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Developments in grapevine trunk diseases research in Australia

Ian Pascoe and Eve Cottral

Institute for Horticultural Development, Agriculture Victoria, Knoxfield, Private Bag 15, South Eastern Mail Centre, Victoria 3176, Australia

Summary. In Australia, trunk diseases of grapevines are becoming increasingly important. *Phaeoacremonium chlamydosporum* causes black goo decline and is very common in newly planted vineyards in which establishment is slow and uneven. It is apparently introduced to these vineyards as infections of the propagating material. We have isolated the fungus many times from rootstock mother vines and speculate that it spreads from the trunk of the mother vine to the canes which are later harvested as cuttings. Histological studies of inoculated vine shoots in tissue culture, show that the fungus infects the xylem parenchyma cells as intracellular hyphae, and these cells produce tyloses in the adjacent xylem vessels. The hyphae also penetrate the vessels, often by way of the tyloses. Brown deposits (black goo) are seen in vessels and cells, often some distance from active hyphae. *Phaeoacremonium chlamydosporum* has also been found to produce pycnidia on the surfaces of infected wood when moist incubated. The same pycnidia were also found on the surfaces of inoculated tissue culture vines, proving that they are pycnidia of *P. chlamydosporum*. The spores produced by the pycnidia appear to be conidial rather than spermatial as they germinate readily on agar and develop normal colonies. Esca was formally diagnosed for the first time in Australia in 1999, although it is believed to have been present for many years. In addition to *Phellinus (Fomitiporia) punctatus*, we have also found a second species of *Phellinus* associated with similar symptoms. This species differs from *P. punctatus* in the production of setae on host tissue and in culture. Like *P. punctatus* it has a close association with *Phaeoacremonium chlamydosporum*. Eutypa dieback appears to be increasing in severity in Australian vineyards and may be a major limiting factor in the sustainability of Australian wine production. A national trunk disease project has commenced to develop management strategies for black goo decline, esca, Eutypa dieback and other trunk diseases.

Key words: grapevine, black goo decline, esca, trunk diseases, *Phaeoacremonium*, *Phellinus*.

Introduction

Grapevine trunk diseases represent a new area of interest in grapevine pathology in Australia. We have only just begun to understand the importance of trunk diseases in the long-term sustainability of grape and wine production. Most of our current knowledge of trunk diseases is based on diagnostic experience and laboratory observations, and apart from a few years of research on control of Eutypa dieback, no active research has so far been completed.

Much of our attention in the last few years has been focused on species of *Phaeoacremonium* and their involvement in decline of young vines. So far we have only found *P. chlamydosporum* and *P. aleophilum* in Australian vines. We have found that *P. chlamydosporum* is very common in young vineyards showing poor establishment and there is major concern that the fungus may be carried in propagating material. In Australia the decline associated with *P. chlamydospo-*

Corresponding author: I. Pascoe
Fax: +61 3 9800 3521
E-mail: ian.pascoe@nre.vic.gov.au

rum has become known as "black goo decline".

We frequently isolate *P. aleophilum* from necrotic sectors in vine wood, or from dark pith. However, *P. aleophilum* is much less frequently encountered than *P. chlamydosporum* and does not appear to be associated consistently with black goo symptoms or with severely declining vines.

Esca was not formally recorded in Australia until early in 1999, when we isolated *Phellinus* (*Fomitiporia*) *punctatus* from a vine with black measles fruit symptoms. This is not a new outbreak of esca, however, because we know that symptoms have been seen for many years and herbarium records show that *P. punctatus* has been present in Australia since at least 1890.

In the discussions below, all specimens discussed are lodged in the plant disease herbarium (VPRI) of Agriculture Victoria at Knoxfield, and VPRI numbers are cited accordingly.

Black goo decline

Since 1997, our diagnostic service has isolated *P. chlamydosporum* from almost all areas, with the exception of the granite belt in Queensland, and Tasmania. The fungus has been found in a wide variety of *Vitis vinifera* cultivars and rootstock varieties. However, we have not perceived any difference in varietal susceptibility. The fungus appears to have been present in Australia for many years.

Black goo decline in young vines

Our experience with *P. chlamydosporum* shows that it is very common as an invader of wounds in mature vines, but that it does not cause a serious disease in that context (although it obviously increases susceptibility to other diseases). In one case we have isolated the fungus from five tissue cultured vines (assumed to be clean at the time of planting) that had been planted in the field for 8 years, indicating that infection will eventually occur in initially clean vines planted in an infested area. However, it is as a disease of young vines that black goo decline appears to cause very severe losses in vineyards planted with infected propagating material.

Vines propagated from infected cuttings are slow to establish, or may never show satisfactory growth. Young vines appear to be particularly severely affected if the rootstock is infected prior to grafting. Grafts may fail in severe cases. In other cases the scion may grow better than the stock and become conspicuously larger in diameter. Because the growth of the stock is inhibited, ultimately the vine will not do well. We know of many cases where unsatisfactory establishment of young vines is consistently associated with *P. chlamydosporum* infections. In some of these cases vineyards have lost 50% of vines, and there is evidence that many of the survivors are infected and declining.

If infected vines do grow well initially, they may lose productivity later. We have seen cases of apparently healthy vines which suddenly failed to produce fruit in the 4th or 5th year. Dissection of these vines usually shows a very high level of black goo wood symptoms. It was concluded that the vines were infected but not severely affected at the time of planting, and grew vigorously until the fungus had grown throughout the vine, when perhaps an episode of stress caused a sudden loss of vigour.

We do see variations in the severity of the decline relative to the severity of the internal wood symptom – sometimes the decline might be more severe than the amount of black goo would suggest. On other occasions we see severe black goo symptoms in the wood, but little evidence of decline. There may be other influences on the severity of decline symptoms, including the severity of stress factors or the presence of other disease agents.

Black goo decline in mature vines

Many of our diagnoses have been from mature vines in which the fungus has been associated with randomly scattered black dots in the wood, or with well established crescents of black dots, or with necrotic sectors inside those crescents. Many vines with these symptoms seem healthy, but the same symptoms are seen in vines with varying degrees of decline symptoms. The fungus is also consistently associated with esca wood symptoms.

We have found it easy to visualise the distribution of the fungus in wood by moist incubation of transverse or longitudinal sections of infected wood. In this way it is frequently possible to determine the position of *P. chlamydosporum* relative to black goo symptoms and other fungi such as *P. aleophilum*, *Phellinus* and *Botryosphaeria*. *P. chlamydosporum* seems to grow and sporulate more abun-

Fig. 1. Black goo decline symptoms in trunk of Schwartzmann rootstock mother vine, positive for *Phaeoacremonium chlamydosporum*.

dantly in areas where there is substantial necrosis of wood in and around aggregations of black goo vessels, than on single black goo vessels. In longitudinal sections it can be seen that some black streaks are associated with abundant *Phaeoacremonium*, while others are not. We interpret this to mean that the distribution of the fungus is not continuous through the length of an affected vessel (or group of vessels), but located at a particular point, and that the black goo symptom is therefore capable of appearing at points remote from the actual infection. This may explain our occasional failure to isolate the fungus from typical black goo symptoms.

Black goo decline in rootstock vines

In 1998, we conducted a preliminary survey of rootstock mother vines used by the Australian Vine Improvement Association. Eleven out of 13 black goo symptomatic vines tested positive for *P. chlamydosporum*. None of these vines showed any apparent decline.

Since then we have tested many rootstock mother vines from official source blocks and from nurseries. Almost all have been positive for *P. chlamydosporum* (Fig. 1). In one case 7 samples covering 5 rootstock varieties from a single nursery all tested positive.

Table 1. Presence of *Phaeoacremonium chlamydosporum* in samples of rootstock material tested from 3 states in Australia (VIC=Victoria, SA=South Australia, NSW=New South Wales). Data has been obtained from grapevine material tested by Crop Health Services, Institute for Horticultural Development, Knoxfield, Victoria, Australia.

Rootstock	Locality	No. of vines tested	No. of samples tested positive for *P. chlamydosporum*
Ramsey	Irymple (VIC)	27	27/27
Schwartzmann	Rowlands Flat (SA)	2	2/2
140 Ruggeri	Rowlands Flat (SA)	1	1/1
1103 Paulsen	Mildura (VIC)	1	1/1
5A Teleki	Mildura (VIC)	2	2/2
Schwartzmann	Mildura (VIC)	1	1/1
140 Ruggeri	Mildura (VIC)	1	1/1
101-14	Mildura (VIC)	1	1/1
Ramsey	Mildura (VIC)	1	1/1
Schwartzmann	Irymple (VIC)	1	1/1
K51-52	Irymple (VIC)	1	1/1
5C Teleki	Irymple (VIC)	1	1/1
K51-40	Irymple (VIC)	1	1/1
Harmony	Irymple (VIC)	1	1/1
5BB Kober	Irymple (VIC)	1	1/1
1103 Paulsen	Irymple (VIC)	1	1/1
99 Richter 2-10-285	Irymple (VIC)	1	1/1
110 Richter	Irymple (VIC)	1	1/1
Ramsey	Irymple (VIC)	1	1/1
140 Ruggeri	Gol Gol (NSW)	2	0/2

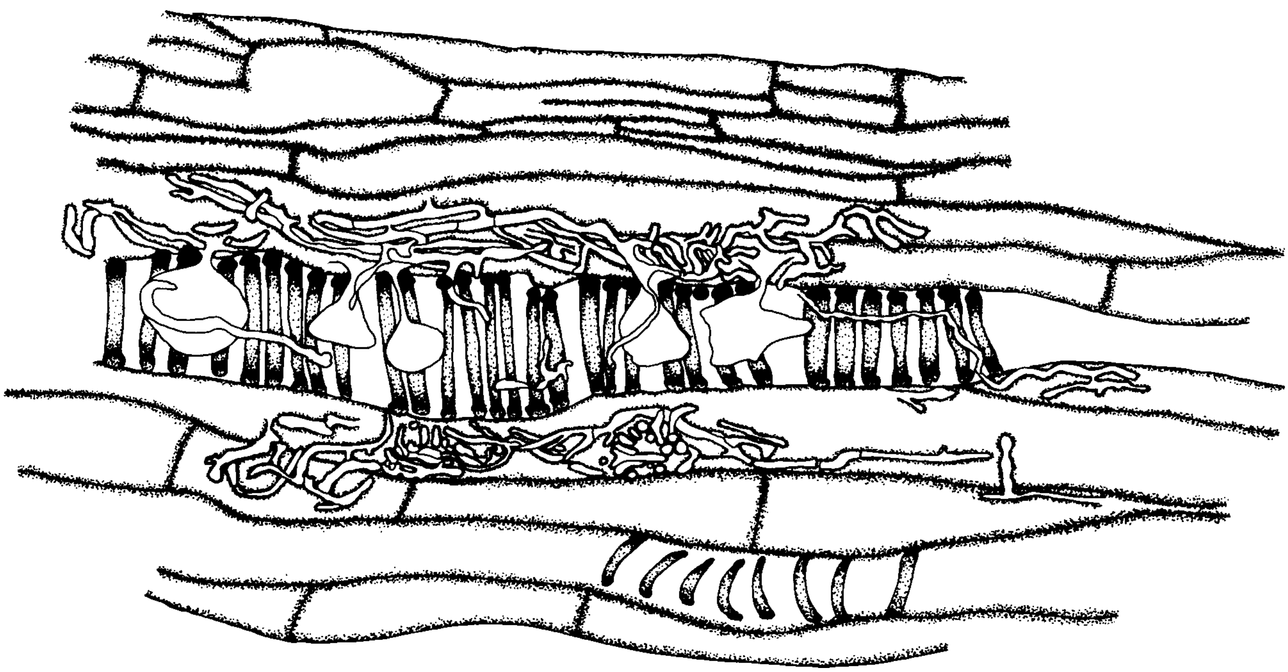

Fig. 2. *Phaeoacremonium chlamydosporum* - mycelium and tyloses in xylem parenchyma cells and vessels of artificially inoculated tissue cultured Chardonnay plantlet.

It seems reasonable to assume that almost all rootstock mother vines are infected. There is no apparent difference in varietal susceptibility because almost all of the common rootstock cultivars have tested positive to *P. chlamydosporum* (Table 1). We have also found the fungus in *V. vinifera* source material, suggesting that the source of the infection in newly planted vines may be either stock or scion.

Histopathology of *Phaeoacremonium chlamydosporum* infections in vine wood

In order to get some idea of the infection process in the absence of other fungi, and of the morphology of hyphae (and hopefully sporulating structures) in the vessels, we chose initially to inoculate tiny tissue cultured shoots (about 1 mm in diameter) of *Vitis vinifera* cv. Chardonnay. We were then able to study the histopathology of *P. chlamydosporum* in an otherwise sterile vine.

We did this by cutting off a single leaf from each shoot and inoculating the wound stub with a suspension of *P. chlamydosporum* conidia (VPRI 22079). At three and six weeks respectively, shoots were harvested and sectioned for microscopic study.

Initially the fungus was only found in the xylem parenchyma cells adjacent to vessels in the inoculated area (Fig. 2). Hyphae were intracellular and densely packed the interiors of infected cells. These cells could be seen producing tyloses which intruded into the vessel lumen. There was frequently a hypha entering the vessel at the point of intrusion of the tylosis, but it is not known whether the tylosis or the hypha came first.

Later, the hyphae were found more widely spread through the stem, in parenchyma, cortical and pith cells remote from the point of inoculation. Hyphae were readily seen travelling along the inside of the vessels, but we have not yet seen any evidence of spore production in vessels. Brown deposits in the vessels and cells (assumed to be accumulations of phenolic compounds, possibly representing early development of "black goo" symptoms) appeared in vessels and other tissues, but not always close to heavy concentrations of hyphae. It appears that the "goo" may be produced at some distance from infected cells, and this suggests that a toxin may be involved and also explains why we are sometimes unable to isolate *P.*

chlamydosporum from perfectly typical symptoms.

Although we have not yet been able to prove it, we still believe that the fungus may spread into canes by movement of spores produced in vessels. A better understanding of the morphology of the fungus under different conditions may help us to know what to look for.

Morphology of *Phaeoacremonium chlamydosporum*

Morphology in culture

We know *P. chlamydosporum* mostly as a fungus in culture. All of the existing descriptions and illustrations of the fungus are based on its appearance in the aerial mycelium on rich media.

The aerial mycelium is pigmented green and the conidiophores are produced on these green hyphae, and are characteristically bicoloured, with a thick walled pigmented conidiophore and an always hyaline phialide (may become pigmented when very old).

Vertical sections of cultures show that only the aerial hyphae are pigmented - below the surface the hyphae are completely hyaline (Fig. 3). Large numbers of bacilliform conidia are produced below the agar surface, and these are produced from less distinctive, hyaline conidiophores. The conidiogenous cells are acropleurogenous (with conidia produced from phialidic conidiogenous loci below the septa of a multiseptate conidiophore), or they are produced as simple lateral conidiogenous loci (adelophialides), or simple, short phialides. These are what we might expect to see in vessels, if the fungus sporulates there.

There has been some speculation about the role of chlamydospores of *P. chlamydosporum* in survival of the fungus in soil. However, we doubt if the chlamydospores of *P. chlamydosporum* really are chlamydospores in the strict sense and we doubt if they really have a survival function. This is because they are produced on rich media such as malt extract agar (MEA) (most fungi produce chlamydospores more abundantly on weak media). By the time these assumed chlamydospores become pigmented, many appear empty and thin-walled. They are very rare on potato dextrose agar (PDA) where they appear more like hyphal swellings, and they are not produced on water agar (WA).

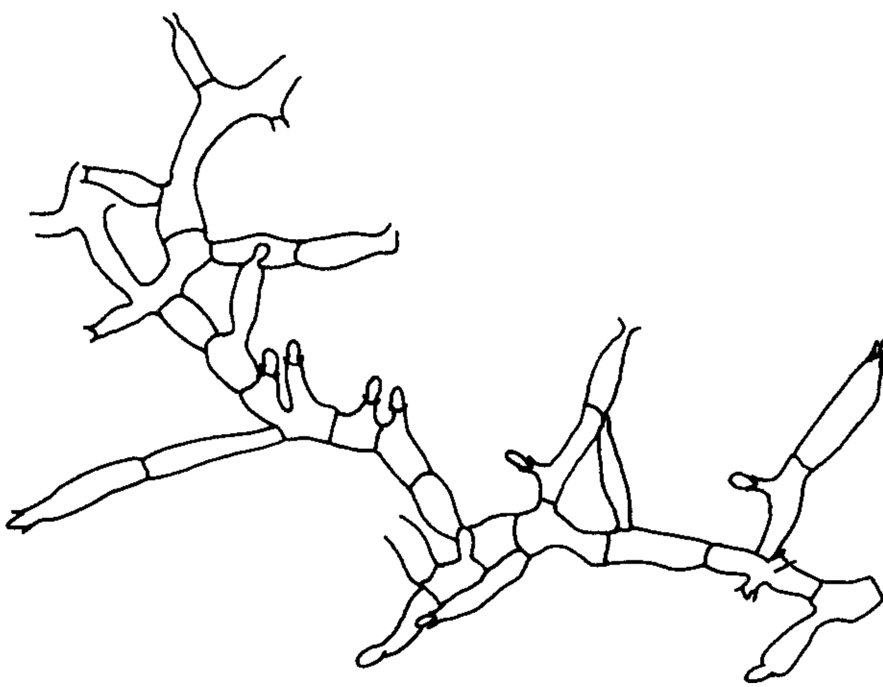

Fig. 3. *Phaeoacremonium chlamydosporum* – sub-surface hyphae and conidiogenous cells from below the surface of PDA culture (VPRI 21811a)

Fig. 4. *Phaeoacremonium chlamydosporum* – pycnidia produced on surface of naturally infected, moist incubated wood (VPRI 22081b).

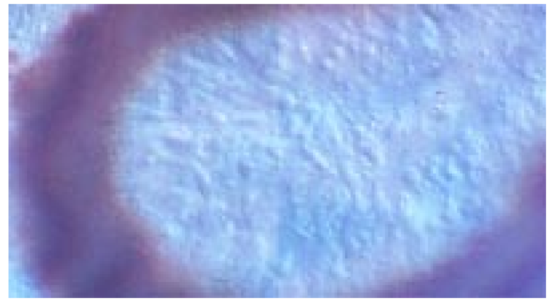

Fig. 5. *Phaeoacremonium chlamydosporum* – longitudinal section of pycnidium produced on moist incubated wood. (VPRI 22081b).

Morphology on moist incubated vine wood - presence of pycnidia

In moist incubations of wood *P. chlamydosporum* sporulates abundantly and produces conidiophores in the aerial mycelium which are identical to those produced in culture. However, over time we have also observed the production of small black pycnidia associated with sporulating mycelium of *P. chlamydosporum* (Fig. 4). These pycnidia first appear about 3-4 weeks after the commencement of moist incubation, often at the margins of active sporulation of the hyphomycete state, and frequently on the outer bark of the trunk where the necrotic wood reaches the outer perimeter of the trunk (VPRI 22081b). They are initially subglobose to pyriform, but gradually become "ten-pin" shaped with age. They contain many small ellipsoid to bacilliform spores (4-4.2 µm x 1.5-2 µm) which are produced from an unusual network of acropleurogenous phialidic conidiogenous cells (Fig. 5, 6).

We initially speculated that the pycnidia might be spermatial in function. Consequently we expected that we might soon discover a teleomorph. However, culturing of spore suspensions from the spores produced by the pycnidia, consistently produced high proportions of germinating spores and these spores produced typical colonies of *P. chlamydosporum*. The spores are thus conidial in function and the pycnidia may be regarded as a pycnidial synanamorph of *P. chlamydosporum*. The pycnidia have not been aligned with any known coelomycete genus, although candidate genera include *Dendrophoma* and related genera.

To further prove that these fruiting bodies are pycnidia of *P. chlamydosporum*, we have observed

Fig. 6. *Phaeoacremonium chlamydosporum* – conidiogenous cells from squash preparation of pycnidium (VPRI 22081b).

them on the surfaces of our inoculated tissue cultured plants.

Esca in Australia

Esca was not formally recorded in Australia until early in 1999, when we isolated *Phellinus* (*Fomitiporia*) *punctatus* from a vine with black measles fruit symptoms (Pascoe, 1999) (culture lodged as VPRI 22080). This is not a new outbreak of esca, however, because we know that symptoms have been seen for many years and herbarium records show that *P. punctatus* has been present in Australia since at least 1890.

Fig. 7. *Phellinus* sp. (non *P. punctatus*) isolated from esca-like heart rot – setae produced in MEA culture (VPRI 22081a).

Since this first specimen, we have found esca wood symptoms to be common in mature vines with slow decline symptoms, but there are frequently not associated leaf symptoms. We have examined many vines with white heart rots in trunk tissue, and isolated *Phellinus* every time.

To our surprise, about half of these isolations are not *P. punctatus* but another, setose species (VPRI 22174, 22173 & 22081a). This species produces abundant, long brown setae in culture (Fig. 7) and on moist incubated wood, in contrast to *P. punctatus* which does not produce setae either in basidiomata or in culture. The setose species is associated with the same type of white heart rot as *P. punctatus*, and is associated with decline in at least some cases, but we have not been able to confirm that it causes the same leaf and fruit symptoms. The fungus is intimately associated with *P. chlamydosporum* and the white

Fig. 8. Esca-like wood rot symptoms caused by *Phellinus* sp. (setose) and *Phaeoacremonium chlamydosporum* (VPRI 22081).

heart rot is always surrounded by a black line which indicates the presence of *P. chlamydosporum* (Fig. 8). This is easily seen in moist incubations of wood cross sections where mycelium of both *Phellinus* and *Phaeoacremonium* can be seen growing close together, and *P. chlamydosporum* can be seen to be consistently associated with the dark lines around the margins of the *Phellinus* rot. We can not be sure at this stage that it is a single species, and we have to accept that a range of *Phellinus*/*Fomitiporia* species may be involved in esca.

Although esca is not currently a major disease in Australia, we expect that with the large number of newly planted vines in Australia which are infected with *P. chlamydosporum*, we can expect a great increase in the incidence and severity of esca in the next 10-20 years.

Eutypa dieback in Australia

Eutypa dieback is emerging as one of the major causes of decline and death of mature vines in Australia. A recent survey by Highet & Wicks (1998) in South Australia found (based on symptoms) that 8% of mature 'Shiraz' vines in South Australia were affected. In some vineyards up to 40% of vines were symptomatic. We believe that Eutypa dieback will be the major constraint on the sustainability of wine production in Australia in the next decade.

Current research in Eutypa dieback is concentrated on development of biological and chemical controls, but new research will also address the epidemiology of the disease.

The outlook for trunk diseases in Australia

We expect trunk diseases to increase greatly in importance over the next 10-20 years as the incidence of Eutypa dieback and esca increases, encouraged by the high level of *P. chlamydosporum* in young vines.

A major national trunk disease research project has commenced, with funding from the Cooperative Research Centre for Viticulture and the Grape & Wine Research & Development Corporation.

The project team includes Ian Pascoe, Jacky Edwards, Bob Emmett and DeAnn Glenn (Agriculture Victoria), Trevor Wicks and Mette Creaser (South Australian Research and Development Institute), Peter Taylor and Helen Waite (University of Melbourne), Mary Cole (Monash University), Eileen Scott (University of Adelaide), and two new PhD students, Eve Cottral (University of Melbourne) and Richard Lardner (University of Adelaide).

We welcome collaborators from the northern hemisphere because we recognise that by collaborating with northern hemisphere workers we can get two sets of results in one year and dramatically increase the speed with which we can deliver management strategies to the wine industry.

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