

## Root diseases of grapevines in California and their control

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**Abstract.** Root rots of grapevine in California are caused by several soilborne pathogens. While root rot in vineyards in general has been considered to be a relative minor problem, nearly all root diseases have increased in incidence and severity since the introduction of rootstocks resistant to Phylloxera. Pathogens such as *Phytophthora* spp. and *Armillaria mellea* have been known to occur for many years. However, seldom did root rot become a serious problem in vineyards. New diseases such as Petri disease caused by *Phaeoconiella chlamydospora*, vine decline caused by *Phaeoacremonium* spp. and black foot caused by *Cylindrocarpon destructans* have only recently been shown to occur in California. Wilt caused by *Verticillium dahliae* has become more prevalent in nearly all grape growing regions in California. Though losses due to actual root disease have been minor over the past 10 years, losses due to vineyard reestablishment have been large.

### Introduction

Root rots of grapevine in California have become more of an issue in recent years. Thirty-five years ago, most of our vineyards were planted on their own roots or were planted on AXR1 rootstock. Vines on AXR1 performed very well in terms of tolerance to major diseases. Phylloxera became prevalent in Californian vineyards in the 1980s but still our vineyards stood up to this debilitating insect. However, with the emergence of Phylloxera biotype B, our vineyards, especially on AXR1 and own-rooted, began showing the ravages of the insect. A search for rootstock that would stand up to biotype B resulted in many new rootstocks being introduced to Californian vineyards. While much research was done on these rootstocks in regard to culture, quality, vigour etc., little work was done in assessing the material for resistance to soilborne pathogens.

While a relatively large number of rootstocks were evaluated in California, the most popular were those that resulted in some devigourating of the vine. On the north coast of California, large-scale replanting of vineyards on new rootstocks began in the early 1990s. Initially, rootstocks such as SO4, 5BB, 5C, 420A and 110R (all crosses of *Vitis berlandieri* × *V. riparia*) were used quite extensively. Later, 3309 and 101-14 (*V. riparia* × *V. rupestris*) and 1103 Paulsen

(*V. berlandieri* × *V. rupestris*) were planted rather extensively. Within a few years, many of the vineyards replanted with these rootstocks began showing signs of plant decline and subsequent death. A severe winter in 1991 injured many vines and much of the vine decline was attributed to this injury. Even the typical symptom of winter injury, a blackened cambium, was present on much of the declining material. However, when symptomatic plants were assessed for pathogen presence, we discovered that a group of fungi, later to be identified as *Phaeoacremonium* spp. and *Phaeoconiella chlamydospora*, were consistently present in the affected vascular tissues. In the 2 years prior to this, we had identified two of these pathogens (*Pm. aleophilum* and *Pa. chlamydospora*) in vines showing symptoms of esca (black measles). Grapevine inoculations of 1 year old rooted cuttings with these pathogens in greenhouse tests had reproduced some of the internal symptoms of dark vascular streaking, presence of gums and tyloses, and extreme drying of the vascular tissue. Symptoms produced in the greenhouse were identical to those observed on young declining grapevines in the vineyards replanted on Phylloxera resistant rootstocks. The diseases caused by *Phaeoacremonium* spp. were called vine decline while the disease caused by *Pa. chlamydospora* was named Petri disease. *Phytophthora*

spp. and *Cylindrocarpon destructans* were also present in some of the samples assessed for pathogens. When these two pathogens were present, the vines showed a more rapid death and symptoms were different, especially for Phytophthora root rot. *Phytophthora*-infected plants typically showed more extensive root rot, which was chocolate-brown and moist, and *Cylindrocarpon destructans*-infected roots typically showed small black lesions scattered on the surface of roots. As the latter pathogen further invaded, the tissues blackened and exhibited a dry cortical rot, which rapidly moved into the butt of the vine causing rapid dieback and death (black foot).

It had become apparent that vine decline, black foot and Petri disease were being expressed preferentially on the rootstocks planted for Phylloxera control. Though nurseries producing grapevine plants were receiving all the blame for spreading the new diseases to various production areas, there appeared to be something else involved in the decline. In every case where Petri disease and Phaeoacremonium vine decline occurred, plants were sampled and found to be showing severe signs of water deficit stress, 'J' rooting or had been fruited heavily in the 2 and 3 years after planting. Root systems of plants showing decline symptoms had not grown much and for the most part were still contained within the plastic or cartons in which they were delivered to the grower, even though a good many had been planted up to 5 years earlier. Although our lab continued to isolate *Cylindrocarpon destructans*, *Pa. chlamydospora* and *Phaeoacremonium* spp., our tests showed that these pathogens only occurred at low incidences in grapevines in Californian nurseries. The results of our vineyard and nursery surveys and lab isolations together with previous work on esca (W. D. Gubler, unpublished data), indicate that inoculum of the pathogens causing black foot, Petri disease and vine decline typically comes from nurseries as well as old vineyard replant sites.

Current and future grape rootstock requirements present important challenges to grape breeders, nursery staff and growers. Improved rootstock resistance to Phylloxera is needed but, in addition, rootstocks should possess desirable horticultural traits and adequate resistance to other soilborne pests such as parasitic nematodes and fungi. The resistance to soilborne pests may become increasingly important due to the scheduled phase out of methyl bromide fumigation.

### **Esca (black measles) and Petri disease**

Esca (black measles) and Petri disease are two of the most destructive diseases in many grape production countries. Esca-diseased vines may show both external and internal symptoms (Mugnai *et al.* 1999). Esca is primarily a disease of mature grapevines. Symptoms can appear in severe or mild forms (Viala 1926; Dubos and Larignon 1988; Gubler and Schnathorst 1992; Scheck *et al.* 1998a). This disease is suspected of being caused by at least three or more fungi in California: *Phaeoacremonium chlamydospora*, *Phaeoacremonium*

*aleophilum*, as well as other species of *Phaeoacremonium* (not discussed here) acting in combination or in succession. They attack the roots, pith and xylem of the plant killing the cambium and cortical tissues. The mild form of esca is characterised by foliage deterioration or fruit spotting. In other cases, infected grape clusters appear normal, but berries may not fill properly and generally do not reach maturity. The severe form of the disease, called apoplexy, is characterised by the sudden wilting and death of bearing vines or cordons in midsummer. Although the pathogens causing esca are the same as those causing Petri disease, esca results primarily from pruning wound infections, and Petri disease results from root infections. Root infections of mature vines do occur but infections remain compartmentalised in the roots and it is doubtful that the infection plays a major role in adult vine esca.

The disease known as Petri Disease (syn. young esca, young vine decline, black goo and black goo decline) has emerged as a significant problem in vineyard establishment. The disease affects grapevines during the first 10 years of establishment and is not specific to any scion/rootstock combinations, though we suspect that the disease is more severe on certain rootstocks. This disease has been diagnosed in many countries around the world (Chicau *et al.* 2000; Larignon and Dubos 1997; Scheck *et al.* 1998a; Mugnai *et al.* 1999; Crous and Gams 2000). In California, *Pa. chlamydospora* and *Phaeoacremonium* spp. have been isolated from 'healthy' vines and have been recovered from inoculated asymptomatic vines as well (Scheck *et al.* 1998a). In Italy, *Pa. chlamydospora* has been isolated from healthy rooted cuttings (Bertelli *et al.* 1998). Researchers around the world now view these fungi as endophytes that may become more virulent under conditions of plant stress (Scheck *et al.* 1998a; Groenwald *et al.* 2001). In this young phase of the esca, it is known that root infection plays a major role in disease expression. Root symptoms include small dark lesions on feeder roots and small lateral roots. Direct penetration of the root epidermis has been shown to occur (Gubler and Feliciano, unpublished data) and the pathogens penetrate into the cortical tissue and subsequently into the vascular tissue. The fungi also have been shown to move from inoculated pith at the butt of the vine into the vascular tissue through parenchyma cells (Gubler and Feliciano, unpublished data). *Pm. aleophilum* produced a brown necrosis of soft texture located in sectors in the vascular tissue of rootstocks. *Pa. chlamydospora* caused dark necrosis in young rootstocks similar to discoloured wood in vineyard vines as described by Larignon and Dubos (1997).

It is likely that the recent high incidence of young vine decline and Petri disease in California has resulted from planting and replanting of large vineyard acreages under circumstances that favour the disease, rather than from recent introduction or spread of the pathogens. We know that

these pathogens of young vines have been present for many years because these same fungi have been known to occur in mature grapevines since 1912 in Italy (Petri 1912) and 1964 in California (Chirappa 1964). However, the planting and replanting of large acreages in California due to *Phylloxera* infestations has increased both the awareness and the magnitude of the problem.

#### Rootstock susceptibility

Inoculation with *Pa. chlamydospora* showed that the rootstocks 3309, 420A, 110R, 5C, Schwarzman, St George and Salt Creek were the least susceptible while 99R 039-16, Freedom, P. Glore, 140Ru, 16-16 and 1103 were the most susceptible (Eskalen *et al.* 2001a). Inoculation with *Pm. aleophilum* showed that AXR1, 1103, 420A, Harmony and Salt Creek were the least susceptible while 110R, SO4, 039-16 and 161-49 were the most susceptible (Eskalen *et al.* 2001a).

#### Presence in soil

Of the *Phaeoacremonium* spp. associated with grapevines, only *Pm. aleophilum* has been isolated from soil or standing water under drip emitters using selective media (Rooney *et al.* 2001). Of the 11 counties in which vineyard soils were tested, four samples in three counties tested positive for *Pm. aleophilum*. One of these soils was from a Contra Costa County vineyard in which vines showed severe stunting and decline symptoms. Isolations from vines in this vineyard showed both *Phaeoacremonium* spp. and *Pa. chlamydospora* to be present in the grapevines. The other location, also in Contra Costa County, was a vineyard showing overall stunted growth. However, the only pathogens previously recovered from these vines were viral in nature. Therefore two sites in Contra Costa County, one with a history of *Phaeoacremonium* spp. and *Pa. chlamydospora*, the other without, both yielded populations of *Pm. aleophilum* in the soil. In Sonoma County, *Pm. aleophilum* was also recovered from a vineyard soil where declining vines, known to have a history of *Pa. chlamydospora* and *Pm. aleophilum* were present.

Surprisingly, the standing water samples that were taken from beneath symptomatic esca vines in Kern County also contained high populations of *Pm. aleophilum*. Water taken from irrigation tubes before they reached the ground tested negative for *Pm. aleophilum*, indicating inoculum probably originated in the soil or grapevine debris rather than the water source. Different water puddles were tested during separate visits to the vineyard with positive results.

*Pa. chlamydospora* was not recovered from any of the soils tested in our studies but *Pa. chlamydospora* was found to occur in vineyard soil in New Zealand, based on PCR tests (Whiteman *et al.* 2002). Previously, our laboratory showed that this species could survive for 5 months in sterile soil. Of the 11 counties surveyed, three of the counties had positive

isolations for *Pa. chlamydospora*, either from bark or plant sap.

The importance of *Pm. aleophilum* as a soilborne pathogen in California vineyards has been documented (Scheck *et al.* 1998a). The successful recovery of *Pm. aleophilum* from soil and from standing water in vineyards using selective media and subsequent positive confirmation of *Pm. aleophilum* from soil using PCR tests (Eskalen *et al.* 2001b) illustrates the diverse nature of these fungi in grapevines and vineyards.

#### Epidemiology

The presence of *Pa. chlamydospora* in sap exuding from girdling wounds and the presence of this pathogen on exfoliating bark also raises some serious new questions about the epidemiology of this fungus. Release of *Pa. chlamydospora* spores from pycnidia has been documented in a number of locations in California and the susceptibility of pruning wounds has been documented; thus we are certain that release of spores with rainfall during the pruning season is one source of new infections each year (Eskalen and Gubler 2001; Gubler *et al.* 2001; Eskalen *et al.* 2003). The teleomorph of *Pm. aleophilum* (*Togninia minima*) has also been produced *in vitro* on exposed vascular tissue (Rooney *et al.* 2002). The release of ascospores is not necessarily related to rainfall, although ascospore release has been documented to occur in spring with rainfall. More commonly, *T. minima* spores are trapped in late spring and up to mid-summer in the absence of rainfall. The mucoid mass of spores at the tip of the ostiole appears to be suited for insect transmission, although this has not yet been proven.

#### Control

Control of both vine decline caused by *Pm. aleophilum* and Petri disease caused by *Pa. chlamydospora* can best be achieved by preventing plant stress. In one study, it was shown that when cuttings of grapevines were vacuum infiltrated with  $9 \times 10^6$  spores then rooted and grown in the greenhouse, there were no differences in rate or amount of growth between inoculated and non-inoculated plants. However, when the inoculated plants used in the study above were cut back then exposed to differential irrigation regimes, those exposed to water stress exhibited symptoms of dieback and decline in 30 days, while the those maintained with regular irrigation did not show symptoms. Studies examining the effect of 'J' rooting and early fruiting are currently underway. Vineyard observations have led us to believe that these two phenomena (water stress and 'J' rooting) are partially responsible for vine decline symptoms in California. These factors coupled with restricted size and vigour of some of the new rootstocks may contribute to devigourate vines, leading to slowed growth and subsequent decline. Rootstock resistance might afford some degree of

control of vine decline and Petri disease but resistance to one pathogen was not associated with resistance to the other pathogens in our tests. Furthermore, rootstock selection is determined by response to Phylloxera and horticultural traits.

### Black foot

Since 1995, black foot caused by *Cylindrocarpon destructans* has become the most serious disease of young grapevines in the north coast production area of California (Scheck *et al.* 1998b). The pathogen is soilborne and causes severe losses when grapevines are grown on susceptible rootstocks in heavy, poorly drained soils. The pathogen invades roots of grapevines and quickly moves into the butt of the rootstock. The vascular tissue turns black and the xylem becomes occluded with fungal tissue, gums and tyloses. When young vines are attacked, they die very quickly but as the vines age, infection results in a more gradual decline and death may take more than 1 year to occur. However, death seems to be inevitable when vines less than 10 years of age are infected with *C. destructans* and we have observed vines up to 10 years of age succumb to the disease. Black foot is most prevalent on the north coast of California. Though usually affecting only small areas of vineyards, the disease can cause more severe losses when the overall soil type in the vineyard is heavy and wet. There is evidence that this disease also has a link with nursery production but presence of the pathogen in vineyards probably plays a larger role in disease. Many studies have assessed disease levels as vines are delivered to the grower. In California, we have observed up to 5% of vines from some nursery lots carrying the pathogen and showing vascular symptoms. However, researchers in Portugal have confirmed that, in one study, the amount of disease on plants as they left the nursery was 40%. Obviously this level is too high and it would be disastrous for growers to plant such a lot of infected plants.

### Control

Control of black foot can be achieved by planting clean stock and by not planting in heavy poorly drained soils. Drainage in heavy soils can be achieved by planting on berms and moving drip irrigation emitters away from the vine. In regard to rootstock, *Vitis riparia* O39-16 and Freedom have a good degree of resistance to *C. destructans* (Petit and Gubler, unpublished data). Recent research has also shown that use of the mycorrhizal fungus *Glomus intraradices*, in advance of inoculation, resulted in excellent control of black foot (Petit and Gubler, unpublished data).

### Armillaria root rot

Armillaria root rot is an often, fatal disease of grapevines in California, the southeastern USA, Europe, South America and Australia. *Armillaria mellea*, the causal fungus in the

northern hemisphere, infects grapevine roots, killing the cambium and decaying underlying xylem. *A. mellea* has a broad host range, infecting over 500 species of woody plants. The common occurrence of Armillaria root rot on grapevines and orchard trees planted on sites previously inhabited by *Quercus* species (oaks) in California has been known since the disease was first described in the 1880s. Several *Armillaria* species are native to California, but only *A. mellea* kills grapevines.

Reports of Armillaria root rot on grapevines outside California include descriptions of identical symptoms, but sometimes identify different *Armillaria* species. In the southeastern USA and Europe, grapevine infections are most frequently associated with *A. mellea*. *A. tabescens*, another virulent *Armillaria* species reported from orchard trees in both the southeastern USA and Europe, may also infect grapevines. Unresolved taxonomy of southern hemisphere *Armillaria* species complicates reports of Armillaria root rot on grapevines from Australia and South America. In Australia, Armillaria root rot of grapevine is caused by *A. luteobubalina* and possibly, *A. novae-zealandiae*. Based on current knowledge of *Armillaria* species in the southern hemisphere, *A. novae-zealandiae* is likely to be the causal species in South America.

*Armillaria* species differ slightly in their host range and fruiting body morphology, but they share the same life cycle and infection cycle. Regardless of the *Armillaria* species involved, symptoms, disease development, epidemiology and control are all similar.

### Symptoms

Armillaria root rot symptoms include stunted shoots, dwarfed leaves, wilting, premature defoliation and raisining of berries. The most apparent course of symptom development (rapid desiccation of all leaves and berries followed by grapevine death) occurs mainly between veraison and harvest on apparently healthy, vigorous grapevines and on grapevines with noticeably stunted shoots. Grapevines with severely stunted shoots that do not succumb to Armillaria root rot before harvest usually die during the dormant season. Armillaria root rot occurs in circular patches in a vineyard (disease centres) that expand radially over time, which is due to the localised distribution of primary inoculum (infected roots from trees that previously inhabited the site) and the spread of *Armillaria* between neighbouring grapevines.

*A. mellea* produces mushroom fruiting bodies that are light yellowish-brown in colour, resulting in their common name 'honey mushrooms'. Cap diameter ranges in size from ~3–28 cm. Caps are light yellowish-brown with a slightly darker centre. Scales are sometimes present on the cap. Gills are pale yellow to pink. Stipe size ranges from 5–20 cm long and 0.5–3 cm thick. Stipes are pale yellow to pink at the top and light yellowish-brown at the base. The stipe usually has

a fleshy pale yellow to pink annulus. Clusters of *A. mellea* may contain up to 50 mushrooms. The hyaline, cylindrical basidiospores are white en masse and are sometimes deposited by the tallest mushrooms onto the top of the caps of shorter mushrooms. Mushroom size, colour and the number of mushrooms per cluster are highly variable.

Other *Armillaria* species associated with Armillaria root rot on grapevines outside California include *A. luteobubalina*, *A. novae-zealandiae* and *A. tabescens*. Their mushrooms are very similar to that of *A. mellea* in size, shape and colour, with the exception of *A. tabescens* which has no annulus. *Armillaria* species mushrooms mainly differ in sizes of subtle microscopic features and are best identified using mating tests in Petri plates or with the aid of molecular identification techniques.

For identification purposes, belowground signs of *Armillaria* are much more diagnostic than aboveground symptoms. *Armillaria* forms thick, white mats of vegetative fungal tissue (mycelial fans) beneath the bark of infected roots. The most convenient part of the root system to sample for mycelial fans is the base of the trunk, starting at several centimetres below the soil line and extending down to the bottom of the trunk where main roots originate (the root collar). In a disease centre, mycelial fans are often found on the root collars of dead and dying grapevines. Apparently-healthy grapevines and grapevines with slightly stunted shoots may not have mycelial fans at their root collars, although *Armillaria* may exist on deeper parts of their root systems.

*Armillaria* also forms rhizomorphs, which are black, shoestring-like structures that contain mycelium. Rhizomorphs are occasionally found within the root bark covering mycelial fans. They grow out from infected roots and through the soil to colonize additional roots. Rhizomorphs may grow on infected grapevine roots, but they are not thought to be a significant means of spread, due to their infrequent occurrence.

*Armillaria* is easily cultured from mycelial fans, decayed wood, surface-sterilised rhizomorphs, or the stipes of mushrooms on water agar amended with benomyl and streptomycin. *Armillaria* mycelium cultured from these sources is unique among other basidiomycete fungi in that it is diploid and has no clamp connections.

*Armillaria* may be present on a site before a vineyard is established. It can survive as a saprobe on woody host roots long after the host dies. Its mycelium decomposes cellulose, hemicellulose and lignin for nutrients as it grows, thereby decaying the root wood. After land is cleared of diseased forest trees or orchard trees, any infected roots that remain belowground serve as a source of inoculum for grapevines planted in place of the trees. *Armillaria* can attack any woody part of a grapevine's root system. Infection occurs when grapevine roots come in direct contact with partially decayed tree roots and they are colonised by *Armillaria* mycelium.

Infection can also occur when grapevine roots contact *Armillaria* rhizomorphs that grow out from partially decayed roots. Once infected, grapevine roots serve as a source of inoculum for neighbouring grapevines whether they are living or dead. *Armillaria* mushrooms release wind-blown spores, but spores are not a significant means of spread of *Armillaria* root rot.

Regardless of how a grapevine becomes infected, disease development is the same. Once *Armillaria* meets a root, it secretes lytic enzymes that dissolve root bark. Below the root bark, *Armillaria* colonises and kills a small section of cambial tissue, then forms a mycelial fan. The mycelial fan expands beneath the root bark, colonising and killing more cambial tissue. After the cambial tissue is destroyed, *Armillaria* decays the underlying root wood.

Grapevines succumb to *Armillaria* root rot when the remaining, functional vascular tissue in the roots can no longer accommodate the demand for water from the shoots. This occurs when mycelial fans surround the root collar and ~50% of the main roots. Foliar symptoms soon follow. The presence of a mycelial fan at the root collar signifies that *Armillaria* has destroyed some of the underlying cambium and is in the process of girdling the host's trunk.

Severity of *Armillaria* root rot is exacerbated by wet soil conditions. In Mediterranean climates, the relationship between frequent, shallow irrigation during the dry season and *Armillaria* root rot on street trees and orchard trees has been documented. The mechanism of this relationship is not known. Possible mechanisms include decreased host defence response of roots in water-logged soil and/or a more favourable soil environment for growth of mycelium between roots that are in direct contact.

### Control

The best way to avoid *Armillaria* root rot is to plant on land with no *Armillaria*. When clearing an orchard or vineyard, look for foliar symptoms (most obvious just before harvest) and disease centres. Examine the root collars of dead and dying plants in disease centres for mycelial fans. Unfortunately, *Armillaria* is harder to detect on forest trees because aboveground symptoms are rare and mycelial fans and mushrooms, when encountered, are also made by all native *Armillaria* species and look identical.

The most effective control of *Armillaria* root rot is pre-plant removal of partially decayed tree roots. Whether *Armillaria* is positively identified on a site or not, thorough land-clearing is recommended. Absence of symptoms and mycelial fans does not ensure absence of *Armillaria*. Infections below the root collar may escape detection. Deep-ripping of the soil in several directions after clearing tree trunks and stumps will bring large roots to the surface where they can be collected and removed from the site or burned. Ability of *Armillaria* to survive in partially decayed tree roots depends on several factors, including root

diameter, soil temperature and moisture, and the presence of other fungi in the soil. The smaller the root diameter and the closer it is to the soil surface, where soil is warmer and drier, the lower the probability of *Armillaria* survival. In hot and dry soil, *Armillaria* mycelium within partially decayed roots is either killed directly or weakened, encouraging parasitism of *Armillaria* mycelium by soilborne fungi like *Trichoderma*. Soil solarisation may achieve high soil temperatures faster, but its beneficial effects may be restricted to small diameter roots at a shallow depth.

Pre-plant soil fumigation with methyl bromide or treatment with sodium tetrathiocarbonate, a liquid that breaks down into carbon disulfide gas, may improve control of *Armillaria* root rot. Pre-plant soil fumigation is effective as long as the fumigant reaches the majority of partially decayed tree roots. Efficacy of methyl bromide is increased by fumigating during the dry season and tarping after fumigation. Soil can be further dried by planting a vigorous cover crop, such as *Sorghum vulgare* (Sudan grass), several months before the dry season begins. There are few available post-plant control treatments for *Armillaria* root rot. One option is to remove dead grapevines and chemically treat the soil with methyl bromide or sodium tetrathiocarbonate on a spot basis and/or mechanically remove buried partially decayed tree roots before replanting. Few therapeutic treatments are available for control of *Armillaria* root rot on infected grapevines. The success of a therapeutic treatment depends on how much damage a grapevine has already suffered, in addition to the therapeutic treatment's ability to decrease further colonization of healthy root tissue by *Armillaria*.

Cultural controls that decrease soil moisture at the root collar may help prevent *Armillaria* from girdling an infected grapevine's trunk. These include moving drip-line emitters away from the base of the trunk and excavating the root collar. Moving drip-line emitters away from the base of the trunk moves the frequently irrigated area of soil directly beneath the emitter away from the root collar. Root collar excavation involves permanent removal of soil from the base of the grapevine, in an effort to protect this important part of the root system from colonization by *Armillaria*. Root collar excavation may cause a mycelial fan at the root collar to recede before it starts to decay underlying wood. This technique has been shown to increase yields of moderately symptomatic grapevines. The mechanism of this method's success is unclear. Root collar excavation may have several effects (i) keep root collar bark dry and offset the influence of excessive moisture, (ii) prevent initial root collar colonisation by mycelial fans and restrict infections to peripheral roots, and (iii) allow infected tissues to recover. Root collar excavation is unlikely to eradicate an *Armillaria* infection. However, it may allow an infected grapevine to tolerate *Armillaria* root rot, thereby keeping the grapevine in production.

## **Phytophthora and Pythium root and crown rots**

### *Symptoms*

The symptoms of disease caused by *Phytophthora* and *Pythium* spp. include grapevine stunting, decline and death associated with mild to severe root rot and/or crown rot. Young vines in their first year of growth as well as full-size vines many years old can be affected. The root rot can cause vine stunting without killing the vines.

### *Pathogens*

*Phytophthora* and *Pythium* spp. cause disease on many annual and perennial crops in California, including grapevines. Previous work implicated several unidentified *Phytophthora* spp. and *Pythium ultimum* as causes of root rot of grapevines in California (Chiarrapa 1964). Later surveys associated *Phytophthora cambivora*, *P. cinnamomi*, *P. megasperma* and *P. parasitica* with root and crown rot of vines in California, although pathogenicity and relative virulence of these species on grape were not determined (S. N. Mircetich, unpublished data). *Phytophthora cactorum*, *P. cinnamomi*, *P. cryptogea*, *P. parasitica* and several *Pythium* spp. were implicated in root rots of grapes in vineyards and nurseries in South Africa (Marais 1979, 1980). During the last 5 years in California, we occasionally have isolated *P. cinnamomi*, *Pythium ultimum* and an unidentified *Pythium* sp. from rotted roots of declining grapevines. Our field observations and supporting laboratory and greenhouse experiments suggest that only *P. cinnamomi* commonly causes severe root and crown rot and death of full-size vines in California. The other species of *Phytophthora* are less virulent than *P. cinnamomi* on grape. *Pythium* spp. commonly infect roots of grapevines in California, but most are weak grape pathogens, causing less root system damage than *P. cinnamomi*.

### *Regional importance*

Our results suggest that in California, decline of vines associated with *Phytophthora* and *Pythium* spp. is localised, but important where it occurs (G. T. Browne and L. A. Bayramian, unpublished data). For example, for many years *P. cinnamomi* has caused high incidence of severe root and crown rot in several vineyards of varying age in California's north coast districts, but the pathogen is not known to be a common or important problem in other grape growing regions of the state. Compared with *P. cinnamomi*, the other species of *Phytophthora* and *Pythium* are not known to occur as frequently as pathogens on grapevines in California.

### *Pathogen spread, disease incidence, and control*

*Phytophthora* and *Pythium* species can be spread in many ways, including movement of infested soil, plant materials or water. Field observations and results of greenhouse tests indicate that soil moisture conditions, rootstock resistance and pathogen virulence are all important factors influencing

incidence of root disease caused by *Phytophthora* and *Pythium* spp. in California. In our greenhouse tests, flooding generally favoured disease development. Our rootstock screens consistently have indicated that St George rootstock is relatively susceptible to *P. cinnamomi*. Furthermore, additional *V. rupestris* selections as well as 5BB and c9033 exhibited some susceptibility to the pathogen. The susceptibility of St George in our resistance screens and its frequent problems with root rot induced by *P. cinnamomi* in north coast vineyards indicate that it is a poor rootstock choice for heavy soils infested by *P. cinnamomi*. The other selections of *V. rupestris* as well as 5BB and c9033 may possess similar susceptibility to *P. cinnamomi*, but they performed less consistently than St George in greenhouse screening.

It is intriguing to note that in our greenhouse tests, an unidentified *Pythium* sp. (1578) was highly virulent (i.e. caused severe root and crown rot and vine death) on *V. vinifera* cv. Aramon as well as on *V. rupestris* cv. St George. In the same experiments, *P. cinnamomi* only caused severe disease on St George. It is not known whether *Pythium* sp. 1578 damages well-established (several years old) vines as *P. cinnamomi* does; to date, we have only isolated *Pythium* sp. 1578 from 1 to 2 year old vines. We intend to determine the identity, distribution and pathogenicity of *Pythium* sp. 1578 in future research.

In vineyards subject to damage by *Phytophthora* or *Pythium*, careful soil water management and careful choice of rootstock should help minimise the disease losses. Applications of phosphonate fungicides (i.e. phosphorous acid-containing products, fosetyl-Al) have not been thoroughly tested for control of *Phytophthora*- or *Pythium*-induced diseases in California vineyards, although they are effective for other crops.

### Verticillium wilt

Verticillium wilt caused by *V. dahliae* has become more prevalent in Californian vineyards during the past 10 years. Typically, the disease occurs only in grapevines less than 6–7 years old. Symptoms include wilting of leaves and shoots soon after the temperatures increase in early summer. Some leaves may show marginal burning, but wilting is the predominant symptom. The symptoms will nearly always appear as one-sided on the vine, with leaves and shoots on one cordon showing wilting. Fruit on affected shoots dry up and often will remain attached to the pedicel, the result of rapid desiccation before the abscission zone formed.

Infection occurs through the roots of young vines and the consequence is vascular plugging due to gums and tyloses as well as fungal spores and mycelium. Foliar symptom expression occurs as plugging and death of roots become more extensive.

### Discussion

The susceptibility of rootstocks to Petri disease and vine decline pathogens in our studies appears to correlate with incidence and severity of these same diseases in California. For example, 3309, 101-14, 5C and 110R seem to show the diseases most prevalently in Californian vineyards. These rootstocks though, are some of the most widely planted in California and the natural occurrence may be skewed towards these rootstocks simply because of the numbers planted. AXR1 exhibited the most resistance to the Petri disease and vine decline pathogens and though the pathogens were capable of entrance into AXR1, their movement in the rootstock was limited. Even though AXR1 rootstock was capable of being infected by *C. destructans*, *Pa. chlamydospora* and *Pm. aleophilum*, the fungi apparently could not move extensively in the vascular tissue as they were observed to do in the more susceptible varieties (Eskalen *et al.* 2001a).

Research has shown that there are differences in susceptibility to *Pa. chlamydospora* and *Phaeoacremonium* spp., and these differences may be important even though there is no true resistance to the group of pathogens. Furthermore, we know that these fungi are endophytic in both rootstock and scion wood and as such are well adapted to colonization and growth in grapevine. They appear to be highly capable of infecting wood through various tissues including roots, pith, vascular tissue, pruning wounds, green cortical tissue and berries. Prior to the early 1990s, most of the vineyards in California were planted on AXR1 or were own-rooted. The fact that AXR1 was always in the least susceptible group (Eskalen *et al.* 2001a) adds some basic understanding as to why we did not have a serious problem with Petri disease prior to planting on the new rootstock selections. There is good correlation between use of the new rootstocks and the major replanting in Californian vineyards for combating Phylloxera. Though the fungi implicated in Petri disease have been documented to occur in California and other vineyards worldwide for nearly a century, they caused no apparent major problem until many of the new Phylloxera resistant rootstocks were introduced.

Research has shown that esca (black measles) and Petri disease caused black or brown streaking in grapevine trunks. Research has also demonstrated that *Pm. aleophilum*, when inoculated into the roots of cvv. Thompson Seedless, Red Globe, Cabernet Sauvignon, Zinfandel and Chardonnay is capable of causing foliar symptoms identical to those observed naturally in vineyards in all of California's production areas. While vines with Petri disease may recover and go on to produce a near normal crop, the prevalence on young infected vines may indeed result in increased disease incidence in future years.

While it was evident that grapevine roots were susceptible to infection by *Phaeoacremonium* spp. and *Pa. chlamy-*

*dospora*, the question of whether these pathogens were soilborne in vineyards was raised. Development of a semi-selective medium and PCR tests showed the presence of *Pa. chlamydospora* and *Phaeoacremonium* spp. in vineyard soil (Eskalen *et al.* 2001b; Rooney *et al.* 2001).

Fungi associated with Petri disease have been documented in all major grape-growing regions in California. Work by Khan *et al.* (1999) and Whiting *et al.* (2001) demonstrated that *Phaeoacremonium* spp. are effective root pathogens. *Pa. chlamydospora* was a more effective wound pathogen of scion wood but still had the capacity to infect through roots. Recent studies have also shown that spores of these fungi are present in vineyards, as aerial spore traps have confirmed their presence on spurs and cordons in nearly all production regions in California (Eskalen and Gubler 2001). Subsequent work has shown the presence of pycnidia of *Pa. chlamydospora* in exposed vascular tissue on grapevines (Edwards and Pascoe 2001; Eskalen *et al.* 2003). These pathogens can gain entrance by several means into grapevine wood and become established. They are also endophytes with the ability to cause disease when vines are stressed.

Black foot caused by *C. destructans* is by far the most serious pathogen of those recently discovered. Infected vines die quickly and it seems to occur more extensively in affected vineyards. As vineyards become older, they become less prone to die even when infection occurs. This appears to be a good case of plant vigour playing a role in tolerance to disease.

Armillaria root rot has also become more of a problem in vineyards in the North Coast production area of California. As more vineyards are established higher on hillsides where they replace natural forests, we see more disease caused by this pathogen. Although *Armillaria* is not considered to be one of the limiting diseases on grapevine, it has caused considerable loss in some vineyards. Infected roots are difficult to remove from the soil, leaving vines in infected areas of vineyards that continually suffer losses. Current research on control of *Armillaria* root disease focuses on therapeutic treatments that will, hopefully, extend the productive life of infected vines.

We consider *Phytophthora* root and crown rot to be important where they occur but they are rather limited in distribution among California's vineyards. Although some of the rootstocks with *V. rupestris* heritage present increased risk of infection by *P. cinnamomi*, vine death caused by this pathogen has not been widespread to date. The severe disease caused by *Pythium* sp. 1578 on *V. vinifera* cv. Oramon suggests that this pathogen and its distribution should be characterised more completely. Phosphonate treatments are worthy of testing at vineyard sites affected by *P. cinnamomi*.

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