spore production the following season. Thus, although the presence of *P. viticola* as mycelium has been reported it has not been established whether this contributes to the reinfection of healthy tissue the following spring (Hall, 1989).

Whilst population genetic studies on *P. viticola* have highlighted the importance of oospores to disease development (Gobbin et al., 2005, Matasci et al., 2010), they also indicate
the potential ability of *P. viticola* to overwinter asexually (Rumbou and Gessler, 2006). Microsatellite data from the Greek islands revealed three genotypes present over two consecutive epidemics (Rumbou and Gessler, 2006). Similarly, a single genotype was found over consecutive years in South African populations of *P. viticola* (Koopman et al., 2007). Molecular studies conducted on *P. viticola* populations in WA showed a low genetic diversity (Hug, 2005), indicating the possibility of asexual overwintering.

The ability of *P. viticola* to overwinter in dormant buds would not be unique for an obligate biotrophic grapevine disease. Powdery mildew of grapevines (*Erysiphe necator*) can overwinter in grapevine buds and produce shoots in the following season covered in inoculum, known as ‘flag shoots’ (Rumbolz and Gubler, 2005, Ypema and Gubler, 2000, Halleen and Holz, 2000). Flag shoots are the main source of powdery mildew primary infection in most vineyards in Australia, with the presence of sexual bodies, chasmothecia, only being detected in the mid 1980’s, despite *E. necator* being present in Australia since 1866 (Wicks et al., 1985, Emmett et al., 2006).

After the detection of *P. viticola* in WA, extension programs delivered to growers for management were the same as those used in other viticulture regions of Australia. These programs are based on oospore germination as the source of primary infection within vineyards. Therefore, any presence of downy mildew flag shoots would require disease management consideration and change. The overall aim of this chapter was to investigate the survival of *P. viticola* between growing seasons in WA. We sought to test if:

1. *P. viticola* infection leads to flag shoot development the following season under WA weather conditions experienced within vineyards?

2. cuttings with buds from diseased vineyards used as planting material are able to cause disease?
3. *P. viticola* present in buds can be eradicated using post infection fungicides?

4. artificially inoculated nodes can give rise to diseased and sporulating shoots the following season?

5. downy mildew infected nodes remain viable enough to produce shoots?

6. young green buds susceptible to infection by downy mildew?

4. Materials and Methods

*Can natural *P. viticola* *infection lead to flag shoot development? Site 1*

An unmanaged commercial vineyard block of *Vitis vinifera* cv. Perlette in the Swan Valley region of WA was identified in 2014 to determine if grape downy mildew flag shoots occurred under natural conditions. The block consisted of 6 rows of vines (192 total) with plant spacings of 2.8m within row and 3.5m between rows. In past years the vines were cane pruned (Fig. 4.1) with an average of 4 canes being laid down per vine but the vines had not been pruned during the 2014 winter and no fungicide applications were applied. No irrigation was applied. Downy mildew infection was evident in late August and had reached severe disease levels by December of 2014.
In late December 575 shoots and or lateral shoots from the first 17 vines of each of the 6 rows (102 total vines) exhibiting downy mildew infections on the leaves and shoots were tagged at the point of the infected node to determine if the infection would lead to flag shoot development the following season. The block was visited monthly after tagging to observe the loss of any tags or if premature shoot growth occurred. At the first sign of shoot growth the following spring (August) the block was visited twice a month.

**Experiment 1: Can infected cuttings cause disease? Site 2**

A site of *Vitis vinifera* cv. Flame Seedless exhibiting natural shoot and node infection of downy mildew from a commercial table grape vineyard in the Swan Valley region of Western Australia was used as source material. Whilst the vines had been cane pruned the infection had resulted from a lack of fungicide application throughout the growing season. The site consisted of 5 rows (330 vines total) and infections were apparent on shoots amongst
vines throughout each row. With shoots able to reach over 2m in length due to trellising, multiple lesions on a single shoot were evident, indicating weather conditions had been suitable for multiple infection events. In January of 2015 (E-L 36) all node lesions from the first 13 vines of each row were tagged with flagging tape at the site of the infection to be collected at dormancy.

Fungicide Treatments: On the 25th of March 2015 the first 5 vines of row 2 at site 2 were sprayed to run off with 640 g/kg mancozeb plus 40 g/kg metalaxyl-M (mefenoxam) (Ridomil Gold® MZ WG). The first 5 vines of row 4 were sprayed to run off with 600 g/L of phosphorous acid (phosphonic acid) as mono and di phosponate (Agri-fos® 600). The metalaxyl-M + mancozeb was applied at the label rate of 250g/100L water whereas the phosphorous acid was applied at a rate of 1.2g/L based on the effectiveness reported in Wicks et al. (1991). The treatments were applied with a backpack sprayer (15L) to determine if this had an eradicant effect on any downy mildew present within the buds. Whilst Ridomil Gold® MZ WG has a mancozeb component it would have no impact on P. viticola present within the bud as mancozeb has little post infection activity (Wong and Wilcox 2001). The vines at this Eichhorn-Lorenz (E-L) growth stage were between 39 and 41, with bud scales having already been developed (Coombe 1995) (Appendix 1).

Collecting canes: On the 9th of July 2015 (E-L 47) visually infected and non-infected canes were pruned from the tagged vines and placed in tubs consisting of each source row. The canes were pruned to approximately 45cm with at least one node kept above the actual tagged infected node. The canes were hydrated in distilled water for 24 hours prior to them being placed in plastic bags to prevent moisture loss and stored at 4°C.

Trial layout: There were four treatments as part of the trial; visually healthy nodes (control), phosphorous acid application, metalaxyl-M (mefenoxam) application and visually
infected nodes. Treatments were arranged in a randomized block design consisting of two blocks of four raised benches based at the Department of Primary Industries and Regional Development Bunbury District office, several kilometres away from any potential windblown source of inoculum. 400 cuttings were potted into black PVC cylinder pots with commercial general purpose potting mix (Soils Aint Soils) containing no grape marc on the 20th of October 2015. The cylinders were 0.55m high with and 0.08m radius and a volume 0.011m$^3$ (11L). Treatment replicates consisted of 25 (5x5) pots with each raised bench containing two replicates with 1.2m separation in the centre of the bench (Fig. 4.2a). Each block contained one replicate of visually healthy nodes (control), one replicate of phosphorous acid, one replicate of metalaxyl- M (mefenoxam) and five replicates of visually infected nodes. A greater number of infected node replicates were included in comparison to other treatments as incidence of downy mildew flag shoots was expected to be low, for example *E. necator* flag shoots are only found in 1.6% of grapevine shoots (Rumbolz and Gubler 2005). For experimental balance, the total 50 cuttings per treatment consisted of 10 cuttings from the source vineyard of rows 1, 4 and 5, 15 cuttings from row 3 and 5 cuttings from row 2. This was multiplied by five for the infected node treatment.

At planting each cutting was trimmed to 30cm long leaving the infected node as the apical node. The infected node was left as the apical node as shoot growth begins in the apical buds in preference to others on the cane (Keller 2010). To prevent potential water splash from the potting mix, drippers (Philmac True Drip 2 L/h) were used for irrigation (Fig. 4.2b). The base covering of the PVC cylinder was drilled to allow water flow and prevent water logging of the roots. The vines were irrigated 3 times a day, dawn (5am), midday (12pm) and dusk (6pm) for 5 minutes until dormancy the following season (0.5L/day).
Measurements: Each shoot in the experiment was assessed for both budburst and any visual presence of *P. viticola* sporangia. To test if the disease was latent and therefore not visible each raised bench was covered with a tent and a humidifier (Condair 505) was run overnight when the shoots were approximately E-L stage 9 (2 to 3 leaves separated, shoots 2-4cm long) (Fig. 4.3). Budburst and bud death incidence were recorded for all shoots. At the end of the experiment the vines were removed from the PVC tubes and destroyed.

Statistical analysis: Analysis of variance and Fisher’s unprotected least significant difference test were conducted to determine whether there was any difference between treatments in terms of percentage of healthy shoots developing from the buds. All measurements were analysed using Genstat statistical package version 18 (VSN International).
Figure 4.3. A) A tent was placed over each bench at night B) with a humidifier providing ideal sporulation conditions to detect any latent shoot infection.

Experiment 2: Can inoculation of buds give rise to downy mildew flag shoots? Eppendorf method

35 potted vines of cv Flame Seedless were purchased from a local supplier and placed over night in a plastic tent with a humidifier (Condair 505) to determine if they had any pre-existing visual downy mildew infections. Each of the vines had 4 or more shoots, approximately E-L 8, arising from the potted vine.

One of the three youngest nodes, prior to bud scale development, of four shoots from each potted vine was point inoculated with 1 ml of approximately 25,000 *P. viticola* spores per mL, haemocytometer adjusted. The inoculations occurred on the 28th January 2016. The downy mildew was sourced from natural infections and bulked up on successive occasions by inoculating detached leaves incubated in petri dishes. The three youngest nodes were chosen as Killigrew (2006) found these were all highly susceptible to *P. viticola* infection. Studies of *E. necator* also indicate these younger buds are the most likely to produce powdery mildew flag shoots the following season (Emmett et al. 2006). The shoots were between E-L 13 and 15 (6 and 8 leaves separated) at the time of inoculation.
Inoculations were done by using a modified 1.5mL Eppendorf DNA LoBind 1.5mL tube cut vertically with the bottom tip removed. This was placed around the stem, petiole and tendril of the node to be inoculated. The Eppendorf tube was wrapped in parafilm and positioned on the stem with bluetac to prevent the spore solution from leaking (Fig. 4.4). A control inoculation using distilled water was conducted on a single shoot per vine. To ensure the inoculum was viable the spore solution was sprayed onto detached leaf discs for observation of sporangiophore production. Control shoots were tagged with a red tag and treatment shoots with a white tag.

Within an hour of placing the spore solution in the Eppendorf each vine was placed in a high humidity chamber, using a humidifier (Condair 505), and maintained at 20°C overnight to aid zoospore encystment. The following morning the Eppendorf tube and bluetac were removed from the shoots. The potted vines were then placed on the raised bench used in Experiment 1 and after one week returned to the humid chamber to assess if the inoculation had been successful. The potted vines were drip irrigated as per above. Assessment of the nodes occurred the following spring to determine if they were dead, alive and healthy or alive and infected with *P. viticola*. The vines were destroyed by incineration after assessment.

Figure 4.4. Bud inoculations using a modified Eppendorf attached to the shoot with parafilm and bluetac to prevent leakage at the base.
Experiment 3: Can inoculation of buds give rise to downy mildew flag shoots? Droplet method

In July of 2016, 50 dormant cuttings ~45cm each of Vitis vinifera cvs. Sultana (Thompson seedless) and Crimson seedless were taken from a commercial table grape vineyard in the Swan Valley. The cuttings were hydrated overnight in distilled water, placed in plastics bags and kept at 4°C.

In November the cuttings were planted in the same tubes as Experiment 1. A single shoot per cutting was allowed to develop. At E-L 9 (2-3 leaves separated) all vines were placed in a humid chamber (as previously mentioned) and assessed to ensure no prior downy mildew infection.

The third node on 20 shoots of each of the Sultana and Crimson seedless at E-L 14 (7 leaves separated, 21st of Dec 2016) and E-L 17 (12 leaves separated, 21st of Jan 2017) were inoculated. Inoculations were made via placing a 20µL droplet of spore solution containing 25,000 P. viticola spores per mL, haemocytometer adjusted, onto each node using a pipette (Fig. 4.5). The nodes of 5 control shoots per cultivar were inoculated with distilled water in a similar way per inoculation time. Each shoot was then covered with an opened zip-lock bag and placed overnight in a humid chamber. The zip-lock bag prevented water condensation from causing the spore solution from running off the node whilst maintaining high humidity for infection. The pots were placed in the field on the raised benches and irrigation reattached.

To prevent a widespread disease outbreak occurring during this experiment a wooden frame was constructed above the benches allowing Grape Guard™ plastic (Gundle Gelpack Pty Ltd) to cover the top of the vines and the western side of the benches (Fig. 4.6). Plastic rain shelters delay P. viticola epidemics and reduce disease severity (Shuyi et al. 2017).
allowed the vines to receive morning sunlight and subsequent ambient temperature but prevented rain and dew from moistening the leaves and preventing widespread dispersal of inoculum. The vines were regularly assessed for evidence of *P. viticola* infection on the green tissue and for potential node disease progression. In June of 2017 any remaining leaves were removed from the vines and from the pots to prevent any carryover of inoculum. In July 2017 the shoots were pruned so the node receiving the inoculation was the apical node to encourage shoot growth. The percentage of shoot emergence and dead buds were recorded after bud burst (E-L 4 or later).

*Statistical analysis:* Pearson’s chi-squared tests of association ($\chi^2$) were conducted to determine whether any differences in infection percentage between the two cultivars and infection between the control and test inoculations occurred. All measurements were analysed using Genstat statistical package version 18 (VSN International).

![Figure 4.5. A droplet of spore solution was pipetted onto the node.](image)
Experiment 4: Is P. viticola associated with the disease symptoms? Molecular testing

To confirm the presence of P. viticola in symptomatic plant tissue, 36 samples were taken and subjected to microsatellite analysis as detailed in Chapter 3. DNA extraction and processing was performed as described in Chapter 3. The samples included buds from shoots with visible lesions (6), buds from shoots with no visible lesions (6), scrapings from dark lesions (6) and scrapings from cracked lesions (6) from the Flame Seedless vines at Site 2 (Experiment 1). Dead shoots (12) from the Perlette vines of Site 1 were also tested to determine if P. viticola was present.

4. Results

Field observations

At the time of tagging infected nodes at field Site 1, many of the infections had begun with infected leaves or inflorescences and had spread to the shoot and node (Fig. 4.7b). Shoot infections often presented as dark lesions and as the season progressed began to swell, causing large longitudinal cracks in the bark tissue (Fig. 4.7a, c and d). Nodes close to the vine cordon (arm) exhibited infection indicating an infection event occurred when the shoots
were young and had continued to grow after the infection had occurred (Fig. 4.7d). The level of downy mildew infection progressed throughout the season and weakened or killed many of the tagged shoots to the point they were snapped off by winds experienced within the vineyard (Fig. 4.7e and f). A total of 199 tagged shoots (34.6% of the total) were collected on the vineyard floor by February of 2015. The high level of infection caused widespread defoliation amongst the vines with new growth observed at the apical tip of many of the shoots. The leaves of these new shoots were subsequently infected by downy mildew also. Full senescence occurred in July of 2015. The number of dead or snapped off tagged shoots had increased to 296 tagged shoots (51.4%) by this time.
Figure 4.7. A) oily infected node B) a node infected as the lesion travelled from an infected inflorescence C) longitudinal cracks develop in the epidermis D) the lesion can girdle the shoot and can be close to the cordon (vine arm) E) lesions can spread across a number of internodes F) infection can lead to the death of the shoot.
As the vines were not pruned during the winter of 2015 variable bud burst occurred, beginning in August. The first observation of downy mildew infection occurred on the 30th of August on the older leaves of a shoot at E-L stage 13 (6 leaves separated). The shoot had developed from an untagged node with no visual symptoms of infection from the previous year. Of the remaining tagged shoots, 154 produced new season healthy shoots and 87 of the tagged nodes from the previous season produced no shoot at all indicating downy mildew had killed the bud (Fig. 4.8). Incidence of the healthy shoots and dead buds could not be established as a number of the tagged shoots were lost, either on snapped off shoots or had the tag lost. There was no evidence of new season shoots being systemically infected from any of the tagged nodes.

**Experiment 1: Can infected cuttings cause disease? Site 2**

Budburst was first observed in the control vines on the 28th of October 2015 whilst the visually infected cuttings began a week later. As the infected nodes were from different positions on the source canes it is difficult to make any relationship from this observation.

Shoot growth was evident from the apical bud on both visually healthy shoots (Fig. 4.9a) and those appearing to be infected (Fig. 4.9b). Whilst a number of apical buds had been killed by the infection the cutting itself had not been killed with growth appearing from the buds below the dead bud (Fig. 4.9c and d). The control cuttings, visibly healthy cuttings, produced a significantly greater percentage of budburst than any other treatment ($p < 0.05$) (Table 4.1). The application of fungicides, with budburst percentages combined as a single treatment, prior to pruning had no significant effect on the percentage of budburst (Table 4.1). The fungicide treatments were combined as there were no significant differences in budburst percentages with individual fungicide applications.
Figure 4.8. A,B,C) Localised natural field infection of the node caused the death of the bud and no shoot developed the following season (arrows). Cane death did not occur as shoots before and after the dead node are viable D) although visible lesions were evident in the previous season some buds still produced healthy shoots.
Table 4.1. Percentage of successful budburst from the apical bud of each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Apical bud growth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>94 a</td>
</tr>
<tr>
<td>Spray</td>
<td>58 b</td>
</tr>
<tr>
<td>Infected node (lesion)</td>
<td>47 b</td>
</tr>
</tbody>
</table>

Percentages followed by the same letter are not significantly different at ($p=0.05$).
Experiment 2: Can inoculation of buds give rise to downy mildew flag shoots? Eppendorf method

The inoculation of the nodes using the Eppendorf method was successful as all inoculated shoots exhibited sporulation after being placed overnight in the humid chamber two weeks after being inoculated. Sporulation was observed on shoots, leaf petioles, tendrils, new lateral shoots and on the green buds (Fig. 4.10). This indicates P. viticola can successfully colonise and sporulate from new season green buds. It also indicates if conditions are conducive for disease development spread can be rapid. There was no sporulation observed on the control shoot inoculated with distilled water.

Whilst there was very little rainfall observed in Bunbury during February of 2016, there was above average rainfall observed in March of 2016 (65.8mm vs mean of 19.4mm, monthly rainfall for Bunbury, Bureau of Meteorology). Combined with ideal temperatures this created perfect conditions for the secondary spread of P. viticola to all vines and shoots within the trial. The disease progression was so severe it led to the complete defoliation of some of the potted vines and death of entire shoots (Fig. 4.10c and d). The defoliation led to the potted vines producing new shoots late in the growing season (Autumn to Winter) of 2016. The mild winters in WA in comparison to Northern Hemisphere production systems mean late season growth can occur. With new shoots and leaves being available P. viticola was able to remain active and viable throughout the entire winter period. Due to active lesions and continued inoculum no flag shoot observations based upon previous seasons infection could be made.
**Experiment 3: Can inoculation of buds give rise to downy mildew flag shoots? Droplet method**

Visual symptoms of node infection similar to natural field infections were observed within a week of both inoculation dates (Fig. 4.11). Symptoms included both oily and distinct dark lesions (Fig. 4.11a and b). The darker lesions appeared to spread for 1-2cm above and below the inoculated node but some progressed to cover entire internodes (Fig. 4.11c and d). As the season progressed several of the lesions began to develop longitudinal cracks as observed in the naturally infected sites. The Grape Guard™ plastic covers prevented or minimised sporulation from the lesions and there was no secondary spread amongst the vines.

Budburst of the shoots began on 8th of August 2017. There were no significant differences between the cultivars in terms of the percentage of canes infected and the number of dead buds at budburst so the recordings were combined for statistical analysis. The percentage of cuttings exhibiting node lesions and the percentage of dead buds at budburst was not significantly different from the control inoculations on the December inoculation date but both were significantly higher on the January inoculation based on the $\chi^2$ tests (Table 4.2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>21 Dec 2016</th>
<th>21 Jan 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculated</td>
<td>Visually infected</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Dead Bud</td>
<td>17.5</td>
</tr>
<tr>
<td>Control</td>
<td>Visually infected</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Dead Bud</td>
<td>0</td>
</tr>
</tbody>
</table>

*Significant at $p < 0.05$
Figure 4.10. A) sporangiophores evident on the shoot, leaf petiole, tendril and dormant bud (white arrows) B) sporulation on a young developing lateral shoot at E-L 7 C) a young shoot completely infected D) the infection led to the death of the inoculated shoots E) the potted vines two weeks after inoculation E-L 9 F) the same vines after the disease epidemic caused defoliated and the growth of shoots from the apical buds.
Figure 4.11 A) after a week oily lesions appeared on the nodes using the droplet method B) dark lesions developed later C) longitudinal cracks began to appear where the lesion was present D) occasionally the lesion progressed over entire internodes E and F) the infection lead to the death of the bud but did not kill the cutting.
Experiment 4: Is *P. viticola* associated with the disease symptoms? Molecular testing

All samples from both sites tested for *P. viticola* via microsatellites were successfully amplified, including 33% of the visually healthy buds (Table 3).

**Table 4.3. Detection of *P. viticola* DNA via microsatellite primers from different sample types from the two naturally infected sites. Data presented as a percentage of successful microsatellite amplification from each sample type.**

<table>
<thead>
<tr>
<th>Cultivar (vineyard site)</th>
<th>Sample</th>
<th># Samples</th>
<th>DNA present %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perlette (1)</td>
<td>Dead shoots</td>
<td>12</td>
<td>75</td>
</tr>
<tr>
<td>Flame seedless (2)</td>
<td>Bud visually healthy</td>
<td>6</td>
<td>33.3</td>
</tr>
<tr>
<td>Flame seedless (2)</td>
<td>Bud visually infected</td>
<td>6</td>
<td>83.3</td>
</tr>
<tr>
<td>Flame seedless (2)</td>
<td>Dark lesion scraping</td>
<td>6</td>
<td>83.3</td>
</tr>
<tr>
<td>Flame seedless (2)</td>
<td>Cracked lesion scraping</td>
<td>6</td>
<td>83.3</td>
</tr>
</tbody>
</table>

4. Discussion

In this study, there was no evidence of the ability of *P. viticola* to survive overwintering in grapevine buds and produce flag shoots in WA vineyards. In addition, there was no evidence of successful sporulation from lesions on lignified canes. Despite young, entire shoots covered in *P. viticola* sporangiophores being observed in field situations, it cannot be concluded this resulted from infected latent buds, as shoots are susceptible as early as growth stage E-L 5 (rosette of leaf tips visible), and therefore infections may have resulted from oospore germinations (Kennelly et al., 2007). Surveys conducted at the same time as this study revealed *P. viticola* oospores are now widespread amongst viticulture regions of WA (Chapter 5). Oospore germination can occur throughout the grape growing season when conditions are suitable for infection (Rossi et al., 2013). Artificial inoculation experiments conducted as part of this study showed lateral shoots were capable of being infected at growth stages E-L 5-7.

Although flag shoots were not observed, this study has found infection and colonisation of young green buds by *P. viticola* is possible, confirming the observations made
by Killigrew (2006), Barrett (1939) and Chrelashvili (1993). With high humidity and temperatures at 20°C the formation of sporangiophores from colonised buds were observed in artificial inoculation experiments. These observations were only made on green buds and no evidence of sporulation occurred once bud scale maturation was complete. Similar observations have been recorded with bud infection of grape powdery mildew (*E. necator*) (Rumbolz and Gubler, 2005). The bud inoculations in this study occurred on the third node at E-L stages 14 and 17; further experiments would need to be conducted to determine specific age related susceptibility of grapevine buds to *P. viticola* infection. Ontogenic resistance against *P. viticola* is known to occur in grapevine berries (Kennelly et al., 2005).

Both artificially inoculated and naturally infected grapevine buds were found to be killed by *P. viticola* infections. This agrees with previous observations where *P. viticola* has been shown to infect all green structures of grapevines causing necrosis (Wilcox et al., 2015). The presence of *P. viticola* in the dead tissue was confirmed by molecular analysis during the study. It is unknown why a greater percentage of buds from a naturally infected vineyard were killed by *P. viticola* than the point inoculated experiments. It may be a result of the level of the inoculum load and the period of the epidemic within the vineyard scenario compared to a specific amount of inoculum applied once during the point inoculation experiments. The point inoculation experiment did however show the progression of the infection within a growing season; from oily discoloured tissue which darkens and finally longitudinal cracking appears within the lesion boundaries. The development of longitudinal streaks on internodes caused by *P. viticola* infection have previously been described by Lafon and Bulit (1981).

Despite the aggressiveness of the *P. viticola* on infected grapevine buds, the present study identified visible external lesions on nodes do not always correspond to bud death and healthy shoots are produced from these apparently infected nodes. A number of plant defense
responses including callose deposition, stilbene production and programmed cell death around the infection site have been described for differences in susceptibility between *Vitis* species (Boso et al., 2014, Yu et al., 2012, Alonso-Villaverde et al., 2011). These defense mechanisms could explain why no significant difference between the fungicide treatments and the non-treated controls were observed in this study. The two fungicides applied during this study are known to exhibit strong post infection control of *P. viticola* (Wong and Wilcox, 2001, Underdown et al., 2008, Wicks et al., 1991). However, the efficacy of post infection fungicides reduces in time after the infection event, usually before the visual symptoms of the disease (Wicks et al., 1991), and it may be the case in this experiment that the use of the post infection fungicides occurred too late to eradicate the pathogen. Therefore, it is suggested post-harvest or late season applications of post-infection fungicides are not economically practical due in part to timing issues in providing for adequate control and risk of resistance development.

The ability of another grapevine obligate biotroph in *E. necator* to remain viable overwinter in comparison to *P. viticola* may result due to the differing infection mechanisms of these pathogens. Besides the feeding haustoria, the entire structure of *E. necator* is external to the host tissue and therefore even under epidemic conditions only the epidermal cells of the host are killed (Rumbolz et al. 2000; Wilcox et al. 2015). Upon bud infection it is believed the fungus remains viable by living on the prophylls and leaf primordia until bud burst the following spring (Rumbolz and Gubler 2005). *P. viticola* invades the host via stomata, with mycelia extensively colonizing the leaf tissue to the upper epidermis layer, where haustoria are formed to penetrate the cell walls (Kiefer et al. 2002; Langcake and Lovell 1980). The greater penetration of the host tissue by *P. viticola* may mean the pathogen exhausts its food reserves, subsequently killing the infected tissue, prior to bud burst occurring the following season.
Death of grapevine buds by *P. viticola* has received little attention in regards to yield impact assessments in comparison to bunch and leaf infection. With yield reduction due to loss of flower clusters or young berries ranging from 50-100%, depending on weather conditions, bio-economic models and warning systems have been developed investigating the effectiveness of the control strategies available (Emmett et al., 1992, Leroy et al., 2013, Caffi et al., 2010). These models do not take into account the loss of crop yielding shoots in the subsequent year after the loss of buds from downy mildew infection. The impact is likely to be larger in production systems with cane pruning rather than spur pruning as they maintain a greater proportion of buds. However, infected nodes of varying ages were observed in field situations so yield loss can occur in both production systems. Whilst this study has observed the loss of buds in the subsequent growing season the impact of downy mildew infections can result in loss of vine vigour and yield up to three seasons after the infection event (Rives and Lafon, 1972).

Despite no presence of flag shoots, *P. viticola* can remain active throughout the year in WA vineyards due to climate and cultivar mix. This is particularly evident in the warm growing area of the Swan Valley which consists of both wine and table grape production systems in close proximity. Both production systems have early and late maturing cultivars and this means vegetative growth is present in the region year round. Chardonnay vines in the Margaret River region have occasionally been known to undergo budburst in winter due to the warm climatic conditions experienced (Robinson and Harding, 2015). With individual *P. viticola* oilspots maintaining maximum sporulation potential for a minimum 24 days (Kennelly et al., 2007) and the potential to survive for up to 2-3 months (Hill, 1989) there is sufficient vegetative growth for continued disease progression. However, although the potential of year round active *P. viticola* inoculum exists long range secondary spread of the
disease is very limited and therefore it is unlikely to be a source of a region wide disease epidemic (Gobbin et al., 2005).

Based on molecular analysis, taxonomically different P. viticola clades infect different Vitis host species (Rouzel et al., 2014, Rouxel et al., 2013). The cultivars used in this study are all V. vinifera and these are considered susceptible to the P. viticola clades aestivalis and vinifera (Rouxel et al., 2013), although there are differences in the level of susceptibility and severity of infection within them (Boso et al., 2011, Boso and Kassemeyer, 2008). The ability to successfully overwinter in buds may therefore be a combination of a less aggressive clade of P. viticola or more resistant host plant. For example, the observations of bud infection by Barrett (1939) were made on V. californica and not V. vinifera; therefore, the host/pathogen interaction may be different.

4. Conclusion

Current management for P. viticola in WA is to target oospores as the primary infection source with sprays based on the assumption of weather conditions suitable for germination and infection. This study has confirmed management based on this premise provides the greatest likelihood of successful control as downy mildew flag shoots are not a source of primary infections within WA vineyards. However, care still needs to be taken when establishing new production areas as incubation periods and latent infections within green tissue can remain undetected until suitable conditions exist for sporulation to occur. It is likely the movement of plant material aided the rapid spread of the disease within WA viticulture regions. Although flag shoots were not detected, it was found green developing buds are susceptible to infection at a young age. Infection often resulted in the death of the buds, but occasionally it appears the infection is prevented from killing the dormant shoot. Further research is required to understand the mechanism by which this is occurring.
Chapter 5

Detection of grape downy mildew oospores in Western Australian vineyards and mating type crosses

5. Abstract

Oospores are important for both the overwintering and primary infection of *Plasmopara viticola* within vineyards. As *P. viticola* is heterothallic, oospores develop when two distinct mating types fuse within grapevine leaves. However, in WA surveys of vineyards revealed a lack of oospores within grape growing regions in the years after the initial detection in 1998. In Autumn 2015 vineyards in the Swan Valley and Margaret River regions of WA were surveyed for the presence of oospores by targeting mosaic leaf symptoms. Oospores were detected in all vineyards sampled and now appear widespread. Using detached leaf discs co-inoculated with 11 isolates from geographically separate vineyards the mating type ratio of *P. viticola* present in WA was assessed. Oospores were detected in three of the 726 total co-inoculated combinations with one being a co-inoculation of the same isolate. This has raised questions about the flexibility of *P. viticola* to produce survival structures in the absence of both mating types as observed in other oomycetes.

5. Introduction

Grapevine downy mildew, caused by the oomycete *Plasmopara viticola*, is an economically significant disease in Western Australian vineyards (Chapter 6). *P. viticola* is dimorphic, having the ability to produce both asexual and sexual reproductive structures during a growing season (2013; Rossi et al. 2009). Oospores, the sexual spores, overwinter within fallen and decaying grape leaf material on and within the vineyard soil and result in
the primary infection of the disease the following spring (Burruano 2000; Gregory 1912). It was once thought oospores were only important for primary infection with continued secondary infection leading to disease epidemics. However, studies involving molecular markers have highlighted the importance of oospore germination in driving downy mildew epidemics throughout the season and the long distance spread of the disease (Gobbin et al. 2005; 2006; Rossi et al. 2013).

As a heterothallic organism *P. viticola* requires the fusion of the antheridia and oogonia of two different mating types for the formation of oospores to occur (Burruano 2000; Wong et al. 2001). Oospores can develop within a vineyard at any time during the vegetative stage of the growing season (Ronzon-Tran Manh Sung and Clerjeau 1988), but are most prominent in Autumn and often associated with the presence of mosaic leaf symptoms (Burruano 2000; Rossi and Caffi 2007; Vercesi et al. 1999). Grapevine leaves are less susceptible to infection as they age, restricting *P. viticola* infections within the leaf veins forming a mosaic of infected and uninfected sections within the leaf (Reuveni 1998). During winter oospores undergo morphological maturity whilst on the vineyard floor but may not germinate, even under favourable conditions, due to undetermined internal physiological dormancy (Rossi and Caffi 2007; Vercesi et al. 1999). This creates cohorts of oospores with germination occurring at different times throughout the growing season, with some able to survive several years in the soil (Caffi et al. 2011; Jermini et al. 2003).

Temperature and water availability in the leaf litter are key factors in breaking oospore dormancy and for germination (Burruano 2000; Rossi and Caffi 2007). Supply of water to the leaf litter can occur via rainfall, dew or absorption from atmospheric humidity and therefore oospore development can occur without the specific requirement of rainfall, however water splash is required to transport the inoculum to leaves in the canopy (Rossi and
Caffi 2007; Rossi and Caffi 2012). The process of oospore germination occurs from a minimum of 10°C to 28°C, with optimum of between 20-22°C (Burrano et al. 2006). Optimal germination occurs if the outer wall is ruptured, possibly after a light freeze and sufficient humidity (Gessler et al. 2011).

In Australia, downy mildew was first detected on the east coast in 1917 but commercial Western Australian (WA) viticulture regions remained free from the disease until the 1998/1999 season (de Castella and Brittlebank 1917; McKirdy et al. 1999). It is unknown where the WA P. viticola originated, as strict quarantine measures were in place and the region is too isolated for natural invasion. Even if introduced, the absence of favourable weather conditions for disease development was predicted to prevent its establishment (Williams et al. 2007b). Between 2001-2003 a survey of 16 WA vineyards only found oospores in one vineyard (Killigrew et al. 2005), and it was hypothesised the rarity of oospores was the result of only a single mating type dominating with the pathogen overwintering as mycelium (Killigrew 2006). In both American and European populations of P. viticola a 1:1 ratio of P1 and P2 mating types exist (Scherer and Gisi 2006; Wong et al. 2001). To date no studies of P. viticola mating type ratios have been conducted in Australia and therefore no reference isolates exist.

Since the 2001-03 survey Killigrew et al. (2005) P. viticola outbreaks have occurred across WA grape growing regions during wet seasons such as the 2008/09 season. The aims of this study were to: a) determine the current incidence of P. viticola oospores in WA grape growing regions, b) establishing the most efficient method to detect P. viticola oospores in grapevine leaves, c) identify and culturing reference isolates of both mating types of P. viticola. Understanding these aspects of the epidemiology of P. viticola in a temperate growing climate will ensure more efficient management of the pathogen.
5. Materials and Methods

Determining the current incidence of P. viticola oospores in WA vineyards

In Autumn (April) of 2015 ten vineyards in the Swan Valley (Swan District), Pemberton and Margaret River regions of WA which had experienced downy mildew infections of various levels of severity during the Spring of 2014 were revisited for a single sampling event (Fig. 5.1). The sampling was targeted towards leaves exhibiting mosaic symptoms (Fig. 5.2), as these are indicative of infection on older leaves where the infection is limited by leaf veins and have shown to harbor oospores in other studies (Emmett et al. 1992b; Vercesi et al. 2000; Vercesi et al. 1999). Approximately 50 whole leaves were removed from arbitrarily selected vines, placed in a plastic zip-lock bag and returned to the laboratory.
Each leaf was cut into strips the size of a microscope slide (7.6 x 2.5cm) and hydrated in warm water (35°C) containing one or two drops of a surfactant (600g/L Nonyl Phenol Ethylene Oxide condensate) to assist in hydrating the mesophyll layer (Georg Hill, pers comm.). The leaf strips were left on the laboratory bench for 24-48 hours before examining with a compound microscope at 4 and 10 times magnification (Olympus BX 41) for the presence of oospores.

In November 2015 mosaic leaf symptoms from a table grape vineyard left unmanaged were observed (Site 1 of Chapter 4). Leaf samples were taken and using the procedure previously described assessed for *P. viticola* oospores.
Establishing the most efficient method to detect *P. viticola* oospores in grapevine leaves

To aid in the detection of oospores and intracellular structures of *P. viticola* two leaf clearing procedures were tested. Using 1M KOH at 100°C and staining with aniline blue, Díez-Navajas et al. (2007) were able to successfully observe the invasive structures of *P. viticola* within host tissue. Initially this was attempted with the use of an open lid metal saucer of 1M KOH heated on a dry heat block in a fume hood. Leaf clearing created handling problems with the leaf material becoming very brittle and difficult to move onto a glass slide for observation. It was found moving the leaf strips to 2mL clear microcentrifuge screw capped tubes and heating in a dry tube block heater removed some of the handling issues and was easier to maintain temperature control. The higher the temperature the faster the leaf
clearing occurred. The clear tubes allowed for observations of how clear the leaf strips became and therefore allowed the strips to be removed when deemed suitable for staining. Prior to staining the KOH was removed from the tubes and the leaf strips were washed three times in distilled water. Aniline blue (C.I Acid blue 22, ThermoFisher, Australia) 0.05% was placed in the tubes and left overnight before being removed and the leaf discs were then washed three times with distilled water. Leaf strips were mounted in distilled water and observed using light microscopy. This method highlighted the presence of oospores in the leaf material.

Whilst the KOH leaf clearing was suitable for oospore detection it appeared to be damaging to other structures of *P. viticola*. A 2:2:1 clearing solution of glacial 100% acetic acid: 100% ethanol: deionised water was trialled based on observations of Williams (2005). Similarly, to the KOH technique the leaf strips were placed in 2mL clear microcentrifuge screw capped tubes and heated in a dry tube block heater. It was found the technique was less effective in removing all the leaf content but this meant *P. viticola* structures including mycelium could clearly be differentiated when stained with 0.05% aniline blue as previously detailed.

*Identifying and culturing reference isolates of both mating types of *P. viticola***

During the 2015/16 growing season naturally infected leaves from ten geographically distinct vineyards were collected (Fig 5.1). Two samples were selected from different blocks within a single vineyard making a total of eleven isolates tested. Only distinct individual oilspots were sourced and each leaf was incubated overnight in a zip-lock bag with moisture to promote the production of sporangia. An individual sporangiophore with multiple sporangia was picked off a sporulating oilspot using the tip of fine point tweezers and transferred to a drop of distilled water placed on a detached leaf in a petri dish with the
abaxial side facing upwards. The detached leaves were sourced from rooted vines (cv. Chardonnay), grown cuttings from a vineyard with no evidence of *P. viticola* infection for over 10 years. Stomata for which *P. viticola* zoospores encyst are located almost exclusively on the abaxial side of grapevine leaves (Keller 2010). The petri dish was lined with a moistened filter paper and sealed with a strip of cling wrap. The petri dish was left on the laboratory bench overnight with air conditioning temperature set at 20°C. The following morning the lid of the petri dish was removed and any remaining liquid on the leaf evaporated. The petri dish was then resealed with cling wrap and maintained on the laboratory bench.

Approximately 5 days later sporiangophores were apparent on the detached leaf. To bulk up the sporangia small amounts of distilled water was used to wash the sporangia off the sporangiophores inside the petri dish. The liquid solution inside the petri dish was then sprayed onto the abaxial side of new leaves within petri dishes set up as described previously, using a modified micro-sprayer. The micro-sprayer was the spraying head and tube removed from retail breath mint spray bottles flushed several times with distilled water. To avoid cross contaminating isolates, the micro-sprayer was flushed with 70% ethanol and then distilled water between isolates. The isolates were bulked up at least three times prior to conducting the mating type tests.

A 7 μL droplet containing $2.5 \times 10^4 \, \text{mL}^{-1}$ sporangia, haemocytometer adjusted, of each isolate was co-inoculated on each of six replicate leaf discs for a total of 726 crosses (Fig. 5.3a and b). Only WA isolates could be used due to quarantine restrictions preventing use of eastern states isolates. Leaf discs were created by taking the 3rd leaf of visually healthy chardonnay vines grown at the Bunbury district office and had not been subject to any *P. viticola* infections. A corer with 1cm radius was used to core the leaf discs from the source.
leaf. The discs were placed abaxial side up in new petri dishes lined with moistened filter paper. After inoculation the petri dishes were sealed with cling wrap and left overnight on the laboratory bench at 20°C. The following morning the lid of the petri dishes were removed and the droplet was allowed to evaporate or dry. The lids were replaced and the petri dishes were resealed to maintain humidity and remained on the laboratory bench for the length of the experiment. The temperature in the laboratory was reduced and maintained at 16°C for the length of the experiment. This temperature is the balance between the Scherer and Gisi (2006) (18°C) and Wong and Wilcox (2001) (12°C) studies on the development of P. viticola oospores. Any remaining sporangia solution of the isolates was sprayed onto fresh leaves to ascertain if they were viable.

![Figure 5.3. A) Leaf discs were co-inoculated with spore solutions B) a total 726 leaf discs were inoculated.](image)

After 40 days the leaf discs were removed from the petri dish, washed with 70% ethanol to remove surface contaminants, and assessed for the presence of oospores using light microscopy. After an initial assessment the leaf discs were then cleared using the KOH-aniline blue staining technique to determine whether any oospores were missed.
**Light microscopy and photography**

An Olympus BX 41 light microscope was used to assess for oospores in both the naturally infected leaf strips and leaf discs used in the mating tests. Unless otherwise stated an Olympus BX 51 with MicroPublisher 3.3 RTV (QImaging) mounted camera and cellSens standard software (Olympus) were used for photography purposes.

**5. Results**

*Determining the current incidence of P. viticola oospores in WA vineyards*

Oospores were detected in all vineyards sampled and were visible in leaf tissue without the need for leaf clearing or staining (Fig. 5.4a), however clearing and staining enabled a more defined assessment (Fig. 5.4b). A large range of densities occurred within individual leaf strips with infected areas within the strip containing as little as 4-10 oospores whilst others had several hundred constricted within leaf veins alone. The earliest observation of oospores occurred in November in the unmanaged vineyard. Not all leaves with mosaic symptoms were found to present with oospores.
Figure 5.4. a) Oospores could be detected without the need for staining (Bar = 300µm) b) however, staining did improve the detection efficiency (Bar = 100 µm). Note a) taken on an Olympus DP20 camera.

Observations and Measurements

All oospores presented as double walled (Fig. 5.5) with a mean diameter of 30.6 µm (± 3.87 standard deviation) from 20 measured using the measurement tool within the cellSens standard software program. This fits the size range of *P. viticola* oospores as described by Wilcox et al. (2015). The development of the oospores occurs with amphigynous antheridia as observed in Fig. 5.5b.

Figure 5.5. A) Oospores presented with double walls (Bar = 20µm B) evidence of amphigynous oospore development (Bar = 10µm). Oospores were stained with aniline blue.
Identifying and culturing reference isolates of both mating types of *P. viticola*

Sporulation of the *P. viticola* isolates used in the mating type test occurred approximately 5-7 days after inoculation (Fig. 5.6a). Eventually the leaf discs were colonised by secondary pathogens, however some leaf discs did produce mosaic symptoms within the time period of the trial (Fig. 5.6b). The presence of mosaic symptoms did not however correspond to oospore detection. Once assessed three leaf discs from the 726 were observed to contain oospores, these included the crosses of AT326 x AT101 and AT326 x AT312. Oospores were also detected in a single leaf disc of a self-cross of isolate AT 324 x AT 324.

Figure 5.6. A) Sporulation occurred on the leaf discs indicating successful inoculation B) mosaic symptoms were observed in a small number of leaf discs.

5. Discussion

This study has confirmed the presence of *P. viticola* oospores in WA vineyards. The observation of oospores in all vineyards surveyed, geographically separated by several hundred kilometres, indicates their presence is widespread within vineyards across the state. This contrasts the findings of Killigrew et al. (2005) who found oospores in only one of 16 vineyards surveyed. The present study was conducted 17 years after the detection of the disease in WA, in comparison to the 3-5 years by the Killigrew et al. (2005) survey, and it is likely the time differential has allowed the disease to become more established for oospore development. It was suggested the lack of oospores was the result of a genetic bottleneck
situation where genetic diversity was so low at a vineyard level sexual reproduction did not occur regularly (Gessler et al. 2011). Whilst this is plausible in the early years of the disease spread in WA, as the genetic diversity remains low (Chapter 3) questions are raised about this hypothesis.

After the detection of oospores in the field and the presence of oospores in the mating type tests it was assumed both mating types were present in WA, as *P. viticola* is only known to be heterothallic (Scherer and Gisi 2006; Wong et al. 2001). However, the results of this study raise questions over the understanding of the mating system of *P. viticola* in WA. Only three leaf discs produced oospores in the mating test and one of those was co-inoculated with the same isolate. The lack of oospores observed in the number of crosses may be explained by the fact the eleven isolates most were of the same mating type and therefore didn’t reproduce sexually. However, as oospores were produced by crosses of two different isolates it would be expected oospores should be produced by crosses of at least one of those isolates with others in the test if they were putatively a different mating type.

One isolate producing oospores when co-inoculated with itself suggests potential for secondary homothallism to occur within the *P. viticola* population. Whilst never being reported in *P. viticola* populations elsewhere, the existence of secondary homothallism or selfing has been reported in related species, including *Bremia lactucae*, *Hyaloperonospora parasitica* and *Phytophthora infestans* (Michelmore and Ingram 1982; Sherriff and Lucas 1989; Smart et al. 2000). Selfing is also known to occur in some oomycetes in the presence of external stimuli (Jayasekera et al. 2007; Judelson 2009). No external stimuli were provided in this study besides reducing the temperature of the laboratory although there is the possibility an unknown exudate from the grape leaves could potentially stimulate oospore production.
To overcome issues in mating type determination future mating type tests on Australian isolates need to be paired with reference isolates of known mating types. Contact was made with overseas collaborators to conduct a test using known mating type isolates from their populations, but this was met with challenges surrounding the obligate biotroph nature of *P. viticola* and the associated issues in long term storage of viable isolates. Movement of viable *P. viticola* isolates between states within Australia also requires quarantine permits and this restricts the laboratory space where these tests can be conducted.

The detection of oospores in WA vineyards has management implications for viticulturists. Oospores are the cause of primary infection of the disease and the sheer number of oospores detected in some of the grape leaf strips in vineyards indicates a large inoculum pool exists. Furthermore, oospores are known to survive for multiple seasons in the soil (Caffi et al. 2011; Kennelly et al. 2007), and this means potential for disease outbreak is ever present, even after seasons where the disease has not been particularly severe.

Oospores are a means of phenotypic inheritance, with particular implications for fungicide resistance in *P. viticola* populations (Blum et al. 2008; Gisi and Sierotzki 2015; Gisi et al. 2007). In oomycetes, a single recessive nuclear gene mutation is responsible for resistance to carboxylic acid amide (CCA) chemistries, a single maternal mitochondrial gene mutation for quinone outside inhibitors (QoI) and a semi dominant nuclear gene mutation for phenylamides (PA) (Gisi and Sierotzki 2015). In European populations of *P. viticola* with 1:1 mating types this produces F1 and F2 progeny with resistant, intermediate and susceptible phenotypes depending on Mendelian distributions (Blum et al. 2008; Gisi and Sierotzki 2015; Gisi et al. 2007). Resistance risk based on the mutation mechanism within *P. viticola*, PA and QoI chemistries are considered high and CCA are considered moderate (Blum et al. 2008). Resistance to the active ingredient metalaxyl (mefenoxam), a PA chemistry, in WA isolates
of *P. viticola* has been confirmed (Hall 2017), and as this is a mutation of a dominant gene it is expected this mutation will become more widespread amongst grape growing regions within the state.

5. Conclusion

*P. viticola* oospores were detected in all viticulture regions sampled in WA and mating crosses also detected oospores, indicating a widespread distribution and the potential for both mating types to exist. More research is required to determine the viability of the oospores detected and the conditions required for germination in WA with a predominantly Mediterranean climate. Further work is also required to investigate the mating type ratios within the WA and Australian populations of *P. viticola*. This will aid in the understanding and management of fungicide resistance.
Chapter 6

An economic assessment of the impact on the Western Australian viticulture industry from the incursion of grapevine downy mildew

6. Abstract

Grapevine downy mildew (Plasmopara viticola) was first detected in commercial vineyards in the Swan Valley region of Western Australia (WA) in 1998 and has now spread to all grape growing regions of the state. This paper uses a bioeconomic model linking weather, spread, infection development, variable production costs and revenue to estimate the costs of P. viticola over time. Results indicate mean annual costs of AUD $7.3 million per year, equivalent to a 1% drop in the gross value of WA grape growing industries. Cumulative losses since the disease became established are estimated to be AUD $140 million.

6. Introduction

Grapevine downy mildew, caused by the oomycete Plasmopara viticola, is a disease of economic importance in most grape-growing regions with relatively warm and humid summers (Wilcox et al. 2015). With suitable conditions, P. viticola infections can cause foliage and inflorescence loss in grape vines of 50-100% (Emmett et al. 1992), while fungicides used to control it represent 54% of the US$1.2 billion global fungicide market for oomycete pathogens (Gisi and Sierotzki 2008). In this paper, we construct a bioeconomic model to provide the first estimate of variable production cost increases and revenue losses attributable to P. viticola in Western Australia (WA).
Various computer models have been developed to predict infection events based on vine phenological stage, rainfall, temperature, humidity, light intensity and leaf wetness (Blaise et al. 1999; Caffi et al. 2010b; Kennelly et al. 2007; Madden et al. 2000; Magarey et al. 1991; Orlandini et al. 2008; Park et al. 1997; Rosa et al. 1993; Rosa et al. 1995; Rossi et al. 2008b; Rossi et al. 2009; Vercesi et al. 2010). However, relatively few studies have combined these biological parameters with economic consequences of infection by developing bioeconomic models (Blaise et al. 1996; Leroy et al. 2013).

Following the first detection of *P. viticola* in Australia, which occurred in the State of Victoria in 1917 (de Castella and Brittlebank 1917), downy mildew spread rapidly through eastern grape growing regions of the country. This occurred through a combination of wind and human-aided dispersal (Osborn and Samuel 1922). The disease has continued to affect growers in these regions. Magarey et al. (1991) estimated resultant costs across eastern Australia in low and high rainfall years to be approximately $13 million and $47 million, respectively. A more recent analysis at an individual vineyard level found profit reduction due to *P. viticola* ranged from AUD 218 to $814/ha/annum depending on regional climatic conditions, implying a national impact of AUD $63m/annum (Scholefield and Morison 2010).

The isolation of WA from eastern Australian viticulture regions by natural barriers and stringent quarantine restrictions on the movement of grape material prevented *P. viticola* from establishing in commercial production regions until 1998 (McKirdy et al. 1999; McLean et al. 1984). Up to this point it was presumed environmental conditions prevented the disease from developing significantly in WA (McLean et al. 1984; Weltzien 1981; Williams et al. 2007b), but it has now spread to all viticulture regions of the state (Williams et al. 2007a).
As there are no definitive records of WA grape area affected by \( P. \) \textit{viticola} since arrival, estimating the damage costs attributable to downy mildew requires the use of a predictive model. The model used in this paper accounts for natural and human mediated \( P. \) \textit{viticola} spread scenarios and sporadic weather events promote periodic severe outbreaks. The financial implications of disease spread are calculated as changes in grape industry revenue and variable production costs.

6. Materials and Methods

Stochastic ‘probability models’ relying on random number generators to simulate random events are commonly used in modelling natural systems with high parameter uncertainty and variability (Hodda and Cook, 2009; Maguire, 2004). We adapt the stochastic model of Cook et al. (2015) to simulate the spread of \( P. \) \textit{viticola} over time, adding a weather variable to simulate climatic conditions conducive to severe damage events.

Parameters within the model represent the ecological processes of spread, establishment, population growth and vine damage, together with their economic consequences in terms of crop yields and control measures (Table 1). Each parameter is given a mean or modal value as well as variation about this value, which may have a number of statistical distributions as listed (Hodda and Cook, 2009).

The model calculates the present value of damage caused by \( P. \) \textit{viticola} in a given grape-growing region \( i \) in time \( t \left( d_{it} \right) \) by converting yield loss and variable production cost increases to present value terms. The estimation period spans 30-years beginning at the time of arrival in 1998, so contains both past and future values converted to present values. Past values are inflated at 2.6% per annum (Australian Bureau of Statistics, 2017), and future
values are discounted using a hyperbolic discount rate of 5% per annum (Commonwealth of Australia, 2006).

In the applied model, \( d_{it} \) is calculated as:

\[
d_{it} = Y_{it}P_{it}A_{it} + V_{it}A_{it} \quad [1]
\]

where: \( Y_{it} \) is the change in yield resulting from \( P. viticola \) becoming established across region \( i \) in year \( t \) despite the efforts of growers to control it; \( P_{it} \) is the prevailing price for an affected host (i.e. wine, table or dried grapes) in year \( t \); \( A_{it} \) is the area affected by \( P. viticola \) in region \( i \) in year \( t \) weighted by the density of infection; and \( V_{it} \) is the increase in variable cost of production per unit of area (i.e. hectares, ha) induced by \( P. viticola \) management methods in region \( i \) in year \( t \). The WA viticulture industry is exclusively based on \( Vitis vinifera \) cultivars with no hybridisation and planting ratio of 1:1 white to red cultivars in the majority of vineyards. Although differences in susceptibility between \( V. vinifera \) cultivars have been reported (Boso et al. 2011), we assume under favourable weather conditions all are susceptible to infection.
In regards to prevailing price of a host in time period $t$, the volume produced affects the price of the commodity in monopolistically competitive markets. For practical purposes, we use predicted production loss $Y_i A_n$ as a proxy for the reduction in output caused by yield loss attributable to $P. viticola$. This is divided by the gross value of production for the host, $G_t$, to approximate proportion of total value lost to the disease, which is converted to a percentage. This percentage change in the value of output is used as a proxy for percentage change in quantity which is combined with the lagged host price (i.e. host price in the previous time step), $P_{t-1}$, to calculate $P_t = P_{t-1} \left[ 1 - \left( \frac{Y_i A_n}{G_t \varepsilon} \right) \right]$. Here, $G_t$ is the gross value of production divided by 100 and $\varepsilon$ is the price elasticity of demand for the affected host.

To describe the dispersal of $P. viticola$ across multiple regions after its arrival, a stratified diffusion model combining both short and long distance dispersal processes is used (Hengeveld, 1989). It is derived from the reaction diffusion models originally developed by Fisher (1937) which have been shown to provide a reasonable approximation of the spread of a diverse range of organisms (Okubo and Levin, 2002, Dwyer, 1992, Holmes, 1993, McCann et al., 2000).

These models assume an organism diffusing from a point source will eventually reach a constant asymptotic radial spread rate of $2\sqrt{r_t D_{ij}}$ in all directions, where $r_t$ describes a growth factor for $P. viticola$ per year in region $i$ (assumed constant over all infected sites) (Lewis, 1997, Shigesada and Kawasaki, 1997, Hengeveld, 1989). $D_{ij}$ is a diffusion coefficient for an infected site with an age index $j$ in region $i$, and is also assumed constant over time for simplicity. Following an initial infection, the area affected at time $t$, $a_{ijt}$, is predicted by:
\[ a_{it} = 4D_{it} \pi r_{ij}^2. \]  

[2]

The density of infection within \( a_{it} \) influences the control measures required to counter the effects of an infection, and thus partially determines the value of \( A_{it} \). Assume that in each site with age index \( j \) in region \( i \) affected, the density \( (N_{ijt}) \) of infection grows over time following a logistic growth curve until the carrying capacity of the environment \( (K_{ij}) \) is reached:

\[ N_{ijt} = \frac{K_{ij}N_{ijt}^{\text{min}} e^{\delta_{ij}t}}{K_{ij} + N_{ijt}^{\text{min}} (e^{\delta_{ij}t} - 1)}. \]  

[3]

Here, \( N_{ijt}^{\text{min}} \) is the size of the original infection at a site with age index \( j \) in region \( i \), and \( \delta_{ij} \) is the rate of density increase in region \( i \).

In addition to \( a_{it} \), the total area affected \( A_{it} \) also depends on the number of nascent foci (or satellite infection sites) in year \( t \), \( s_{it} \), which can take on a maximum value of \( s_{it}^{\text{max}} \) in any year. Satellites are caused by exogenous factors, such as human and machinery movements, which transport \( P. \ viticola \) to new sites. In this analysis, a logistic equation is used to generate changes in \( s_{it} \) as an outbreak continues:

\[ s_{it} = \frac{s_{i}^{\text{max}} s_{ijt}^{\text{min}} e^{\mu_{i}t}}{s_{i}^{\text{max}} s_{ijt}^{\text{min}} + s_{ijt}^{\text{min}} (e^{\mu_{i}t} - 1)} \]  

[4]

where \( \mu_{i} \) is the intrinsic rate of new foci generation in region \( i \) (assumed constant over time), and \( s_{ijt}^{\text{min}} \) is the minimum number of satellite sites generated in region \( i \).
Given the predicted area affected by *P. viticola* in sites having been affected for different lengths of time (i.e. Eq. 2) and the number of satellite sites predicted to have been created (Eq. 4), the total area, $A_i$, is calculated across $m$ sites as:

$$A_i = s_i \sum_{j=1}^{m} d_{ij} \text{ where } 0 \leq A_i \leq A_i^{\text{max}}$$

[5]

The cost resulting from *P. viticola* is calculated in the model as a function of a weather variable, $w$, and $d_{ij}$ such that total cost (TC) over $t$ years ($\text{TC}$) across $n$ regions is:

$$\text{TC} = \left( \sum_{i=1}^{n} d_{it}N_{it}w_t \right) t^{-1}.$$  

[6]

The parameter $w_t$ is a dichotomous weather variable that takes on the value of one if in growing seasons are sufficiently wet, and zero otherwise. Oospore-driven infections occur when vine shoots are $\geq 10\text{cm}$ long, a minimum $10\text{mm}$ of rain and an air temperature of $\geq 10^\circ\text{C}$ has occurred over a 24 to 48 hour period (Rossi and Caffi, 2012). For secondary sporulation and infection $98\%$ humidity, 4 hours of darkness and a minimum temperature of $13^\circ\text{C}$ is required (Gessler et al., 2011). We assume $w = 1$ with an average frequency of one year in three based on infestation frequency data in Scholefield and Morison (2010) and Lantzke (2004).

In the following analysis, TC associated with *P. viticola* in WA is estimated in present value terms using a large number spread scenarios. Each scenario is simulated over a 30-year period beginning in 1998 when the disease was first reported in the state. In forming these scenarios, parameters which cannot be given definitive values are specified within the model as distributions and a Latin hypercube sampling algorithm is used to sample from each distribution using the @Risk™ software package (Palisade Software, Ithaca, New York). In
each of the 10,000 model iterations, one value is sampled from the cumulative distribution function so the sampled parameter values are weighted according to their probability of occurrence. Model calculations use the sampled set of parameters.

Types of distributions used in the model include: (i) PERT, a type of beta distribution specified using minimum, most-likely, and maximum values; (ii) uniform, a rectangular distribution bounded by minimum and maximum values; and (iii) binomial, returning a zero (i.e. failure) or one (i.e. success) based on a number of trials and the probability of a success.

<table>
<thead>
<tr>
<th>Table 6.1. Parameter values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
</tr>
<tr>
<td>Demand elasticity, $\eta$. $^a$</td>
</tr>
<tr>
<td>Increased variable cost of production per ha induced by <em>P. viticola</em> management methods, $V$ ($/ha$). $^b$</td>
</tr>
<tr>
<td>Intrinsic rate of new foci generation per unit area of infection, $\mu$ ($#/m^2$, i.e. 1ha = 10 000$m^2$). $^c$</td>
</tr>
<tr>
<td>Intrinsic rate of infection and density increase, $r$ (yr$^{-1}$). $^c$</td>
</tr>
<tr>
<td>Maximum area affected, $A_{\text{max}}$ ($m^2$). $^d$</td>
</tr>
<tr>
<td>Maximum number of satellite sites generated in a single time step, $S_{\text{max}}$ (#). $^c$</td>
</tr>
<tr>
<td>Maximum population density, $K$ ($#/m^2$). $^c$</td>
</tr>
<tr>
<td>Minimum area affected immediately upon entry, $A_{\text{min}}$ ($m^2$). $^c$</td>
</tr>
<tr>
<td>Minimum number of satellite sites generated in a single time step, $S_{\text{min}}$ (#). $^c$</td>
</tr>
<tr>
<td>Minimum population density, $N_{\text{min}}$ ($#/m^2$). $^c$</td>
</tr>
<tr>
<td>Population diffusion coefficient, $D$ ($m^2$/yr). $^c$</td>
</tr>
<tr>
<td>Price of grapes, $P$ ($$/t$)</td>
</tr>
<tr>
<td>Probability of adverse weather conditions, $w$ (%)</td>
</tr>
</tbody>
</table>

$^a$ Ulubasoglu et al. (2011), $^b$ Cook and Fraser (2014), $^c$ Specified with reference to case studies in Waage et al. (2005), $^d$ ABS (ABS 2014).

6. Results

Economic impact predicted by the model is proportional to the area affected in the initial years post incursion, increasing rapidly as the disease spreads to new production areas. Peak economic impact occurs approximately 20 years post-incursion as the area affected
approaches 12,000 hectares (Fig. 6.1A). The mean annual cost over the 30-year simulations is $7.3 million, with a standard deviation $2.6 million (Fig. 6.1B).

Uncertainty in model predictions increases the further in time we project. Model estimates for both economic impact and area affected are particularly uncertain between 10 and 20 years post-incursion. In the absence of any eradication effort in WA, the area affected by *P. viticola* does not fall from one year to the next. In contrast, the economic impact falls from approximately year 20 as the erosive effects of the discount rate begin to outweigh the yearly increase in impact.
Fig 6.1. Predicted spread and impact of downy mildew in WA. Expected area affected and resultant damage costs are shown in panels A and B, respectively. The box-whisker plots in each pane show the 25th percentile, median, 75th percentile and remaining values up to and including the 5th and 95th percentiles.
6. Discussion

The mean annual economic costs of $7.3 million as a result of the *P. viticola* introduction to WA viticulture is in broad agreement with the $63 million national industry loss caused by the disease as put forward by Scholefield and Morison (2010). The estimated impact of the disease is equivalent to approximately 1% of gross value of both the WA and Australian viticulture industries (Australian Bureau of Statistics 2014).

The model suggests a rapid increase in the area affected occurred during the early years post-incursion with minimal intervention. This appears to provide a fair representation of observed spread in WA following *P. viticola* introduction. Within a week of the initial 1998 detection in the Swan Valley region, *P. viticola* was not considered eradicable due to the extent of infection. Growers were instead educated on management options, including the timing and use of fungicides in addition to their current practices. It took industry several seasons to become confident in how to manage the disease under local environmental conditions. Whilst this occurred *P. viticola* was detected in all major viticulture areas north and south of the Swan Valley region, including areas previously considered unfavourable for disease development (Williams et al. 2007a).

During the 1990’s tax incentives and changing consumer preferences meant viticulture plantings across WA and Australia were expanding rapidly (Anderson and Aryal 2015). It is likely this provided a catalyst for human-assisted spread of *P. viticola* to new grape growing regions where environmental conditions were favourable for disease development. However, impacts were not necessarily felt immediately. This is because in any given population of *P. viticola* oospores variable germination rates occur, with many lying dormant in the soil for several years post-arrival (Caffi et al. 2011; Jermini et al. 2003).
Due to the dependence of downy mildew on weather conditions, the weather variable used in the model is critical. We assume favourable conditions are likely to have occurred one year in every three post-introduction to a region, although exact timing of weather events vary with each model iteration. Favourable conditions are considered the 10:10:24 weather parameters for predicting primary infection. This was introduced to WA growers to assist in management of the disease after the detection in the Swan Valley and is often referred to as a useful guide for viticulture production over a large geographical area, such as experienced in WA (Nicholas et al. 1994; Wilcox et al. 2015). However, being of a generalised nature models based on these parameters have been known to cause under or over estimation of infection events (Gessler et al. 2011; Rossi and Caffi 2012). Whilst we have conservatively used an infection rate of 1 in 3 years a more specific weather parameter for WA conditions could refine the estimates or reduce the standard deviation seen.

The south west region of WA, where the majority of viticulture plantings are located, has experienced a period of declining rainfall and a steady increase in temperatures over the past 40 years (Climate Commission 2011). Comparable modelling of climate change in Italian vineyards suggests higher temperatures counterbalance reductions in precipitation and tend to promote *P. viticola* disease pressure early in the growing season, thus necessitating more intensive (i.e. costly) fungicide treatments (Salinari et al. 2006).

Fungicide purchases and application represent a large proportion of the cost imposed by *P. viticola* in affected areas. Increased costs in disease management represented 58% of the industry-wide reduction in profit amongst the total Australian market (Scholefield and Morison 2010). In this study an addition 1-3 fungicide treatments per season were added to spray regimes specifically for the control of downy mildew. The model does not distinguish between preventive treatments (i.e. applied prior to any infection event) and post- infection
treatments (i.e. applied in an effort to eliminate the disease prior to damage occurring). We therefore understate the costs of preventive sprays as they are assumed to only result in seasons when conditions are favourable, whereas in reality they accrue regardless of whether infection occurs or not.

The implications of fungicide resistance and loss of available chemistries to control the disease warrant further investigation. *P. viticola* is considered a high-risk pathogen for the development of fungicide resistance, particularly those chemistries with single site activities (Fungicide Resistance Action Committee 2014, Gisi and Sierotzki, 2008). Moreover, market access requirements have restricted phosphorous acid use on Australia wine grapes destined for export since the 2006-07 growing season, limiting the chemistries available to be used post-infection (Hoare 2011). The effect this could have on the development of resistance and resultant impacts of the disease over time have yet to be investigated.

The germination of oospores and subsequent infection is more complex than suggested in the use of a binomial weather variable, \( w \) (i.e. eq. 6). A number of factors involving the physiological development of the oospores in combination with environment factors are required for successful infection to occur and even then it can be intermittent (Rossi et al. 2008a). The future addition of variables involving the maturation and germination stages of *P. viticola* oospores requires further investigation.

6. Conclusion

This study has presented a bioeconomic model to be used to estimate the economic effects *P. viticola* has had on the WA viticulture industry since its introduction in the late 1990s. Results indicate the disease has caused intermittent damages due to its dependence on
suitable weather conditions, but average annual economic losses have been in the order of $7.3 million. Despite its prevalence elsewhere in the world, there remains a great deal of uncertainty about important *P. viticola* spread parameters. This is exacerbated by uncertainty in the frequency and severity of weather events triggering the economic effects of the disease, and by the propensity of the pathogen to develop resistance to chemical treatments.
Chapter 7

General Discussion

Despite being present in Australia for over 100 years limited information was known about the source of origin and population structure of *P. viticola* in Australian vineyards prior to the commencement of this study. Given the relatively recent detection of the disease in WA, being some two decades, even less was known about its ability to survive and cause economic impact in a production system considered less than optimal for high yield loss. The development of microsatellite markers specific to *P. viticola* has enabled considerable understanding of the disease and population structure of the pathogen to be established (Rossi et al. 2013). The vast majority of studies have occurred within European and North American production systems where climatic conditions are more favourable for disease development. By identifying microsatellites (Chapter 2) this study has addressed both the source of origin into Australia and the likely introduction into WA (Chapter 3).

*P. viticola* is one of the most studied organisms in plant pathology with over 3000 papers published on the disease since 1910 (Gessler et al. 2011). However, the aspect of overwintering in dormant buds has received little attention. This project is the first to investigate the presence of grape downy mildew flagshoots caused by overwintering mycelium (Chapter 4). Finding *P. viticola* doesn’t produce flagshoots by overwintering within buds, combined with the discovery of non-random (asexual) mating in the WA population (Chapter 3), despite the detection of oospores (Chapter 5) indicates the pathogen is able to cause disease under sub-optimal climatic conditions. It is proposed this adaptation is an ability to self, where oospores are produced by a single mating type. It was once thought the climate in WA was unfavourable for *P. viticola* to become an economic threat to
commercial viticulture production (McLean et al. 1984), the ability to adapt has meant the disease has a high economic impact (Chapter 6).

**Genetic diversity and population studies**

The results of this study have highlighted the genetic diversity of the *P. viticola* populations between countries and within states of Australia differ (Chapter 3). However, it is difficult to compare the results of this study with the genetic diversity seen within other investigations. Whilst the use of microsatellites has enabled a greater understanding of the genetic diversity of *P. viticola*, the fact over 50 microsatellites markers exist has meant researchers have used a number of different combinations of the markers within their studies, leading to different findings. For instance, both Fontaine et al. (2013) and Gobbin et al. (2006) used different microsatellite combinations to study the *P. viticola* populations in Europe, with one finding high genetic diversity and the other indicating a low level of diversity. To overcome this issue in the *P. infestans* global population a set of easily and efficiently reproducible multiplex microsatellite primers has been developed with an accompanying international database of genetic diversity (Li et al. 2013). A similar international standard could be developed for researchers of *P. viticola*. By a stringent comparison of all existing primers (Chapter 2) a set of reproducible, reliable primers were characterized and then used to examine the populations of *P. viticola* in a number of countries (Chapter 3). This set of primers could now be used as a base standard for further population examinations of *P. viticola*.

The genetic diversity within the WA population was the lowest of all populations included in this study (Chapter 3). This is not surprising given the relatively recent introduction of the disease in comparison to other populations examined, the presence of a geographical barrier preventing natural spread (desert) and the requirement of all new
grapevine material to spend 12 months in quarantine, where they are examined for *P. viticola*. Despite the genetic bottleneck situation, *P. viticola* has a high potential for adaptation and low probability of future extinction (Gobbin et al. 2006). There is the possibility adaptation has already begun within the WA population, with the discovery of direct germination of sporangia, via the production of a germ tube, without the presence of free water (Williams et al. 2007c). This adaptation would assist the pathogen in infecting host plants in viticulture regions such as WA where Mediterranean climates with minimal summer rainfall occur.

The study by Fontaine et al. (2013) proposes the European isolates of *P. viticola* are likely to have resulted from a single source population from North America. It is often acknowledged the probable introduction occurred via native American *Vitis* species which were imported for use as rootstocks due to their resistance to grape phylloxera (Gessler et al. 2011; Rossi et al. 2013). The three species *V. riparia*, *V. rupestris* and *V. berlandieri* represent the majority of the introduced rootstocks (Grzegorczyk and Walker 1998). This project has shown *P. viticola* clade *aestivalis* to be the cryptic species of grape downy mildew in South America and Australia and these populations have linkages with isolates from France (Chapter 3). The *aestivalis* clade of downy mildew has host specialization to *V. vinifera*, *V. labrusca*, *V. aestivalis* species and their associated hybrids (Rouxel et al. 2014; Rouxel et al. 2013), and therefore the species used as rootstock would not have hosted the pathogen as assumed. *V. labrusca* is suspected as a potential source for the introduction of phylloxera into Europe as it has low phylloxera tolerance (Robinson 1997), and being a host of the *P. viticola* clade *aestivalis* it is also a potential source of downy mildew.

Further questions are raised about how the pathogen has made its way into Europe and WA from their respective source populations if overwintering in grapevine buds does not occur (Chapter 4). As overwintering mycelium is unsuccessful within dormant cuttings, the
introduction pathway would have to have occurred either via the movement of infected but latent vine material with active growth, soil containing viable oospores or oospores having developed within stems or shoot material. The movement of soil into WA is prohibited and in the cases where it is allowed it has to be sterilized or fumigated prior to entry so it is unlikely to have been the source of the WA population. *P. viticola* oospores are most commonly detected within grape leaves with little to no reports of detection within shoots or stems. As canes can be infected by *P. viticola* then it is plausible oospores could develop within these tissues. The presence of oospores within stem tissue and therefore cuttings would make long distance movement of *P. viticola* feasible. Further studies are required to determine the production of oospores in grapevine structures other than leaves. Regardless, despite strict quarantine efforts the introduction of *P. viticola* to WA is yet another example of introduction of pathogens in the important plants-for-planting pathway (Brasier 2008).

*The P. viticola breeding system and oospore production*

The findings in this study suggest the *P. viticola* population in WA is clonal (Chapter 3), and can produce oospores (sexual survival structures) (Chapter 5). The lack of genetic diversity and the potential of a single isolate crossed with itself to produce oospores (Chapter 5), suggests the potential for a single mating type to be present in WA and selfing is occurring. This contrasts with the currently accepted knowledge of the mating structure of *P. viticola* where is considered to be heterothallic, requiring the presence of two distinct mating types for development (Scherer and Gisi 2006; Wong et al. 2001). Killigrew (2006) previously hypothesised a single mating type being present due to a lack of oospores detected in the early years after the incursion into WA.

The capacity to self has been reported in a number of heterothallic organisms, including *Erysiphe necator* (Gaudoury and Pearson 1991), *Venturia inaequalis* (MacHardy et
al. 2001) and several oomycetes (Michelmore and Ingram 1982; Sherriff and Lucas 1989; Smart et al. 2000). It is believed homothallism and not heterothallism is the ancestral state of oomycete organisms (Judelson 2009). Despite heterothallic oomycetes being bisexual, able to individually produce both anthridia and oogonia, they remain self-sterile unless stimulated by environmental factors or hormones (Judelson 2009; Ko 2007). Once broken, self-fertilisation is possible and has been observed in culture (Clive Brasier pers. comm.). Studies on a number of Phytophthora species, including P. infestans and P. cinnamomi, have shown the presence of antagonistic Trichoderma species, fungicides (chloroneb), aged cultures and physical damage of cultures have stimulated selfing in what are predominantly heterothallic oomycetes (Groves and Ristaino 2000; Judelson 2009; Jung et al. 2013; Ko 1981).

Observations made during this study indicate oospore production occurs late in the grapevine growing season as the vines begin to senesce. The earliest observed oospores in the natural environment occurred mid-season (November, Chapter 5), but these were on water stressed vines undergoing early senescence. Observations of oospores being preferentially produced under dry conditions or as leaves senesce are not new (Gessler et al. 2011). A number of plant hormones are associated with dormancy of grapevines and leaf senescence, in particular ethylene and abscisic acid (Keller 2010; Zhang et al. 2011). To the authors knowledge there have been no investigations of the addition of plant hormones on the stimulation of oospores production in P. viticola.

The use of hormones and external stimuli in inducing the formation of oospores would be a major development for P. viticola research. Unlike other organisms (e.g. true fungi) the mating type gene for oomycetes has not been genetically identified and this makes the determination of mating types in isolates difficult without reference isolates. There are no known reference isolates of P. viticola mating types in Australia and being an obligate
biotroph creates issues for long term storage. However, in the *Phytophthora* breeding system two hormones have been identified, $\alpha^1$ and $\alpha^2$, which stimulate the production of oospores in the alternate mating type ($\alpha^1$ for A2, $\alpha^2$ for A1) (Ko 1988; Ko 2007). The $\alpha^1$ hormone was the first to be artificially synthesised and the addition of it to heterothallic *Phytophthora* species, *P. infestans, P. capsici* and *P. nicotianae*, induced oospore production in laboratory test (Harutyunyan et al. 2008). The $\alpha^2$ hormone was synthesised in 2011 (Ojika et al. 2011). Both the $\alpha^1$ and $\alpha^2$ have been tested against a number of *Phytophthora* and related genus with diverse reactions in the sensitivity and the number of oospores produced (Tomura et al. 2017). As *P. viticola* is related to *Phytophthora* clade 1, including *P. infestans* and *P. nicotianae*, based on genomic analysis (McCarthy and Fitzpatrick 2017), there is the potential the synthesised hormones could also stimulate oospores production. This hormone could therefore be used as a screening method for mating types of *P. viticola* in Australia.

**Economic Impact on industry**

Whilst undertaking sampling across Australia for isolates, several industry members indicated grape downy mildew was occurring more frequently than in the past and therefore was having a greater economic impact. At the time this did seem unusual, as climate change is modelled to reduce total rainfall and increase temperatures, particularly in south-west WA (Climate Commission 2011), resulting in less suitable weather conditions for the development of grape downy mildew. However, climate change has also resulted in the advancement or compaction of the grape growing season across Australia due to the warmer spring and summer temperatures, producing earlier harvest dates (Petrie and Sadras 2008; Petrie and Sadras 2016). Growers are encouraged not to apply post-harvest fungicides due to risk of resistance and unnecessary input costs. Meaning, the period from harvest to senescence is now longer than in the past, resulting in the potential for the unprotected leaves
to be infected by *P. viticola* being greater. Higher incidence of autumn downy mildew has been associated with higher severity of disease the following season (Carisse 2016), so the more frequent observation could be valid.

To estimate the economic impact *P. viticola* has had on the WA viticulture industry a weather parameter was used to estimate when weather conditions were suitable for infection events (Chapter 6). The 10:10:24 rule of thumb predicts oospore driven primary infection events where at least 10mm of rainfall, temperature ≥10°C over a 24hr period is required and is based on observations from European vineyards in the 1940’s and 50’s (Gessler et al. 2011; Rossi and Caffi 2012). At the time of the 1998 detection in the Swan Valley the 10:10:24 rule was widely extended to growers as a way of educating them on controlling a disease they had never encountered. However, being of a generalised nature spray regime models based on these parameters have been known to cause under or over estimation of infection events (Gessler et al. 2011; Rossi and Caffi 2012). As WA growers have become more experienced in dealing with *P. viticola* in their production systems it does appear the 10:10:24 is too simplistic for WA conditions. Greater understanding of the epidemiology of *P. viticola* under Australian conditions is required and this would provide improved accuracy in predicting the economic impact the disease is causing.

**Future direction**

Being an obligate biotroph presents challenges in both conducting experiments with *P. viticola*, but also storage of individual isolates of the pathogen. Laviola et al. (2006) outlined a number of techniques for the long term storage of *P. viticola*. Throughout the period of this study several attempts were made to store *P. viticola* sporangia at -20° using the techniques described by Laviola et al. (2006). Although successful colonisation of sporangia was possible within a year of freezing, the success rate fell away greatly after a
year in storage. Discussions with European researchers on optimal techniques for storage have indicated freshly produced sporangia and a large quantity is required (Pierre-Henri Dubuis *pers comm.*). For applied research on *P. viticola* populations in Australia to continue this aspect of long term storage needs to be developed and standardised.

The use of FTA cards and the success of the PCR amplification from them will be of great use in future studies on *P. viticola*. The use of the cards reduces the risk of quarantine incursions as it removes any importation of leaf material and any potential pest or disease associated with them. Under optimal conditions the DNA trapped on the cards are reported to remain viable for at least a decade at room temperature (Smith and Burgoyne 2004). FTA cards provide scope for further understanding of the pathogen on a molecular level in regards to population studies and the storage will provide long term reference samples for any shift in population dynamics. This will be particularly important in the development of future fungicides as the stored populations can act as baseline samples.

Despite conducting mating crosses as part of this study the viability of the oospores produced was not tested. Further studies are required into oospore development and germination under Australian conditions. This would be particularly informative for fungicide resistance testing as oospores are a means of phenotypic inheritance as discussed in Chapter 5. The potential for isolates of *P. viticola* to self means it will be important to determine if fungicide resistance is passed via the selfed oospores and whether there is any loss of fitness as a result. The phenotypic inheritance also suggests current practice of testing fungicide resistance using isolates obtained from infected vines may not be a true reflection of the actual population. Barring any germination fitness loss with resistance a more representative sampling of a population would result by collecting soil from vineyards and germinating the oospores present under optimal conditions. This sampling technique would
therefore collect all the resistant, intermediate and susceptible phenotypes rather than a specific phenotype at the specific time of sampling.

Current downy mildew management in Australia and most of the world is based around control via fungicides. The European Union has legislated (European Directive 2009/128/EC) to reduce the amount of pesticide applications in their agricultural production systems, and to seek alternatives to current products (Kamoun et al. 2015). This has led to a number of decision support systems (DSS) being developed in Europe to aid growers in deciding on the necessity of applying fungicides. In some instances, this has resulted in a 40% reduction in the number of fungicide applications and an associated reduction in input costs (Caffi et al. 2017). There is the possibility to collaborate with European researchers to ground truth some of these DSS under Australian conditions and requires further investigations.

As this study has found the primary source of *P. viticola* in WA vineyards are oospores a potential cultural method could be implemented to reduce their numbers and therefore the impact *P. viticola* has in WA vineyards. Oospores develop in senescing leaves, falling to the ground in Autumn and Winter where they mature as the leaves break down. As a result, a leaf sweeper and collector adaption for a vineyard tractor could be developed to remove the senesced leaves prior to them breaking down. If performed over several years, it would remove a large proportion of the inoculum source available within the soil and therefore reduce the potential downy mildew risk. This would be particularly useful for vineyards experiencing fungicide resistance issues and high disease pressure. A feasibility trial is required to determine the economic cost benefit behind this theory.
Fig A1.1 Grapevine growth and corresponding Eichorn-Lorenz stage as shown in Coombe (1995).
### Appendix 2

**Table A2.1. Collection details for the 413 isolates used for population analysis in Chapter 3.**

<table>
<thead>
<tr>
<th>Isolate Id</th>
<th>Host species</th>
<th>Cultivar</th>
<th>DNA source</th>
<th>Country</th>
<th>State</th>
<th>Region/Commune</th>
<th>Year</th>
<th>Collector</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT083</td>
<td>V. vinifera</td>
<td>Chardonnay</td>
<td>Fresh leaf</td>
<td>Australia</td>
<td>NSW</td>
<td>Hunter Valley</td>
<td>2015</td>
<td>Liz Riley</td>
</tr>
<tr>
<td>AT084</td>
<td>V. vinifera</td>
<td>unknown wine grape</td>
<td>Fresh leaf</td>
<td>Australia</td>
<td>NSW</td>
<td>Hunter Valley</td>
<td>2015</td>
<td>Jenny Bright</td>
</tr>
<tr>
<td>AT085</td>
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<td>unknown wine grape</td>
<td>Fresh leaf</td>
<td>Australia</td>
<td>NSW</td>
<td>Hunter Valley</td>
<td>2015</td>
<td>Jenny Bright</td>
</tr>
<tr>
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<td>V. vinifera</td>
<td>unknown wine grape</td>
<td>Fresh leaf</td>
<td>Australia</td>
<td>NSW</td>
<td>Hunter Valley</td>
<td>2015</td>
<td>Jenny Bright</td>
</tr>
<tr>
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<td>unknown wine grape</td>
<td>Fresh leaf</td>
<td>Australia</td>
<td>NSW</td>
<td>Hunter Valley</td>
<td>2015</td>
<td>Jenny Bright</td>
</tr>
<tr>
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<td>V. vinifera</td>
<td>unknown wine grape</td>
<td>Fresh leaf</td>
<td>Australia</td>
<td>NSW</td>
<td>Hunter Valley</td>
<td>2015</td>
<td>Jenny Bright</td>
</tr>
<tr>
<td>AT089</td>
<td>V. vinifera</td>
<td>unknown</td>
<td>Fresh leaf</td>
<td>Australia</td>
<td>NSW</td>
<td>Hunter Valley</td>
<td>2015</td>
<td>Len Tesoriero</td>
</tr>
<tr>
<td>AT090</td>
<td>V. vinifera</td>
<td>unknown</td>
<td>Fresh leaf</td>
<td>Australia</td>
<td>NSW</td>
<td>Hunter Valley</td>
<td>2015</td>
<td>Len Tesoriero</td>
</tr>
<tr>
<td>AT091</td>
<td>V. vinifera</td>
<td>unknown</td>
<td>Fresh leaf</td>
<td>Australia</td>
<td>NSW</td>
<td>Hunter Valley</td>
<td>2015</td>
<td>Len Tesoriero</td>
</tr>
<tr>
<td>AT092</td>
<td>V. vinifera</td>
<td>Chardonnay</td>
<td>Fresh leaf</td>
<td>Australia</td>
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<td>Hunter Valley</td>
<td>2015</td>
<td>Liz Riley</td>
</tr>
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<td>AT093</td>
<td>V. vinifera</td>
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<td>Fresh leaf</td>
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<td>Fresh leaf</td>
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<td>Merlot</td>
<td>FTA card</td>
<td>Uruguay</td>
<td>Canelones</td>
<td>Canelones</td>
<td>2017</td>
<td>Eduardo Abreo</td>
</tr>
<tr>
<td>AT830</td>
<td>V. vinifera</td>
<td>Tannat</td>
<td>FTA card</td>
<td>Uruguay</td>
<td>Canelones</td>
<td>Canelones</td>
<td>2017</td>
<td>Eduardo Abreo</td>
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<tr>
<td>AT831</td>
<td>V. vinifera</td>
<td>Tannat</td>
<td>FTA card</td>
<td>Uruguay</td>
<td>Canelones</td>
<td>Canelones</td>
<td>2017</td>
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<tr>
<td>AT832</td>
<td>V. vinifera</td>
<td>Tannat</td>
<td>FTA card</td>
<td>Uruguay</td>
<td>Canelones</td>
<td>Canelones</td>
<td>2017</td>
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<td>AT833</td>
<td>V. vinifera</td>
<td>Tannat</td>
<td>FTA card</td>
<td>Uruguay</td>
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<td>FTA card</td>
<td>Uruguay</td>
<td>Canelones</td>
<td>Canelones</td>
<td>2017</td>
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<td>Tannat</td>
<td>FTA card</td>
<td>Uruguay</td>
<td>Canelones</td>
<td>Canelones</td>
<td>2017</td>
<td>Eduardo Abreo</td>
</tr>
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<td>AT836</td>
<td>V. vinifera</td>
<td>Moscatel</td>
<td>FTA card</td>
<td>Uruguay</td>
<td>Canelones</td>
<td>Canelones</td>
<td>2017</td>
<td>Eduardo Abreo</td>
</tr>
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<td>AT837</td>
<td>V. vinifera</td>
<td>Moscatel</td>
<td>FTA card</td>
<td>Uruguay</td>
<td>Canelones</td>
<td>Canelones</td>
<td>2017</td>
<td>Eduardo Abreo</td>
</tr>
<tr>
<td>AT838</td>
<td>V. vinifera</td>
<td>Moscatel de Hamburg</td>
<td>FTA card</td>
<td>Uruguay</td>
<td>Canelones</td>
<td>Canelones</td>
<td>2017</td>
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<tr>
<td>AT839</td>
<td>V. vinifera</td>
<td>Moscatel de Hamburg</td>
<td>FTA card</td>
<td>Uruguay</td>
<td>Canelones</td>
<td>Canelones</td>
<td>2017</td>
<td>Eduardo Abreo</td>
</tr>
<tr>
<td>AT840</td>
<td>V. vinifera</td>
<td>Moscatel de Hamburg</td>
<td>FTA card</td>
<td>Uruguay</td>
<td>Canelones</td>
<td>Canelones</td>
<td>2017</td>
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</tr>
<tr>
<td>AT841</td>
<td>V. vinifera</td>
<td>Moscatel de Hamburg</td>
<td>FTA card</td>
<td>Uruguay</td>
<td>Canelones</td>
<td>Canelones</td>
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<tr>
<td>AT842</td>
<td>V. vinifera</td>
<td>Merlot</td>
<td>FTA card</td>
<td>Uruguay</td>
<td>Canelones</td>
<td>Canelones</td>
<td>2017</td>
<td>Eduardo Abreo</td>
</tr>
</tbody>
</table>

*Year is considered the year of harvest to differentiate southern hemisphere production systems which run over calendar years.*
Table A2.2. The 16 microsatellites, associated dyes and repeat motif used in this study. The microsatellites were combined into 3 multiplex reactions.

<table>
<thead>
<tr>
<th>Multiplex</th>
<th>Microsatellite</th>
<th>Dye</th>
<th>Repeat</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ISA</td>
<td>6FAM</td>
<td>(TC)$_n$</td>
<td>Gobbin et al. (2003b)</td>
</tr>
<tr>
<td></td>
<td>PV13</td>
<td>VIC</td>
<td>(TG)$_8$</td>
<td>Delmotte et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>PV103</td>
<td>PET</td>
<td>(TG)$_6$</td>
<td>Rouxel et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>PV143</td>
<td>PET</td>
<td>(AT)$_8$</td>
<td>Rouxel et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>PV147</td>
<td>PET</td>
<td>(TCGACT)$_8$</td>
<td>Rouxel et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>BER</td>
<td>NED</td>
<td>(TC)$_n$</td>
<td>Gobbin et al. (2003b)</td>
</tr>
<tr>
<td>2</td>
<td>PV74</td>
<td>PET</td>
<td>(AG)$_7$</td>
<td>Rouxel et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>PV137</td>
<td>6FAM</td>
<td>(AT)$_9$</td>
<td>Rouxel et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>PV140</td>
<td>6FAM</td>
<td>(TA)$_9$</td>
<td>Rouxel et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>PV142</td>
<td>NED</td>
<td>(CT)$_{11}$</td>
<td>Rouxel et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>PV146</td>
<td>VIC</td>
<td>(GAG)$_7$</td>
<td>Rouxel et al. (2012)</td>
</tr>
<tr>
<td>3</td>
<td>PV17</td>
<td>NED</td>
<td>(TC)$_{12}$</td>
<td>Delmotte et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>PV65</td>
<td>6FAM</td>
<td>(TC)$_9$</td>
<td>Rouxel et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>PV148</td>
<td>VIC</td>
<td>(ACA)$_6$</td>
<td>Rouxel et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>CES</td>
<td>PET</td>
<td>(TC)$_n$(AC)$_n$</td>
<td>Gobbin et al. (2003b)</td>
</tr>
<tr>
<td></td>
<td>GOB</td>
<td>VIC</td>
<td>(CT)$_n$(CTAT)$_n$</td>
<td>Gobbin et al. (2003b)</td>
</tr>
<tr>
<td>Population</td>
<td>N</td>
<td>MLG</td>
<td>eMLG</td>
<td>SE</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----</td>
<td>------</td>
<td>------</td>
<td>-----</td>
</tr>
<tr>
<td>AU_Western Australia</td>
<td>110</td>
<td>27</td>
<td>4.64</td>
<td>1.32</td>
</tr>
<tr>
<td>AU_South Australia</td>
<td>65</td>
<td>63</td>
<td>9.96</td>
<td>0.20</td>
</tr>
<tr>
<td>AU_Queensland</td>
<td>29</td>
<td>26</td>
<td>9.67</td>
<td>0.52</td>
</tr>
<tr>
<td>AU_New South Wales</td>
<td>43</td>
<td>39</td>
<td>9.76</td>
<td>0.47</td>
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<tr>
<td>AU_Victoria</td>
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<td>31</td>
<td>10.00</td>
<td>0.00</td>
</tr>
<tr>
<td>AU_Tasmania</td>
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<td>8</td>
<td>8.00</td>
<td>0.00</td>
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<tr>
<td>North America Virginia</td>
<td>28</td>
<td>28</td>
<td>10.00</td>
<td>0.00</td>
</tr>
<tr>
<td>North America Maryland</td>
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<td>23</td>
<td>10.00</td>
<td>0.00</td>
</tr>
<tr>
<td>North America New Jersey</td>
<td>3</td>
<td>3</td>
<td>3.00</td>
<td>0.00</td>
</tr>
<tr>
<td>North America New York</td>
<td>11</td>
<td>11</td>
<td>10.00</td>
<td>0.00</td>
</tr>
<tr>
<td>North America Oregon</td>
<td>4</td>
<td>4</td>
<td>4.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Brazil Sao Paulo</td>
<td>8</td>
<td>8</td>
<td>8.00</td>
<td>0.00</td>
</tr>
<tr>
<td>France Bordeaux</td>
<td>32</td>
<td>32</td>
<td>10.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Uruguay Canelones</td>
<td>18</td>
<td>18</td>
<td>10.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>413</td>
<td>320</td>
<td>9.24</td>
<td>0.93</td>
</tr>
</tbody>
</table>

N = number of isolates in the population, MLG = number of multilocus genotypes, eMLG = expected number of multilocus genotypes at the smallest samples size > 10, SE = Standard error based on the eMLG, H = Shannon-Wiener index of MLG diversity, G = Stoddard and Taylor index of MLG diversity, Lambda = Simpson’s index, Hexp = Nei’s unbiased gene diversity, Ia = Index of association, rbarD = Standardized index of association, p.rD = p value (rbarD) = significance of the standardised index of association (p < 0.01)
Table A2.4. Collection details for 19 isolates tested using ITS sequencing to determine *P. viticola* cryptic species including sample type, host species, cultivar, source location, GenBank accession number and collector.

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>Year</th>
<th>Sample type</th>
<th>Vitaceae Host</th>
<th>Cultivar</th>
<th>Location</th>
<th>Country</th>
<th>Genbank Accession Number</th>
<th>Collector</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT32_WA</td>
<td>2015</td>
<td>Leaf</td>
<td><em>V. vinifera</em></td>
<td>Midnight Beauty</td>
<td>Walkaway, Western Australia</td>
<td>Australia</td>
<td>MG552090</td>
<td>Andrew Taylor</td>
</tr>
<tr>
<td>AT121_WA</td>
<td>2016</td>
<td>Leaf</td>
<td><em>V. vinifera</em></td>
<td>Dawn seedless</td>
<td>Swan Valley, Western Australia</td>
<td>Australia</td>
<td>MG552091</td>
<td>Andrew Taylor</td>
</tr>
<tr>
<td>AT620_WA</td>
<td>1997</td>
<td>Herbarium</td>
<td><em>V. vinifera</em></td>
<td>unknown table grape</td>
<td>Kalumburu, Western Australia</td>
<td>Australia</td>
<td>MG552099</td>
<td>Ian Riley</td>
</tr>
<tr>
<td>AT623_WA</td>
<td>2008</td>
<td>Herbarium</td>
<td><em>V. vinifera</em></td>
<td>unknown table grape</td>
<td>Carnarvon, Western Australia</td>
<td>Australia</td>
<td>MG552100</td>
<td>Amanda Annells</td>
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<tr>
<td>AT156_VIC</td>
<td>2015</td>
<td>Leaf</td>
<td><em>V. vinifera</em></td>
<td>Chardonnay</td>
<td>Yarra Valley, Victoria</td>
<td>Australia</td>
<td>MG552092</td>
<td>David Hughes</td>
</tr>
<tr>
<td>AT209_SA</td>
<td>2015</td>
<td>Leaf</td>
<td><em>V. vinifera</em></td>
<td>Chardonnay</td>
<td>Riverland, South Australia</td>
<td>Australia</td>
<td>MG552093</td>
<td>Peter Magarey</td>
</tr>
<tr>
<td>AT549_SA</td>
<td>2017</td>
<td>Leaf</td>
<td><em>V. vinifera</em></td>
<td>Cabernet Sauvignon</td>
<td>Southern Fleurieu, South Australia</td>
<td>Australia</td>
<td>MG552095</td>
<td>Matthew Wilson</td>
</tr>
<tr>
<td>AT434_NSW</td>
<td>2016</td>
<td>Leaf</td>
<td><em>V. vinifera</em></td>
<td>unknown table grape</td>
<td>Sydney, New South Wales</td>
<td>Australia</td>
<td>MG552094</td>
<td>Andrew Taylor</td>
</tr>
<tr>
<td>AT577_QLD</td>
<td>2017</td>
<td>Leaf</td>
<td><em>V. labrusca</em></td>
<td>Isabella</td>
<td>Mareeba, Queensland</td>
<td>Australia</td>
<td>MG552096</td>
<td>Ricardo Feliciano dos Santos</td>
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<tr>
<td>AT578_QLD</td>
<td>2017</td>
<td>Leaf</td>
<td><em>V. labrusca</em></td>
<td>Isabella</td>
<td>Mareeba, Queensland</td>
<td>Australia</td>
<td>MG552097</td>
<td>Ricardo Feliciano dos Santos</td>
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<tr>
<td>AT616_TAS</td>
<td>2017</td>
<td>Leaf</td>
<td><em>V. vinifera</em></td>
<td>Chardonnay</td>
<td>Tamar Valley, Tasmania</td>
<td>Australia</td>
<td>MG552098</td>
<td>Dennis Patten</td>
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<tr>
<td>AT711_USA</td>
<td>2016</td>
<td>FTA card</td>
<td><em>V. vinifera</em></td>
<td>Cabernet Franc</td>
<td>Shenandoah Valley, Virginia</td>
<td>USA</td>
<td>MG552101</td>
<td>Andrew Taylor</td>
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<tr>
<td>AT730_USA</td>
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<td>FTA card</td>
<td><em>V. aestivalis</em></td>
<td>Norton</td>
<td>Northern Virginia, Virginia</td>
<td>USA</td>
<td>MG552102</td>
<td>Andrew Taylor</td>
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<tr>
<td>AT765_USA</td>
<td>2016</td>
<td>FTA card</td>
<td><em>V. vinifera</em></td>
<td>Chardonnay</td>
<td>Long island, New York</td>
<td>USA</td>
<td>MG552103</td>
<td>Andrew Taylor</td>
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<tr>
<td>AT768_USA</td>
<td>2016</td>
<td>FTA card</td>
<td><em>V. riparia</em></td>
<td>wild vine</td>
<td>Finger lakes, New York</td>
<td>USA</td>
<td>MG552104</td>
<td>Andrew Taylor</td>
</tr>
<tr>
<td>AT786_USA</td>
<td>2017</td>
<td>FTA card</td>
<td><em>P. tricuspidata</em></td>
<td>Boston Ivy</td>
<td>Corvallis, Oregon</td>
<td>USA</td>
<td>MG552107</td>
<td>Andrew Taylor</td>
</tr>
<tr>
<td>AT777_BRA</td>
<td>2016</td>
<td>FTA card</td>
<td><em>V. labrusca</em></td>
<td>Niagara Rosada</td>
<td>Piracicaba, São Paulo</td>
<td>Brazil</td>
<td>MG552105</td>
<td>Ricardo Feliciano dos Santos</td>
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<tr>
<td>AT778_BRA</td>
<td>2016</td>
<td>FTA card</td>
<td><em>V. vinifera</em></td>
<td>Moscato Giallo</td>
<td>Jundial, São Paulo</td>
<td>Brazil</td>
<td>MG552106</td>
<td>Ricardo Feliciano dos Santos</td>
</tr>
<tr>
<td>AT825_URY</td>
<td>2017</td>
<td>FTA card</td>
<td><em>V. vinifera</em></td>
<td>Merlot</td>
<td>Canelones</td>
<td>Uruguay</td>
<td>MG552108</td>
<td>Eduardo Abreo</td>
</tr>
</tbody>
</table>
Figure A2.1. Genotypic accumulation curve across all loci for the clone corrected data for all populations investigated in this study.

Figure A2.2. Counts of the exact test $p$-values for the HWE statistic for all 12 loci for all $P. viticola$ populations in this study. $P$-values below 0.05 (red dotted line) indicate significant deviation from HWE.
Table A2.5. Analysis of molecular variance (AMOVA) summarising the genetic variation in *P. viticola* microsatellite data between countries and between states within countries.

<table>
<thead>
<tr>
<th>AMOVA</th>
<th>SSD</th>
<th>MSD</th>
<th>df</th>
<th>Sigma²</th>
<th>% of variance coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between countries</td>
<td>15.08</td>
<td>3.77</td>
<td>4</td>
<td>0.07</td>
<td>25%</td>
<td>NA</td>
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<tr>
<td>Between states</td>
<td>5.75</td>
<td>0.64</td>
<td>9</td>
<td>0.02</td>
<td>24%</td>
<td>0</td>
</tr>
<tr>
<td>Error</td>
<td>21.71</td>
<td>0.07</td>
<td>307</td>
<td>0.07</td>
<td>51%</td>
<td>NA</td>
</tr>
</tbody>
</table>

SSD = sum of squares, MSD = mean squares, df = degree of freedom, sigma² = standard deviation

Figure A2.3. K-means clustering of *P. viticola* isolates using Bayesian Information Criterion (BIC) to identify the optimal number of clusters or groups (K). Increasing the number of groups from 2 to 4 results in a substantial decrease in BIC while increasing the number of groups beyond 4 did not dramatically decrease the BIC.
Appendix 3

Optimising DNA analysis from FTA® discs.

Introduction

The quality and quantity of P. viticola DNA available on the FTA® cards from the direct leaf press was unknown. A trial test was conducted to determine the optimal preparation procedure for DNA analysis of the FTA® cards.

Materials and Methods

The test involved comparing the Whatman FTA® Protocol BD08: Preparing an FTA® Disc for DNA Analysis with a protocol describing the elution of DNA using virus infected leaf samples (Ndunguru et al. 2005). As the quantity of the DNA trapped on the cards wasn’t known, the test also compared the elution from one disc to three. Included in the evaluation was DNA from the extraction of fresh samples using the Powerplant® Pro DNA Isolating Kit protocol and blank FTA® disc (with no DNA). The test used two primers to determine reproducibility between primers.

Table A3.2. Comparison of the different methods of DNA extraction from FTA cards.

<table>
<thead>
<tr>
<th>Step</th>
<th>Whatman FTA® Protocol BD08</th>
<th>Protocol from [Ndunguru et al. 2005]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Take sample disc from the dried spot following Removing a sample disc from an FTA or clonesaver card for analysis, BD09</td>
<td>Take sample disc from the dried spot following Removing a sample disc from an FTA or clonesaver card for analysis, BD09</td>
</tr>
<tr>
<td>2</td>
<td>Place disc in PCR amplification tube</td>
<td>Place disc in PCR amplification tube</td>
</tr>
<tr>
<td>3</td>
<td>Add 200µL of FTA Purification Reagent to PCR tube.</td>
<td>Add 300 µl of TE buffer to PCR tube. (Tris–HCl buffer (10mM Tris–HCl pH 8))</td>
</tr>
<tr>
<td>4</td>
<td>Incubate for 5 minutes at room temp (the tube should be given moderate manual mixing to disrupt the debris and aid in washing).</td>
<td>Incubate for 5 minutes at room temp (the tube should be given moderate manual mixing to disrupt the debris and aid in washing).</td>
</tr>
<tr>
<td>5</td>
<td>Remove and discard all used FTA Purification Reagent using pipette.</td>
<td>Remove and discard all used TE buffer using pipette.</td>
</tr>
<tr>
<td>6</td>
<td>Add 200µL of FTA Purification Reagent to PCR tube.</td>
<td>Add 300 µl of 70% ethanol to PCR tube.</td>
</tr>
<tr>
<td>7</td>
<td>Incubate for 5 minutes at room temp (the tube should be given moderate manual mixing to disrupt the debris and aid in washing).</td>
<td>Incubate for 5 minutes at room temp (the tube should be given moderate manual mixing to disrupt the debris and aid in washing).</td>
</tr>
</tbody>
</table>
8. Remove and discard all used FTA Purification Reagent using pipette.
9. Add 200µL of FTA Purification Reagent to PCR tube.
10. Incubate for 5 minutes at room temp (the tube should be given moderate manual mixing to disrupt the debris and aid in washing).
11. Remove and discard all used FTA Purification Reagent using pipette.
12. Add 200µL of TE-1 Buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0) to PCR tube.
13. Incubate for 5 minutes at room temperature.
14. Remove and discard all used TE-1 Buffer using pipette.
15. Add 200µL of TE-1 Buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0).
16. Incubate for 5 minutes at room temperature.
17. Remove and discard all used TE-1 Buffer using pipette.
18. Ensure that all the liquid has been removed before performing analysis. The disk may be allowed to dry.
19. Use the dried disc in the PCR reaction.

Results and Discussion.

The test highlighted the use of the Ndunguru et al. (2005) method was the optimal protocol for PCR amplification using the FTA® cards Fig. A2.1. No PCR amplification occurred when using the discs in the PCR reaction as described in the Whatman FTA® Protocol BD08 and this may have resulted from contaminants or the leaf tissue inhibiting the PCR process. The addition of the 70% ethanol wash was found to remove these contaminants and provide uninhibited PCR amplification (Ndunguru et al. 2005). There appears to be no difference between the strength of the bands using a single leaf disc, three leaf discs and the DNA extracted from the fresh leaves. However, three leaf discs were used in future reactions as insurance against selecting areas of the FTA® card having limited DNA pressed onto it.
Fig. A3.1. Agarose gel comparing the different extraction and PCR conditions containing FTA cards for two microsatellite primers.

ISA New microsatellite

Whatman 1 plug
Ndunguru 1 plug liquid
Whatman 3 plugs
Ndunguru 3 plugs liquid
Ndunguru 1 plug only
Standard extraction
Blank plug
Standard extraction and blank plug
Negative control PCR mix

BER New microsatellite

Whatman 1 plug
Ndunguru 1 plug liquid
Whatman 3 plugs
Ndunguru 3 plugs liquid
Ndunguru 1 plug only
Standard extraction
Blank plug
Standard extraction and blank plug
Negative control PCR mix
References


Brasier C. 2008. The biosecurity threat to the UK and global environment from international trade in plants. Plant Pathology 57:792-808


Climate Commission. 2011. The critical decade: Western Australia climate change impacts Department of Climate Change and Energy Efficiency Canberra, Australian Capital Territory, Commonwealth of Australia, pp 16


Cook, DC, Fraser RW. 2014. Eradication versus control of Mediterranean fruit fly in Western Australia. Agricultural and Forest Entomology 17:173-180


Crone M, McComb JA, O’Brien PA, Hardy GESJ. 2013. Survival of Phytophthora cinnamomi as oospores, stromata, and thick-walled chlamydospores in roots of symptomatic and asymptomatic annual and herbaceous perennial plant species. Fungal Biology 117:112-123


Emmett R, Clarke K, Hunt T, Magarey P, Learhinan N. 2006. Final report DNR 02/06 Improved management of grapevine powdery mildew. Grape and Wine Research and Development Corporation, Department of Primary Industries (Victoria) and Primary Industries and Resources South Australia, pp 61


Gregory C. 1912. Spore germination and infection with *Plasmopara viticola*. Phytopathology 2:235-249


Hall B. 2017. Final report SAR 1204 Understanding fungicide resistance in powdery mildew, downy mildew and botrytis. South Australian Research and Development Institute, Adelaide, Australia, pp 170


Hoare T. 2011. Phosphorus acid - can we have it back please? Wine Viticulture Journal 26:45-47


Holleley CE, Geerts PG. 2009. Multiplex Manager 1.0: a cross-platform computer program that plans and optimizes multiplex. PCR Biotechniques 46:511-517

Hug F. 2005. Genetic structure and epidemiology of *Plasmopara viticola* populations from Australian grape growing regions. Diploma Thesis, ETH Zurich

Jayasekera AU, McComb JA, Shearer BL, Hardy GESJ. 2007. *In planta* selfing and oospore production of *Phytophthora cinnamomi* in the presence of *Acacia pulchella*. Mycological Research 111:355-362


Jung T, Colquhoun IJ, Hardy GES. 2013. New insights into the survival strategy of the invasive soilborne pathogen *Phytophthora cinnamomi* in different natural ecosystems in Western Australia. Forest Pathology 43:266-288


Killigrew BX, Sivasithamparam K, Scott ES. 2005. Absence of oospores of downy mildew of grape caused by Plasmopara viticola as the source of primary inoculum in most Western Australian vineyards. Plant Disease 89:777-777
Killigrew BX. 2006. The source of primary inoculum of *Plasmopara viticola*, cause of grapevine downy mildew, in Western Australia. Doctoral Thesis, University of Western Australia


Lantzke N. 2004. Downy mildew infection events in Western Australian viticulture regions. Final Report to the Grape and Wine Research and Development Corporation, Project Number: RT 01/06.


McCarthy CGP, Fitzpatrick DA. 2017. Phylogenomic reconstruction of the Oomycete phylogeny derived from 37 Genomes. mSphere 2:e00095

McKirdy S, Riley I, Cameron I, Magarey P. 1999. First report of grapevine downy mildew (*Plasmopara viticola*) in commercial viticulture in Western Australia. Plant Disease 83:301-301


Quinn DG. 1924. Downy Mildew (Plasmodora viticola) vol 179. Bulletin of South Australian Department of Agriculture. Department of Agriculture of South Australia, Adelaide

(Plasmopara viticola) at Kalumburu, east Kimberley, Western Australia. Agriculture 
Western Australia, South Perth, WA


Ronzon-Tran Manh Sung C, Clerjeau M. 1988. Techniques for formation, maturation, and 
germination of Plasmopara viticola oospores under controlled conditions. Plant 
Disease 72:938-941

program for grapevine downy mildew development forecasting. Computers and 
Electronics in Agriculture 9:205-215

Rosa M, Gozzini B, Orlandini S, Seghi L. 1995. A computer program to improve the control 

Plant Pathology 56:957-966

Rossi V, Caffi T. 2012. The role of rain in dispersal of the primary inoculum of Plasmopara 
viticola. Phytopathology 102:158-165

dynamics of Plasmopara viticola oospores using hydro-thermal time. Plant Pathology 
57:216-226

infections of downy mildew in grapevine. Ecological Modelling 212:480-491


Journal of Phytopathology 165:331-341


Tomura T, Molli SD, Murata R, Ojika M. 2017. Universality of the Phytophthora mating hormones and diversity of their production profile. Scientific Reports 7:5007


Wickham H. 2009. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York, pp 266


Wilcox WF, Gubler WD, Uyemoto JK. 2015. Compendium of grape diseases, disorders, and pests. The American Phytopathological Society, St Paul, Minnesota, USA

Williams MG. 2005. Impact of environmental conditions on the infection behaviour of Western Australian strains of Plasmopara viticola, causal agent of downy mildew in grapevines. Doctoral Thesis, University of Western Australia


Wines of Western Australia. 2014. Western Australian Wine Industry Strategic Plan 2014-2024. Western Australia


Woodfin JC. 1926. Downy Mildew of the Vine (Plasmopara viticola) in New Zealand: The Disease and Its Treatment. New Zealand Journal of Agriculture 33:14-20

