Behaviour of infective propagules of *Plasmopara viticola* (causal agent of grapevine downy mildew) under Western Australian conditions

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1. Abstract

Grape downy mildew (caused by *Plasmopara viticola*), is a significant disease in viticulture worldwide. The absence of downy mildew from viticulture in Western Australia (W.A.) prior to 1998, and its subsequent discovery in almost every viticultural region of W.A., prompted questions regarding the biology of *P.viticola* in W.A.. This study showed that oospores, the dominant over-wintering spore elsewhere, were absent from all but one of the 19 vineyards surveyed. In the absence of oospores, another mechanism of perennation was suspected. It was discovered that the pathogen was able to colonise buds during the growing season and that it may survive over winter within the dormant bud, becoming active again after bud-burst. It is, therefore, possible that the epidemiology of *P. viticola* in W.A. differs from that in other parts of the world, and that management strategies developed elsewhere may fail to control of the disease in W.A. Furthermore, the findings of this study may be relevant to the behaviour of the pathogen in other parts of the world where conditions are similar to those in the viticultural regions of W.A.

2. Executive summary

This study illustrates a number of differences between the biology and epidemiology of *Plasmopara viticola* in W.A. and reports from research elsewhere. These differences are important for understanding the infection process and the epidemiology of the disease in W.A. and, as such, are an essential requirement for developing strategies for the effective management of the disease.

A survey of 19 vineyards in W.A. detected oospores in only a single vineyard. Even where oospores were found, very few of these oospores could be induced to germinate in the laboratory. The absence of oospores from most W.A. vineyards suggests that oospores play little or no role in the over-wintering of the pathogen in these vineyards. In contrast, in Europe, oospores are reported to be the main (if not only) source of inoculum, at the start of the season and throughout the season (Gobbin *et al.*, 2003a). Therefore, disease models and management strategies based on European research may not be directly applicable to viticulture in W.A.

It was thus important to determine the mechanism by which *P. viticola* over-winters on *Vitis vinifera* in W.A. The first symptom observed in vineyards early in the growing season (primary infection) in this study was often that of a single large lesion spread across various distorted tissues (e.g. leaf, petiole, shoot, and tendril). In contrast, typical symptoms of primary infection in other regions are sparsely scattered oilspots on leaves, which are thought to represent a primary infection event arising from oospore-derived inoculum. The initial symptoms in W.A. described above are comparable to “flag-shoots” for powdery mildew, which result from over-wintering of the pathogen, *Erysiphe necator*, from bud infection in the previous growing season. Thus it was suspected that colonisation of buds by *P. viticola* in the previous growing season may have occurred, and may have led to the over-wintering of the pathogen within buds.

Subsequent experiments involving field and glasshouse inoculation showed that bud-scales and primordia were infected and colonised by *P. viticola*. Buds were only susceptible to infection on newly emerged, green shoots, and as they became sclerotised with age, tissue appeared to become resistant to penetration and further colonisation. It was apparent that some buds were killed following colonisation, however, it is likely that some may have remained viable and could have sustained

the pathogen over winter. Such buds may burst in spring, where the pathogen, having colonised much of the tissue of the primordial shoot, could sporulate during favourable weather conditions. As such, symptoms on the emerging, colonised shoot would be similar to the atypical initial infections detected early in the season in this study, rather than the discrete oilspots on leaves that result from primary infection from oospore-derived inoculum

In summary, it is possible that over-wintering of *P. viticola* occurs in buds of *V. vinifera* in W.A. The implication of these results is that the primary inoculum giving rise to the first infection of the season is likely to emerge from within the vine, rather than from oospores in the soil. These findings require confirmation via further field studies and, if confirmed, will require revision of management strategies. New measures may include monitoring for severely infected shoots soon after bud-burst, removal of the same, and immediate application of fungicides to protect surrounding vines.

3. Background

Grape downy mildew, caused by the biotrophic, oomycete pathogen *Plasmopara viticola* is among the most damaging pathogens of grapevines worldwide (Wong *et al.*, 2001). It has been recorded in Australia since arriving on cuttings around 1916 (Lafon and Bulit, 1981) but, until 1998, had not been recorded in W.A. (McKirdy *et al.*, 1999). Since the first report of the disease in W.A., it has since been found in nearly all viticultural regions in the state (Fisher, 2000).

The pathogen uses two propagation strategies in its lifecycle, namely a sexual stage and an asexual stage. Sexual recombination in infected tissue leads to the formation of oospores, which act as over-wintering structures during the period of grapevine dormancy (Büche *et al*., 2002). Oospores mature and germinate in spring, and are the source of inoculum for primary infection during the season (Gregory, 1912). The asexual stage is responsible for the rapid propagation (by numerous secondary infections) of the pathogen during the season, when vegetative growth of vines occurs. Secondary infection and sporulation is reported to occur regularly during the season following favourable (warm and wet) conditions (Emmett *et al.*, 1992).

There have occasionally, over many years, been reports of the pathogens ability to over-winter in bud scales, cortical tissue or even remnant leaves in some *Vitis* species, however many of these reports are anecdotal, or do not provide suitable detail or evidence of such abilities (Chrelashvili, 1984; Barret, 1939).

Verbal reports from advisors and growers in the field indicated that epidemics started very early in the season and to a greater extent than could be explained by primary infection from oospores. Single sporulating lesions were found in vineyards that did not represent a “typical” primary infection (ie a few “oilspots”), after little or no rainfall. Lesions were atypical of a primary infection, in that they spread over a number of tissue types (leaves, petioles, shoots, tendrils and inflorescences) and appeared to have distorted the colonised tissue.

Project Aims and Performance targets:

The original aim of this project was to attempt to inhibit germination of *P. viticola* oospores using soil amendments. Upon discovering that oospores did not form in infected tissues in the glasshouse, even following inoculation with multiple isolates from various regions, a survey of vineyards in various regions of the state was performed. Having found that oospores were absent in most vineyards in W.A., aims were redirected to study the biology of *P. viticola* in W.A.

4. Methods

A number of experiments were conducted to (i) establish the role of oospores in the epidemiology of *P. viticola* in W.A. and (ii) discover the means by which *P. viticola* over-winters.

4.1 Leaf age and colonization

a) Whole vine, leaves attached experiment
Vines of cv. Marsanne were established in the glasshouse. Each leaf on ten shoots, each with leaves between 2 and 60d old, were drop-inoculated at the junction of the main vein and the petiolar sinus using a suspension of *P. viticola* sporangia prepared from lesions collected from various viticultural regions. Leaves were harvested 30d post-inoculation (dpi), leaf and lesion size were measured and lesion characteristics were recorded. Leaves were also analysed microscopically to detect the presence of oospores. Leaf samples were cleared of pigment using 1M KOH, then stained using Aniline Blue (0.05% in 0.067M K2PO4 buffer pH 9) (Kiefer *et al.*, 2002) and assessed for the presence of oospores using a compound microscope (100-400x magnification).

b) Detached leaf experiment
Leaves were detached from ten shoots on Chardonnay vines maintained in the glasshouse. Detached leaves were labeled from 1 (most recently emerged) to 10 (oldest) and six discs of 10 mm diameter were excised from each leaf and incubated in a moistened Petri dish at 22°C. Leaf discs (LD) were drop-inoculated with 20 µl of a mixed suspension of *P. viticola* sporangia (as above). Colony size, indicated by the area of sporulation, was measured over a period of 7d. After 7d each LD was transferred to a 1.5 ml tube and 500 µl of DI H_2O was added and the sporangia suspended by repeated inversion of the tube for 1 min. Sporangial counts were performed using a haemocytometer.

4.2 Survey of W.A. viticultural regions for oospores

Nineteen vineyards in 13 regions of W.A. where downy mildew had been reported were surveyed for the disease between 2001 and 2005. Where signs of the disease were found, colonised leaves were collected and representative samples were assessed microscopically as per the whole vine experiment.

4.3 Diagnosis of bud colonisation by epi-fluorescence microscopy and molecular detection of *P. viticola* DNA in tissue

Buds for this experiment were inoculated using either a point inoculation or spray inocultation method. Point inoculation involved the inoculation of only the bud scales, whilst spray inoculation involved the inoculation of the entire shoot. For point inoculation, *V. vinifera* vines (Chardonnay) were established in the glasshouse. The apical ten buds were point inoculated with a suspension of sporangia held in contact with the bud by a modified pipette tip mounted over the bud and attached using U-tac®. Sultana vines, established in the glasshouse were spray inoculated with a suspension of sporangia. All vines were kept at 100% RH in the dark for 24h and then maintained in the glasshouse for 5 weeks. Buds from colonised nodes were assessed for the presence of *P. viticola* in scales or primordia. Shoots were removed and transferred to a laminar flow cabinet where buds were excised, cut in half longitudinally and cleared in 1M KOH individually for 12 h at 60°C. Cleared bud halves were mounted on slides separately so that one half of each section showed the internal bud with primordia and the other the outer bud scales. Buds were stained with a 0.05 % w/v Aniline Blue solution, as above. Buds were viewed at 100-400x magnification using a Zeiss compound microscope with epi-fluorescence facility.

A small number of buds had burst during winter (late July) after a warm period of at least three concurrent days over 20°C maximum temperature and some appeared to be systemically colonised by *P. viticola*. Some shoots had sparsely scattered sporangiophores on them. Shoots, attached to canes, were removed and transferred to the laboratory where they were incubated at 22°C at 100% RH for 12 h in the dark to promote sporulation. Buds were removed and treated as above, and viewed at 100-400x magnification using a Zeiss compound microscope with epi-fluorescence facility.

Dormant buds were collected from a vineyard that had experienced an epidemic of downy mildew in the previous growing season. Buds were collected at two times, from dormant cane during winter and from shoots showing signs of infection during summer. Buds were analysed for the presence of *P. viticola* DNA using DNA markers developed by Gobbin *et al.* (2001), buds from shoot tips that had been inoculated with sporangia of *P. viticola* were analysed using microsattelite PCR. Sporulating leaves were used for the positive control and uninfected buds as the negative control.

PCR was conducted by Dr John Stephen at the Australian Genome Research Facility (AGRF) Adelaide, SA.

5. Results and Discussion

5.1 Leaf age and colonization

a)Whole vine, leaves attached experiment

Yellow-brown lesions, typical of colonisation by *P. viticola*, appeared on leaves from all nodes. The time taken for first appearance of symptoms ranged from 7 d for apical leaves to 14 d for basal leaves. Larger oilspots were observed on apical leaves (generally the first three emerged), whereas smaller, mosaic-like lesions were observed leaves towards the base.

Spread of the pathogen was more extensive at the point of inoculation in apical leaves than in basal leaves, as oilspots formed in apical leaves had spread extensively from the point of inoculation, whereas in basal leaves, lesions had generally formed only below the inoculated area.

Oilspot lesions continued to expand radially on apical leaves (generally at nodes 1 - 5) until approximately 20 dpi, after which lesions became necrotic. Lesions on basal leaves also became necrotic after 20 dpi. Leaves at the three most apical, inoculated nodes (i.e. the three youngest leaves at the time of inoculation), had significantly larger lesions (Leaf 1: 12.2 ± 4.1 cm^2; Leaf 2: 4.5 ± 1.6 cm^2 and Leaf 3: 2.2 ± 0.7 cm^2), than leaves from nodes 4 - 10 on the shoot, on which lesion size (all <1 cm^2) did not differ significantly from one another (Figure 1).

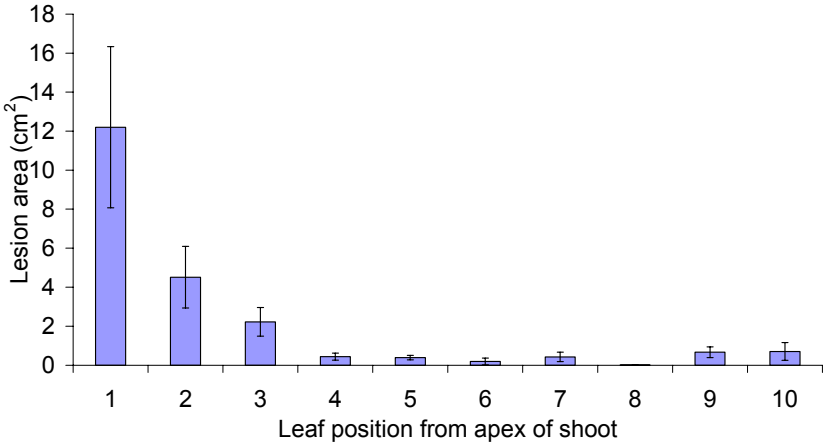

Figure 1. Lesion area (cm^2) in relation to leaf position from the apex of the shoot for *Vitis vinifera* cv. Marsanne leaves colonised by *Plasmopara viticola*. Data represent means of 10 replicate shoots and vertical bars indicate standard error.

b) Detached leaf experiment

The droplet of inoculum placed in the centre of each LD covered approximately 10% of the disc surface. Colony area was recorded in terms of the proportion of LD surface area occupied by sporangiophores, representing colonization in underlying tissue. Colony spread was measured as the appearance/amount of sporulation from tissue beyond the original 10% area inoculated.

No chlorotic, oilspot-like lesions were detected on any LD post-inoculation, even after sporulation. Sporulation was the first sign of infection and appeared 4 dpi in LD from node positions 4 and 5. Discs from all other leaves showed sporulation at 5 dpi, with the exception of those from leaves at node position 1, where sporulation was not detected (Table 1).

In discs from leaves 4 and 5, the colonies spread and occupied the entire disc by 7 dpi (i.e. from 10% of LD SA at inoculation to 100% at 7 dpi). In contrast, spread from discs from leaves 3, 6 and 7 was intermediate (final colonization of 40% LD SA), and in discs from other leaves (2, 8, 9, 10 and 11) colony spread was only slight (less than 30% of final LD SA).

In discs from node positions 4 and 5, 80% of the LD was colonised at 4 dpi and increased to 100% by 6 dpi. In comparison, discs from leaves at other node positions were not completely colonised by 7 dpi. Generally, the maximum surface area occupied by sporulation (i.e. at 7 dpi) was reached 1 day after sporulation was

initiated (except for discs from leaf 10; where coverage increased from 10% at 5 dpi to 30% at 7 dpi).

Table 1. Assessment of the spread of *Plasmopara viticola* colonies 1 - 7 dpi in Marsanne. LDs from node positions 1 - 11 (for three shoots, results combined), representing leaves of increasing age from apical (1) to basal (11). Note: The droplet of inoculum covered 10% of the central surface area of each LD.

Node position	Coverage of leaf disc area (%) by sporulation at dpi							Lesion spread
	1	2	3	4	5	6	7	
1 (apical)	0	0	0	0	0	0	0	n/a
2	0	0	0	0	10	10	10	Nil
3	0	0	0	0	30	40	40	Low
4	0	0	0	80	90	100	100	High
5	0	0	0	80	90	100	100	High
6	0	0	0	0	30	40	40	Low
7	0	0	0	0	30	40	40	Low
8	0	0	0	0	20	30	30	Low
9	0	0	0	0	10	20	20	Low
10	0	0	0	0	10	20	30	Low
11 (basal)	0	0	0	0	20	30	30	Low

NB: Values for lesion spread are for colony size at 7 dpi: 10% = Nil (inoculated area only), 20% - 40% = Low/Slight, 50% - 70% = Medium, >80% = High.

The susceptibility of grapevine leaves to the spread of *P. viticola* was related to leaf ontogeny/nodal position and age. A similar relationship was shown for tobacco leaves colonised by *Peronospora tabacina* (Matthews, 1981). In point-inoculated leaves attached to vines, leaves nearest the apex, i.e. leaves 1 - 3 at the time of inoculation, developed the largest lesions. There appeared to be a general linear decrease in lesion size on leaves at nodes 1 to 5, i.e. for leaves 1 - 20 days old. Leaves greater than 10 days old generally produced very small lesions (<2 cm^2) that were not significantly different in size (p <0.05). In comparison, colonies of *V. inaequalis* on leaves of apple were restricted to infection points by defence mechanisms until leaves began to senesce, after which the pathogen spread. In old apple leaves, some colonies remained latent, but could still produce small amounts of sporulation in favourable conditions (Li and Xu, 2002). This breakdown in resistance is due to physiological changes (such as cell wall thickening and lignification) that occur at the onset of senescence (Olivier and Lespinasse, 1981; Valsangiacomo and Gessler., 1988; Koller *et al.*, 1992) and, as a result, old leaves may become susceptible to further colonization or infection late in the season (MacHardy, 1996; Li and Xu, 2002). Such a relationship was not found in the literature for any downy mildew disease, and the results of this study suggest that *P. viticola* probably does not remain latent in older leaves, as symptoms were observed in leaves of all ages. However, lesions were very small in some leaves, where only a

single or a few leaf units (the area between minor leaf veins, LU) were colonised (smallest: 1 LU, 0.08 cm^2), such lesions would be difficult to detect in the field and may contribute to secondary infection.

Examining lesion surface area as a proportion of total leaf surface area revealed significant differences among leaves at node positions 1 - 3 nearest the shoot apex. When lesion size relative to leaf surface area was assessed, differences in lesion size between apical and basal leaves were amplified due to the relatively small apical leaves. For example, the largest lesion on a leaf at node 2 occupied 50% of total leaf area (average lesion area for node 2: 23.6% ± 9.6). It is expected that such extensive colonization of leaves would cause a considerable reduction in the photosynthetically active area of the leaf, and that such leaves would contribute little to overall carbon assimilation, thus affecting potential yield and quality.

Young leaf tissue appeared to become extensively colonised, illustrated by progressive, radial colonization from the point of inoculation through all tissues (including veins) to an adjacent, uninfected LU. Lesion expansion ceased with the onset of ontogenic resistance (at leaf age of 6 - 7 d after unfurling or at node position ca 6). This type of colonization was characterised by rounded oilspots. In contrast, colonies in basal leaves appeared to be restricted to the LU under the point of inoculation, from where the pathogen was unable to progress past the veins of colonised LU into surrounding units. This type of colonization in basal leaves was characterised by single or few defined, chlorotic or necrotic LUs.

The LD experiment indicated trends similar to, yet distinct from, point-inoculation of attached leaves. Generally, discs from leaves nearest the apex were more susceptible than those from basal leaves to colonization by *P. viticola*, expressed as colony spread beyond the point of inoculation. However, sporulation, representing colony area in LD, was sparse in discs from leaves 1 and 2 from the shoot apex, extensive in discs from leaves 3 and 4, and slight in those from 5 to 11. These results contrast with those for attached leaves, where those closest to the apex were the most susceptible. It is possible that this phenomenon is related to the leaf nutrient status, such that attached leaves nearest to the apex continued to function and remained a strong sink for nutrients, but when excised leaf function was compromised. Thus pathogen development was less in discs from leaves closest to the apex and was greatest in discs from leaves at nodes 4 and 5, with higher nutrient status when excised. This finding may have ramifications for studies utilizing LDs or

detached leaves to examine pathogen behaviour, as the colonization of the pathogen in detached apical leaves appears not to be related to colonization of apical leaves in the field.

5.2 Survey of W.A. viticultural regions for oospores

Oospores were detected in colonised leaf tissue from only a single vineyard in Manjimup with a history of relocation.

An epidemic of downy mildew occurred in the 2001/2 season Manjimup vineyard. Oospores were detected in symptomatic leaves collected during the epidemic. The vineyard comprised a cultivar collection managed by the Department of Agriculture, Western Australia and the West Australian Vine improvement Association (WAVIA). The vines at the site (4 vines of each cultivar and 4 of each clone, of 80 wine and table grape cultivars, some cultivars with several clones), originated from the Swan Valley, W.A. (31.75S, 116.02E, mean annual rainfall: 740 mm), where vines derived from local and imported cuttings had been planted periodically (as a cultivar collection) over a number of decades from the mid-1950s (pers. comm. J. Campbell-Clause, 2005). In 1995/6, cuttings from the vines in the Swan Valley vineyard were planted at Wokalup (33.07S, 115.53E, mean annual rainfall: 964 mm) (Campbell-Clause, 1995a;b). In 2000, whole vines including roots were translocated from Wokalup to the vineyard in Manjimup (34.15S, 116.09E, mean annual rainfall: 1022 mm) (Harding, 2001).

The re-location of these vines may have influenced the number of mating types of *P. viticola* present. Oospores were associated with both mosaic lesions in old leaves and with oilspots on younger leaves, although oospore density appeared to be greater in the former. Inoculation of potted vines with sporangia from the Manjimup collection produced oospores in potted vines in the glasshouse. Thus it was concluded that the sexually reproducing pathogen population in the Manjimup vineyard was atypical and that oospores were either absent from or uncommon in most viticultural regions of W.A.

Michelmore (1981) discussed the infrequent and sometimes localised formation of oospores in many downy mildews and how this attribute may be a consequence of heterothallism (that is, the need for two mating types to recombine). Where heterothallism was known to occur, sexual reproduction had been observed in any

tissue where mycelia of both compatibility types were found, e.g. in *Bremia lactucae* in lettuce (Michelmore, 1981).The heterothallic nature of *P. viticola* (Wong *et al.*, 2001),. and the observation of oospores in only one vineyard in W.A. in this study suggest that the absence of oospores may be due to the presence of only one mating type in the leaves assessed, or that both mating types were present but were unable to mate, perhaps because of unfavourable physiological, epidemiological or environmental factors.

It has been reported that symptom expression, leaf age or date of collection may play a role in the ability of the pathogen to produce oospores (Michelmore, 1981). The possible effect of these factors was accounted for in this study by repeated sampling of leaves with a variety of symptoms, in several vineyards, at various stages of the season, over four growing seasons. However, these factors appeared not to influence oospore formation. All types of lesions (single oilspots, coalesced oilspots and mosaic lesions) were represented in leaves sampled from the Manjimup vineyard, and oospores were detected in all of them. Furthermore, leaves from potted vines inoculated with a mixture of isolates from this vineyard contained oospores, but leaves inoculated with mixtures of isolates from other vineyards did not, perhaps indicating that most vineyards contained a single mating type and that only the Manjimup vineyard contained more than one mating type

In years when downy mildew is sporadic in vineyards, it is likely that individual oilspots (each formed by a separate mating type) would be too infrequent to make contact with another oilspot (or mating type). This scenario would preclude the formation of oospores and may reflect effective management and hot, dry conditions unfavourable for sporulation and dissemination. During an epidemic, as occurred in several untended vineyards during the study period, the large number of oilspots would increase the chance of them coalescing and forming oospores. However, this did not occur, suggesting that a single mating type was present, sexual reproduction was unsuccessful, or that oospore formation was rare and not detected in the leaves sampled.

6. Diagnosis of bud colonization by epi-fluorescence microscopy and molecular detection of *P. viticola* DNA in tissue

Colonisation of the dormant bud, either the scales or primordium, by *P. viticola* was common on severely diseased shoots of *V. vinifera*. Many of the point- and spray-

inoculated buds, particularly those near the apex, became colonised in all treatments (Figure 2). Bud scales from vines naturally infected in the field were also frequently colonised. Colonisation of bud primordia was less common than that of the bud scale, and also occurred most commonly near the shoot apex. The colonisation of bud primordia was related to the presence of oily-brown lesions caused by *P. viticola* on the node subtending the dormant bud (Figure 3). Thus lesions at nodes appeared to be indicative of colonisation of the bud primordium.

Lignification of bud scales upon maturity protects the bud from the environment and from pathogens (Mullins *et al.*, 1992). This was supported in this study, in which basal buds were more resistant to colonisation by *P. viticola* than were apical buds. Infection of bud scales and spread of the colony appeared to cease with the maturation of the bud scales, preventing the pathogen from progressing to the bud primordium. Colonisation of the bud primordium appeared more likely to occur in younger tissue (i.e. near the apex of the shoot), in which systemic infection of the primordium appeared to originate from colonies within the subtending node tissue (Figure 4). A zone of suberisation was present between shoot and bud in mature buds which may restrict the spread of the pathogen into the bud. Thus there appeared to be a brief period of time, whilst the shoot and bud are young, when systemic colonisation of the bud primordium may occur.

The presence of the pathogen on the primordial shoot may lead to either necrosis or death of the shoot, or the survival, over winter, of the pathogen within the shoot. In this study, necrotic primordia were observed in buds excised from nodes colonised by *P. viticola*. Some bud primordia appeared to be colonised by hyphae similar to those of *P. viticola*. Additionally, buds with a necrotic primary bud, but with apparently viable (i.e. not necrotic), colonised secondary buds were observed. Thus it is possible that the shoots emerging from such buds may be colonised by *P. viticola*, and have an epidemiological function similar to flag-shoots in powdery mildew.

The difficulty in confirming mycelium in dormant buds as to be *P. viticola* necessitated the use of molecular markers for detection of pathogen DNA within buds. The detection of pathogen DNA in all bud primordia (12) from current season's shoots and the majority (8 out of 13) of those collected in mid-winter showed that coenocytic hyphae observed by microscopy in similar buds could have been *P. viticola*, and that it was possible that the pathogen was surviving in buds over winter.

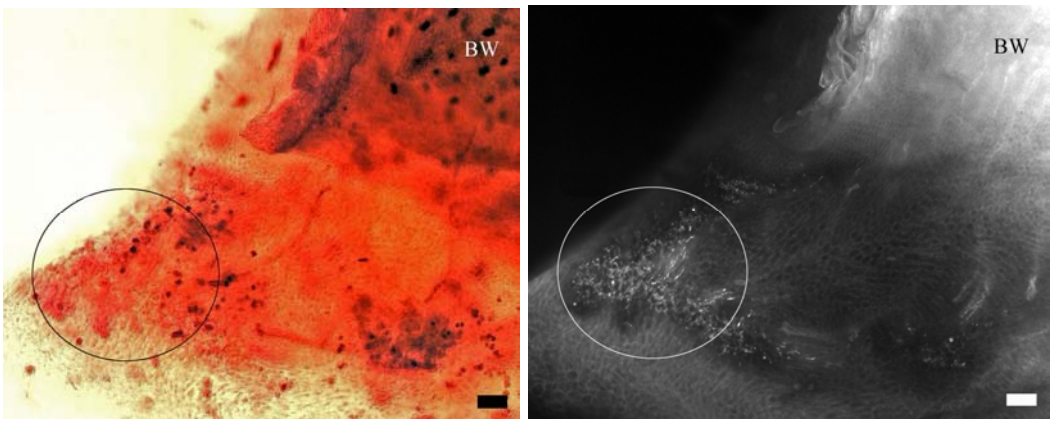

Figure 2. Small colony of *Plasmopara viticola* in the outer bud scale of a latent bud left: light microscopy and **right**: epi-fluorescence microscopy. Circle indicates area colonised. BW indicates bud-wool (Bar = 100 µm).

Figure 3. Oily-brown lesions on nodes and internodes (circles), an indicator typical of colonisation of the bud primordium by *Plasmopara viticola* at each node. Note the necrosis of the leaf at the central node.

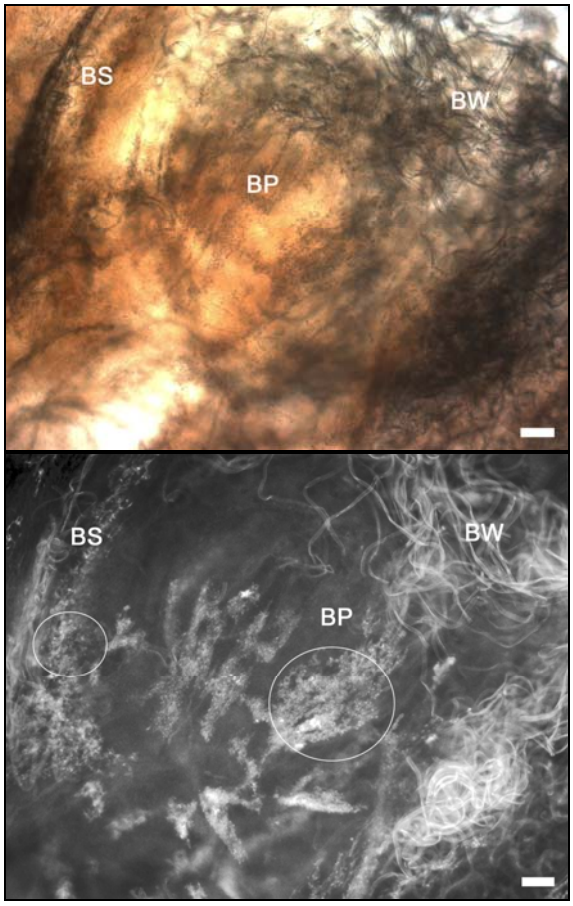

Figure 4. Microscopy (Light: **left** and Fluorescent: **right**) of cv. Chardonnay bud primordium colonised by *Plasmopara viticola*. (BW: bud wool, BP: bud primordia, BS: bud scale). In B note mycelium colonising both the bud primordium (large circle) and bud scale (small circle) (Bar = 100µm).

Stunted, distorted shoots with many lesions were observed on several occasions. These had formed from buds at nodes with lesions indicative of colonisation by *P. viticola* in the previous season. Additionally, observations of bud burst during a warm period in mid-winter in a vineyard that had suffered an epidemic of downy mildew in the previous season showed that many colonised shoots had emerged. These shoots appeared yellow and oily compared to uncolonised green shoots (Figure 5 A, 5 B) and bore small tufts of sporangiophores of *P. viticola* (Figure 5 C). When transferred to the laboratory and incubated at 100% RH overnight, abundant sporulation appeared on the entire shoot surface (Figure 5 D) and thus shoots appeared to have been systemically colonised. These shoots were very small (<10 cm long) and oospores had not been detected in leaf tissue from this vineyard in the previous growing season. It is therefore likely that such colonisation arose from the asexual state of the pathogen within the dormant compound bud. It is also unlikely that oosporic primary infection would lead to systemic colonisation of such tissue, as

infection caused by one or several zoospores is more likely to form only localised lesions in the several days immediately following bud burst.

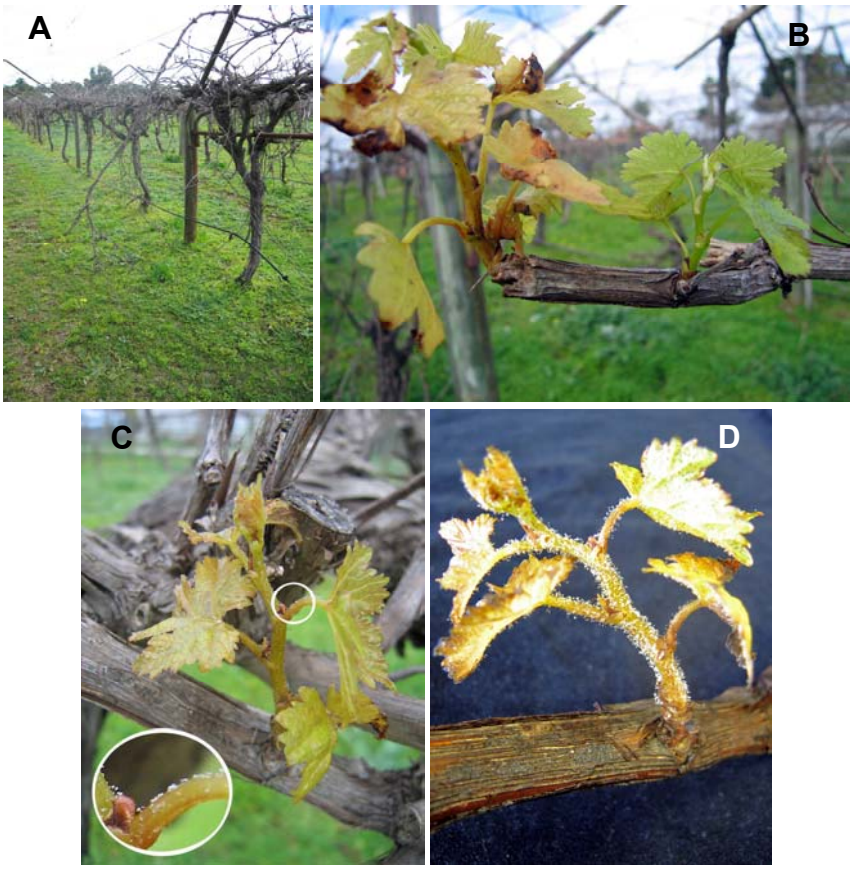

Figure 5. A: The vineyard in which sporadic bud-burst was observed during a brief warm period at the end of winter (late July) in the Swan Valley. The vineyard had suffered an epidemic in the previous season. **B**: Systemically colonised shoot (left) next to an uncolonised shoot. Note the difference in colour between colonised (yellow) and uncolonised (green) shoots. **C**: Systemically colonised shoots had produced some sporulation (circle, see close-up in insert). **D**: Systemically colonised shoot in C produced masses of sporangia when incubated *ex situ* at 100% RH at 22°C overnight.

DNA of *P. viticola* was detected in all of the 12 dormant buds excised from colonised nodes during the growing season and in 8 of 13 buds collected during winter (Table 2).

Table 2. Microsatellite primed PCR analysis of *Vitis vinifera* cv. Red Globe bud primordia for *Plasmopara viticola* DNA. Buds collected during the growing season and in winter. Analysis performed by the Australian Genome Research Facility using ISA, CES and BER primers (Gobbin *et al.*, 2003b).

		DNA amplified	DNA not amplified
Positive Leaf Control		X	
Negative Bud Control			X
Buds collected during growing season	1	X	
	2	X	
	3	X	
	4	X	
	5	X	
	6	X	
	7	X	
	8	X	
	9	X	
	10	X	
	11	X	
	12	X	
Buds collected during winter	1	X	
	2		X
	3	X	
	4	X	
	5	X	
	6		X
	7		X
	8		X
	9	X	
	10	X	
	11		X
	12	X	
	13	X	

The survival, over winter, of *P. viticola* in buds may play a role in the disease cycle when colonised buds remain in the vineyard over winter, and burst in the following season, providing primary inoculum. All buds, except the basal two, are removed in spur-pruned vines, thus survival of the pathogen by this means is likely only if those buds are colonised, which could occur only at the start of the season when these buds are closest to the shoot apex and susceptible. However, in management systems where large numbers of buds are retained, such as cane pruned, minimally pruned or untended vines, survival of the pathogen by this means is more likely than in spur-pruned vines.

In some regions, for example where there is the risk of early season frost, spur-pruning is completed very late in winter or early in spring and commonly after buds

have burst from the apex of the cane. In this situation, those shoots, if colonised by *P. viticola*, may provide primary inoculum. Vineyards generally consist of a number of cultivars, and bud burst occurs several weeks earlier in some cultivars (i.e. Chardonnay) than others (i.e. Shiraz). Thus inoculum may be provided by an un-pruned late cultivar with a retained, colonised bud, leading to infection of an early cultivar with an established canopy.

To our knowledge, this is the first report of infection of dormant buds using epi-fluorescence microscopy, the presence of sporangia within the bud scales and detection of DNA of *P. viticola* in dormant buds during the growing season and in the middle of winter. Also, this appears to be the first report of shoots systemically colonised by *P. viticola* at bud burst in a vineyard. If this phenomenon is confirmed and found to be common in the vineyard, it would have ramifications for the early season control of the disease. Systemically colonised shoots within the canopy would provide asexual inoculum when RH was greater than 95% and temperature greater than 13°C (Lafon and Bulit, 1981), whereas oospore germination for primary infection in Australia requires temperatures of greater than 10°C and 10 mm of rain over a 24 h period. Additionally, the amount of inoculum produced by colonised shoots is potentially far greater than that from oospore germination and may provide more inoculum within the canopy than would otherwise be provided by oospores within decomposing leaf matter on the vineyard floor.

7. Outcome/Conclusion

The project changed considerably after oospores could not be found in most viticultural regions of W.A. where downy mildew had occurred. Subsequently, the research focused on discovering the origin of primary inoculum, if not from oospores. The colonization of buds in one season by the pathogen, and the subsequent formation of a downy mildew “flag shoot” is suggested as a means of overwintering of the pathogen in W.A. and possibly elsewhere.

If confirmed, these findings have practical implications for disease control where *P. viticola* survives over winter in buds. Growers currently apply preventative, copper-based fungicides at the start of the growing season; these would not inhibit the pathogen within the buds or emerging “flag-shoots”, but would, by analogy with powdery mildew, protect neighbouring foliage While it is possible that curative sprays at the start of the growing season would kill the pathogen within emerging flag shoots

and prevent further spread of inoculum, this would increase selection pressure for resistance to the single-site active fungicides. It would be important to prevent the colonisation of buds, by preventing the colonization of young tissue surrounding the developing bud. This is especially important at the beginning of the season in spur-pruned vineyards where the first nodes to emerge are those that will produce shoots in the following season.

8. Recommendations

This project was focused on the W.A. wine industry. The possibility that the pathogen survives over winter in buds in other parts of Australia and in Europe should be examined also.

Extension of this research to the broader industry would be of benefit to those vineyards affected by downy mildew. It is important to train vineyard workers and monitoring personnel to identify lesions on all tissue types caused by the pathogen that differ from the “oilspots” on leaves that are the most commonly recognised symptom. This is especially important as such atypical lesions may lead to colonization of buds.

9. Communication

Findings have been presented at grower workshops in the Swan Valley, Albany, the Great Southern and Margaret River, W.A. and early results on effect of leaf age on colonisation were presented at scientific conferences in Milwaukee and the Napa Valley, USA. The novel and unique nature of the findings on survival of the pathogen in colonised buds means that this report should be kept confidential until the research is published in refereed scientific journals.

Presentations at conferences

Killigrew, B., Sivasithamparam, K and Scott, E.S. (2002) Sporangial and oospore production by *Plasmopara viticola* varies with age. Proceedings of the 4th International Workshop on Grapevine Powdery and Downy Mildew (eds D.M. Gadoury, C. Gessler, G. Grove, W.D. Gubler, G.K Hill, H.H. Kassemeyer, W.K. Kast, J. Rumbolz and E.S. Scott), University of California Davis, California, p 22. (ISBN 0 9724157 0 X).

Scientific publications

Killigrew, B.X., Sivasithamparam, K. and Scott, E.S. (2005) Absence of oospores of downy mildew of grape (*Plasmopara viticola*) as the source of primary inoculum in most Western Australian vineyards. Plant Disease **89**, 777.

10. References

Barret, J. T. (1939). Overwintering mycelium of *Plasmopara viticola* in the California wild grape *Vitis californica*. Phytopathology **29**: 822-823.

Campbell-Clause, J. (1995a). Grape Vine Improvement Program. Wine Industry Newsletter **43 & 44**: 9.

Campbell-Clause, J. (1995b). The Western Australian Vine Improvement Association. Wine Industry Newsletter **45**: 4.

Chrelashvili, L. G. (1984). Overwintering of fungus *Plasmopara viticola* Berle et de Toni causing downy mildew of grapes. Soobshch Akad Nauk Gruz SSR Bull Acad Sci Ga SSR. Tbilisi : "Metsniereba". **115**: 173-175.

Emmett, R. W., T. J. Wicks and P. A. Magarey (1992). Downy mildew of grapes. in: Plant diseases of international importance. Volume III. Diseases of fruit crops. Ed. Prentice Hall, Englewood Cliffs, USA.

Fisher, D. (2000). Downy mildew in vineyards. Department of Agriculture Western Australia.

Gobbin, D., I. Pertot and C. Gessler (2003a). Genetic structure of a *Plasmopara viticola* population in an isolated Italian mountain vineyard. Journal of Phytopathology-Phytopathologische Zeitschrift **151**: 636-646.

Gobbin, D., I. Pertot and C. Gessler (2003b). Identification of microsatellite markers for *Plasmopara viticola* and establishment of high throughput method for SSR analysis. European Journal of Plant Pathology **109**: 153-164.

Gobbin, D., G. Valsesia and C. Gessler (2001). Genetic variability of *Plasmopara viticola* between and within selected populations. Bulletin Oilb/Srop **24**: 19-23.

Harding, C. (2001). WAVIA took some positive steps in 2000. The Australian Grapegrower & Winemaker **April**: 38-39.

Kiefer, B., M. Riemann, C. Buche, H. H. Kassemeyer and P. Nick (2002). The host guides morphogenesis and stomatal targeting in the grapevine pathogen *Plasmopara viticola*. Planta **215**: 387-393.

Koller, B., M. Muller, C. Valsangiacomo and C. Gessler (1992). Cell wall degrading enzymes and inhibitor involved in the interaction between *Venturia inaequalis* and *Malus domestica*. Acta Phytopathologica et Entomologia **27**: 353–359.

Lafon, R. and J. Bulit (1981). Downy Mildew of the Vine. in: The Downy Mildews. Ed. D. M. Spencer. London. Academic Press.

Li, B. and X. Xu (2002). Infection and development of apple scab (*Venturia inaequalis*) on old leaves. Journal of Phytopathology **150**: 687–691.

MacHardy, W. E. (1996). Apple Scab: biology, epidemiology, and management. St Paul, MN. American Phytopathological Society.

Matthews, P. (1981). Breeding for resistance to downy mildews. in: The Downy Mildews. Ed. D. M. Spencer. London. Academic Press.

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

Michelmore, R. W. (1981). Sexual and asexual sporulation in the downy mildews. in: The Downy Mildews. Ed. D. M. Spencer. London. Academic Press.
Mullins, M. G., A. Bouquet and L. E. Williams (1992). Biology of the grapevine. Cambridge, England;. Cambridge University Press.
Olivier, J. M. and Y. Lespinasse (1981). Evolution des recherché sur la resistance du pomier a la tavelure. II. Etude du parasite et strategies
de lutte. Ler Colloque sur les Recherches fruitieres, Bordeaux, France.
Valsangiacomo, C. and G. C. (1988). Role of the cuticular membrane in ontogenic and Vf-resistance of apple leaves against *Venturia inaequalis*. Phytopathology **78**: 1066–1069.
Wong, F. P., H. N. Burr and W. F. Wilcox (2001). Heterothallism in *Plasmopara viticola*. Plant Pathology **50**: 427-432.

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12. Budget reconciliation

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