

***In situ* sporulation of *Phaeomoniella chlamydospora* in the vineyard**

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Summary. The fungus *Phaeomoniella chlamydospora* causes black goo decline of grapevines and is associated with esca, but little is known about its epidemiology. Sporulation was rarely observed on transversely-cut surfaces of naturally-infected roostocks and cordons of ten-year-old 'Pinot Noir' on 'Ramsey' grown near Geelong, Australia, over the winter months of 2000. However, abundant sporulation of the hyphomycete and pycnidial synanamorphs of *P. chlamydospora* were observed on protected wood surfaces inside deep cracks at the trial site. Sporulation in cracks was also observed on several grapevine varieties from other regions of south eastern Australia. Collembolans and mites were usually associated with the sporulation, but it is not known whether they have a role in dissemination of conidia.

Key words: black goo decline, Petri grapevine decline.

Introduction

The anamorphic fungus *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams (syn. *Phaeoacremonium chlamydosporum* W. Gams, Crous, M.J. Wingf. & L. Mugnai) is strongly suspected to be the causal agent of the grapevine disease commonly known as black goo decline, also known as Petri grapevine decline (Crous and Gams, 2000) and is also associated with esca (Mugnai *et al.*, 1996; Larignon and Dubos, 1997) Although *P. chlamydospora* has been recorded in grape-growing regions all over the world, for example in South Africa (Ferreira *et al.*, 1994), France (Larignon and Dubos, 1997), the USA (Scheck *et al.*, 1998), Australia (Pascoe, 1999), Italy (Mugnai *et al.*, 1999), Argentina (Gatica *et al.*, 2000), Austria (Reisenzein *et al.*, 2000), Portugal

(Chicau *et al.*, 2000; Rego *et al.*, 2000) and Turkey (Erkan Ari, 2000), the source of inoculum remains unknown. In young vineyards it is believed that the planting material is already infected, either systemically from infected mother vines (Ferreira, 1999; Pascoe and Cottral, 2000) or by contamination during the propagation process (Bertelli *et al.*, 1998; Scheck *et al.*, 1998). In mature vineyards, it is known that infection can occur through wounds (Larignon and Dubos, 2000). However, the fundamental questions remain unanswered: how do mother vines become infected and where is the original source of inoculum in the field? It has been suggested that *P. chlamydospora* is soilborne (Ferreira *et al.*, 1994; Bertelli *et al.*, 1998; Mugnai *et al.*, 1999; Sidoti *et al.*, 2000) or that sporulation occurs on dead wood and debris in the vineyard (Mugnai *et al.*, 1999; Ferreira 1999), but neither hypothesis has been supported by conclusive evidence.

Little is known of the reproductive and dispersal behaviour of *P. chlamydospora* in the field although *in vitro* ontogeny and morphology of coni-

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dia have been well documented (Crous *et al.*, 1996; Crous and Gams, 2000; Pascoe and Cottrall, 2000) and Edwards and Pascoe (2001) described the occurrence of pycnidia on grapevine wood in the field. Obviously, further information on reproductive behaviour is critical to a full understanding of the epidemiology of the fungus in vineyards, particularly with respect to the timing, site and mode of spore production and dispersal and the role of these different spore types. The knowledge that *P. chlamydospora* sporulates readily on moist incubated, freshly-cut grapevine wood in the laboratory led us to speculate that the fungus may sporulate on fresh wounds in the vineyard. However, sporulation on wound surfaces in the field has never been reported. Larignon and Dubos (2000) were able to demonstrate infection via wounds and trapped *P. chlamydospora* spores on vaseline-coated glass slides in vineyards, but they failed to demonstrate the source of these spores.

This paper reports on a field trial designed to determine the timing and environmental conditions for spore production on wounds in the vineyard and describes observations of *in situ* sporulation on living vines.

Materials and methods

P. chlamydospora sporulation on cut surfaces of infected scion and rootstock wood

The trial site consisted of 100 vines: 25 vines in each of four adjacent rows of 10-year-old 'Pinot Noir' grapevines grafted onto 'Ramsey' rootstocks growing in a vineyard near Geelong, Victoria, Australia. The vines had declined rapidly over the previous three years, the decline being triggered by undetected faulty irrigation drippers that resulted in prolonged waterlogging. Associated symptoms included reddening of the leaves, poor vigour and fruit set, dieback and death of the vines. Inspection of internal wood symptoms in May 2000 revealed symptoms of black goo decline (Fig. 1). Subsequently, the fungus was consistently isolated from symptomatic wood in both rootstock and cordons of all vines tested.

On May 25 (week 0), 88 vines were cut off either at the cordons to expose infected scion wood (40 vines, two cordon cuts each), or below the graft union (48 vines) to expose infected rootstock wood. The vines were assigned to 8 treatments (Treat-

ment 1, one-week old cut surface; treatment 2, two week old cut surface, and so on consecutively).

At regular intervals (from May 25 to September 14) 1 cm thick slices were removed from the relevant scion or rootstock cut surfaces (following the scheme in Table 1), every week in Treatment 1, every 2 weeks in Treatment 2, and so on, thus obtaining a series of one- to eight-week-old cut surfaces over the winter months at a time when the vines would normally be pruned. There were ten replicates per each survey date for the scion and six replicates per each survey date for the rootstock.

The slices were microscopically examined (x50) immediately on return to the laboratory on the day of collection and the presence or absence of *P. chlamydospora* sporulation on the exposed surface was recorded. They were then moist incubated for up to four months at room temperature (20–22°C) and re-examined several times for the presence of *P. chlamydospora* to determine if the wood was infected.

In addition, spore dispersal was monitored by placing ten spore traps consisting of vaseline-coated slides (Larignon, 1999) among the vines each week and collecting them the following week. The vaseline was plated onto potato dextrose agar amended with 50 ppm Achromycin® (a.i. tetracycline hydrochloride; American Cyanamid Company, USA) and examined after two weeks incubation (20–22°C) for the presence of *P. chlamydospora*.

Rainfall and temperature data were collected over the period of the trial. We were unable to extend or repeat the trial as the site was unavailable after this period.

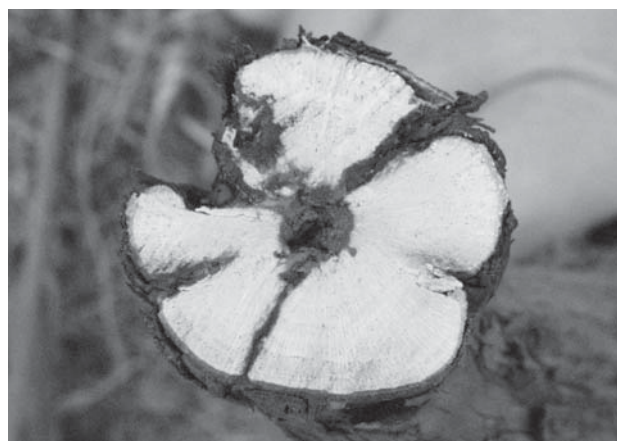


Fig. 1. Black goo symptoms in infected 'Ramsey' rootstock, with *P. chlamydospora* sporulation detected in crack.

Table 1. Sampling procedure and results of the investigation on sporulation of *P. chlamydospora* on freshly-cut infected grapevine wood *in situ*. The treatments' number, from 1 to 8, correspond to different age cut surfaces in weeks. At the time of each survey a wood slice to be examined was cut thus causing a new wound that was then left exposed for the due time for each treatment.

Sampling date	Treatments							
	1	2	3	4	5	6	7	8
25 May (week 0) ^a								
1 June (week 1)	-							
8 June (week 2)	-	-						
15 June (week 3)	-		-					
22 June (week 4)	-	-		-				
29 June (week 5)	-				-			
6 July (week 6)	-	-	-			-		
13 July (week 7)	-						-	
20 July (week 8)	-	-		-				-
27 July (week 9)	-		-					
3 August (week 10)	+	-			+			
10 August (week 11)	-							
17 August (week 12)	-	-	-	-		-		
24 August (week 13)	-							
31 August (week 14)	-	-					-	
7 September (week 15)	-		-		-			
14 September (week 16)	-	-		-				-

^a First cut on all the plants (scion or rootstock) of the 8 treatments.

-, No detection of *P. chlamydospora* sporulation.

+, Positive detection of *P. chlamydospora* sporulation.

P. chlamydospora sporulation in cracks and crevices

Deep cracks and crevices were often observed on grapevines where the cordons separated from the trunk (Fig. 2) and also in rootstocks (Fig. 1). These were first noticed on the 10-year-old 'Pinot Noir' scions and 'Ramsey' rootstocks used in the trial described here. The cracks were prised open and the surfaces which had been pressed together microscopically examined (x50). Other vines exhibiting similar symptoms were also examined: these were 10-year-old 'Merlot' on 'Schwarzmann' from Geelong (Victoria), 14-year-old 'Sultana' on 'Ramsey' from Mildura (Victoria) and 6-year-old 'Chardonnay' on 'Ramsey' or 'Teleki' (owner unsure) from Barooga (New South Wales).

Results

P. chlamydospora sporulation on cut surfaces of infected scion and rootstock wood

A total of 410 scion and 252 rootstock cut surfaces from the 88 vines were examined over 4 months, from 1 June to 14 September 2000. De-

spite having carried out 16 surveys, only once, on 3 August, single sporulating *P. chlamydospora* hyphal strands were observed, on 20% of the scion wood slices of Treatment 1, but none of the Treatments 2 and 5 wood slices collected on that date. In addition, sporulating *P. chlamydospora* hyphal strands were observed, only at that same date, in the pith region of the rootstock samples (50% of the Treatment 5 samples, but none of the Treatment 1 and 2 wood samples). Sporulation was only observed on the exposed cut surfaces on this sampling date, and there was no evident relationship with rainfall or temperature (Fig. 3).

Moist incubation of the pieces at room temperature confirmed that all pieces from all 88 vines were infected with *P. chlamydospora*. Examination of the pieces after one and two weeks moist incubation revealed a small amount of growth and sporulation on approximately half of the pieces, but after two to four months moist incubation *P. chlamydospora* had overgrown the surface of more than 90% of the pieces.

No *P. chlamydospora* cultures grew from the plated-out vaseline of the slide traps.



Fig. 2. Cracks in upper trunk at crown of vines in which *P. chlamydospora* was found sporulating.

***P. chlamydospora* sporulation in cracks and crevices**

In all cases, the two surfaces which had been pressed together were dark and covered with abundantly sporulating *P. chlamydospora*, often associated with copious amounts of black sticky 'goo'. A specimen has been lodged in the plant disease herbarium (VPRI) of Agriculture Victoria at Knoxfield as VPRI 22629. At x50 magnification, pycnidia were seen on the wood surface under the hyphomycete state on several of the samples, one of which has been lodged as VPRI 22410. Despite numerous attempts, none of the spores produced by the pycnidial synanamorph, either *in vivo* or *in vitro*, germinated on potato dextrose agar amended with 50 ppm Achromycin®.

Purple collembolans and fast-moving mites were always found among the sporulating hyphae. The collembolans were identified as belonging to *Brachystomella* sp. cf. *platensis* Najt & Massoud from the family Brachystomellidae, members of which are known to feed on various fungi (P. Green-slade, personal communication). The mites were identified as a species of Phytoseiid predatory mite. Both mites and collembolans were observed entering exposed xylem vessels.

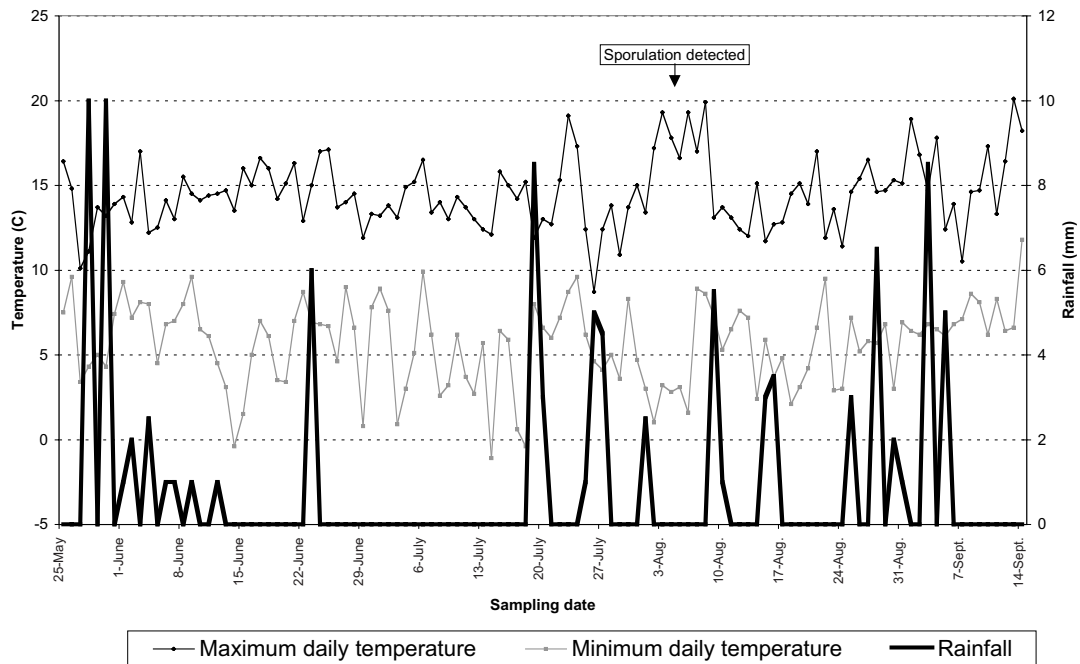


Fig. 3. Daily temperature (maximum and minimum) and rainfall over the duration of the trial (May to September 2000) and date of detection of *Phaeoemoniella chlamydospora* sporulation.

Discussion

Contrary to our expectation, *Phaeoconiella chlamydospora* did not sporulate to any significant extent on exposed transversely-cut surfaces during winter. Winter is the most likely time for new infection as this is when pruning is generally conducted. On the one date (3 August) that sporulation was observed on cut surfaces, it was extremely sparse, only one or two hyphal strands. Instead, *P. chlamydospora* was found to sporulate abundantly in deep cracks and crevices on infected grapevines in the field. The cracks and crevices provided a protected humid environment for egress of the fungus, comparable to the moist incubation conditions provided in the laboratory. *P. chlamydospora* is a very slow-growing fungus (Chiarappa, 1959) and the amount of sporulation observed within the crevices was consistent with that observed on moist incubated wood after several months. Subsequent moist incubation of the sampled wood slices revealed that nearly all were infected with *P. chlamydospora*. The lack of any *P. chlamydospora* spores trapped in the vineyard during the study period implies that the sporulation in the moist chambers was due to internal infection of the wood and not external contamination with spores of the fungus. In view of this, the unexpected low level of sporulation observed on the exposed cut surfaces may be due to the wound surfaces being too dry and exposed to support *P. chlamydospora* growth and sporulation, but *P. chlamydospora* has been shown to be insensitive to decreasing water potential in laboratory tests (Whiting *et al.*, 2001). Another possible explanation is that it was just too cold, as Whiting *et al.* (2001) showed that the temperature range for growth of the fungus in culture was 10–35°C, with an optimum temperature of 25°. This would explain the lack of sporulation observed in this present study, with the amount of *P. chlamydospora* growth and sporulation observed within the trunk crevices suggestive of active growth during the preceding summer months (December–March).

Many vines have deep cracks at the junction of cordons and trunk, reported by growers to be the result of damage caused by mechanical pruning and/or harvesting. Vineyard managers in Australia have suggested that the increased incidence of black goo decline is correlated with increased mechanised viticultural practices. This would be consistent with increased opportunities for egress of the fungus.

Pycnidia were observed among the sporulating hyphae of *P. chlamydospora* in several of the cracks examined. This first occurrence of *P. chlamydospora* pycnidia in the field was reported by Edwards and Pascoe (2001). Attempts to germinate spores from any of the pycnidium-like structures failed, regardless of whether they were produced *in vivo* or on moist incubated wood, and as such pathogenicity tests could not be carried out. This is inconsistent with observations made by Adalat *et al.* (2000) and Pascoe and Cottrill (2000) who reported germination of conidia taken from pycnidia produced on moist incubated wood. It is possible that age of pycnidia has a bearing on the conidial germinability. Alternatively, the pycnidia may be spermogonia and the spores not germinable, in which case Adalat *et al.* (2000) and Pascoe and Cottrill (2000) erroneously reported germination of hyphomycete-state aerial conidia from pycnidial surfaces rather than conidia/spermatia from inside the pycnidium. A recent population genetics study of *P. chlamydospora* using molecular data (Tegli, 2000) revealed sufficient genetic diversity among *P. chlamydospora* isolates to suggest that a sexual state does exist, although it has not been discovered.

We believe that the observed association of collembolans and mites with the sporulation in cracks may be relevant to dispersal of *P. chlamydospora*. The sheltered nature of the cracks provides limited scope for dispersal methods such as rain or wind, and no spores were detected using the vaseline-coated slide traps. Both the phialidic conidial heads and the pycnidial cirrhi of *P. chlamydospora* are presented in such a way as to be readily picked up by small passing arthropods. It is possible that the collembolans and mites could carry fungal spores stuck to their exoskeletons into the cut ends of xylem vessels as they explore fresh wounds. Collembolans and predatory mites have been reported to carry fungal spores on their exoskeletons (Blackwell, 1984; O'Connor, 1984).

The observation that *P. chlamydospora* sporulates in cracks and may be arthropod-dispersed may partially explain the apparent increase in importance of black goo decline, coinciding with an increase in vineyard mechanisation. Avoidance of cracking in vines may reduce the inoculum density of *P. chlamydospora*, but it is first necessary to compare un-mechanised with mechanised vineyards and verify the suggested correlation between

cracks and mechanisation. Further research is also required to determine whether small arthropods are able to transmit this disease.

Acknowledgements

We thank the vineyard owner and manager for their collaboration, Drs Mali Malipatil and Penny Greenslade for identifying the arthropods and the Grape and Wine Research and Development Corporation and Agriculture Victoria for funding the study through the Co-operative Research Centre for Viticulture.

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Accepted for publication: February 15, 2001