Phytopathol. Mediterr. (2006) 45, S12–S29

REVIEWS

A review of *Phaeoacremonium* species involved in Petri disease and esca of grapevines

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Summary. The current status of *Phaeoacremonium* species involved in Petri disease and esca is reviewed. The taxonomical position and classification of *Phaeoacremonium* as well as its teleomorph, *Togninia*, are discussed. The review also provides the currently known distribution and host range of *Phaeoacremonium* species. The epidemiology of *Phaeoacremonium* species together with the more commonly isolated *Phaeomoniella chlamydospora*, is also treated. An overview is given of the molecular methods that have been used thus far to identify and detect the fungi involved in Petri disease. The role that *Phaeoacremonium* species, and the morphologically closely related pathogen *Pa. chlamydospora*, play in disease development and the results obtained with pathogenicity trials are also discussed. Lastly, an overview is given of the effect of management strategies on the *Phaeoacremonium* species associated with Petri disease and esca.

Key words: detection, epidemiology, management, pathogenicity, *Vitis vinifera*.

Introduction

Petri disease causes stunted growth and dieback of grapevines (*Vitis vinifera* L.). Internal symptoms can normally be seen in the trunk and cordons. These include black spots (Fig. 1A) when vines are cut transversely, and dark brown to black streaking (Fig. 1B and C) when trunks or shoots are cut longitudinally. The severed xylem vessels often ooze black xylem sap; hence the popular name 'black goo'. Petri disease is mainly found on young vines and has caused significant losses of young vines in newly planted vineyards (Bertelli *et al.*, 1998; Scheck *et al.*, 1998; Ferreira *et al.*, 1999; Mugnai *et al.*, 1999; Pascoe and Cottral, 2000). Petri disease is caused by a combination of *Phaeomoniella* (*Pa.*) *chlamydospora* (W. Gams, Crous & M.J. Wingf. & L. Mugnai) Crous & W. Gams and several species of *Phaeoacremonium* (*Pm.*) W. Gams, Crous & M.J. Wingf. (Scheck *et al.*, 1998; Mugnai *et al.*, 1999; Groenewald *et al.*, 2001). *Phaeomoniella chlamydospora* has been more often associated with typical Petri disease symptoms than species of *Phaeoacremonium*

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(Mugnai *et al.*, 1999; Chicau *et al.*, 2000; Edwards and Pascoe, 2004).

Esca disease of grapevines, better defined as esca proper following Mugnai *et al.*, 1999, can be typically identified by wood decay (Fig. 1D), symptoms on leaves (Fig. 1E) and in some cases also berries (Fig. 1F). The leaves of affected vines can show symptoms, having interveinal regions of chlorotic and yellowish tissue also described as 'tiger stripes'. Berries can also be affected and develop small, dark brown to purple spots; hence the name 'black measles'. Internal symptoms include an area of white rotted wood surrounded by a dark border line and dark brown to black spots (Mugnai *et al.*, 1999). In severe cases, sudden wilting and death of vines or vine-parts appear during the summer, also called 'apoplexy'. Esca has more often been associated with older vines. However, reports of this disease on younger vines have also been made (Edwards *et al.*, 2001b). Fungi that have been associated with esca symptoms include the wood rotting basidiomycetes, *Fomitiporia* (*F.*) *mediterranea* M. Fischer, to a lesser extent *Stereum hirsutum* (Willd. : Fr) Pers. as well as the hyphomycetes, *Pa. chlamydospora* and *Pm. aleophilum* (Larignon and Dubos, 1997; Mugnai *et al.*, 1999; Ari, 2000; Cortesi *et al.*, 2002; Fischer, 2002). Various reports of *F. punctata* (P. Karst.) Murrill formerly called *Phellinus punctatus* (P. Karst.) Pilát, have been made from grapevines. Even though the fruit bodies of *F. punctata* are indistinguishable from those of *F. mediterranea*, did phylogenetic studies show that these are indeed distinct species and that previous findings of *F. punctata* on grapevines are assignable to *F. mediterranea* (Fisher, 2002). *Phaeoacremonium* strains isolated from esca diseased vines have often not been identified to species level (Serra *et al.*, 2000; Gatica *et al.*, 2001).

Sixteen species of *Phaeoacremonium* have thus far been described (Table 1) (Crous *et al.*, 1996; Dupont *et al.*, 2000; Groenewald *et al.*, 2001; Mostert *et al.*, 2005b). Eleven of these species have been isolated from grapevines (Table 1). Of these, *Pm. aleophilum* (Fig. 2A and B) appears to be the most widely distributed and the most common in grapevines (Larignon and Dubos, 1997; Mugnai *et al.*, 1999; Groenewald *et al.*, 2001). Other species that have also been isolated in relatively high frequencies from grapevines include *Pm. parasiticum* (Fig. 2C and D) in Argentina (Dupont *et al.*, 2002), and *Pm. viticola* (Fig. 2E and F) in France (Dupont *et al.*, 2000). The relative importance of the different *Phaeoacremonium* species in Petri disease and esca has been difficult to assess since strains are often not identified to species level, and several new species have only recently been described.

The genus *Phaeoacremonium* has been confirmed as anamorph of *Togninia* (Mostert *et al.*, 2003). This finding was also confirmed by Pascoe *et al.* (2004) and Rooney-Latham *et al.* (2005a). *In vitro* mating studies done with *Pm. aleophilum* showed that the sexual state of this species was *Togninia minima*, and that it had a heterothallic mating strategy (Mostert *et al.*, 2003; Rooney-Latham *et al.*, 2005a). Perithecia of *T. minima* formed on field samples of grapevines that were incubated in moist chambers (Pascoe *et al.*, 2004; Rooney-Latham *et al.*, 2005b), showing that both mating types occur on the same vine in the field. This was also seen with strains from the same vine forming perithecia with *in vitro* matings (Mostert *et al.*, 2003). The presence of both mating types on a vine indicates that the sexual state could readily form in the field under the right environmental conditions. Recently, perithecia of *Togninia* were also observed on grapevines in the field (Eskalen *et al.*, 2005; Rooney-Latham *et al.*, 2005). Based on morphological studies, the perithecia were identified as *Togninia minima* and *Togninia fraxinopennsylvanica* (Eskalen *et al.*, 2005; Rooney-Latham *et al.*, 2005). DNA sequence data also confirmed the anamorph-telemorph relationship between *Pm. mortoniae* and *Togninia fraxinopennsylvanica* (Eskalen *et al.*, 2005; Mostert *et al.*, 2005a).

Phaeoacremonium species have also been associated with human infections, often causing phaeohyphomycosis (lumps of fungal growth under the skin) (Ajello *et al.*, 1974; Crous *et al.*, 1996; Padhye *et al.*, 1998; Guarro *et al.*, 2003). Observations over several years have shown that species of *Phaeoacremonium* are opportunistic pathogens needing a subcutaneous traumatic inoculation or predisposed host to be able to infect and cause disease. Eight species are currently known to be able to infect humans (Table 1) (Crous *et al.*, 1996; Mostert *et al.*, 2005b). Of these, *Pm. alvesii*, *Pm. krajdenii*, *Pm. parasiticum* and *Pm. venezuelense* have also been isolated from woody hosts. Infected wood splinters could be a source of human infections, but it remains unclear to what extent it contrib-

utes to phaeohyphomycotic cases, since no case has been directly linked to a wood splinter infection.

This review aims to give an overview of the *Phaeoacremonium* species involved in Petri disease and esca. The topics that will be discussed in this paper include the current taxonomical position of *Togninia* / *Phaeoacremonium*, distribution of *Phaeoacremonium* species, alternative hosts, epidemiology, detection tools, pathogenicity studies, toxins produced and efficacy of control methods. On many of these aspects an account of the results and data obtained on *Pa. chlamydospora* will be given, because of its involvement in the same diseases and similar behaviour in epidemiology, pathogenicity and control aspects.

Taxonomic overview

Togninia and its relatives

Togninia has historically been classified in the *Calosphaeriaceae* (*Calosphaeriales*) (Berlese, 1900; Barr, 1985; Mostert *et al.*, 2003). Barr (1983) outlined the history of the *Calosphaeriaceae* and the respective genera, and published the first modern concept of this family (Barr, 1985). Eight genera were included in the first treatment of the *Calosphaeriales*: *Calosphaeria* Tul. & C. Tul., *Scoptria* Nitschke, *Enchnoa* Fr., *Jattaea* Berl., *Romellia* Berl., *Graphostroma*, *Togninia* Berl. and *Pleurostoma* Tul. & C. Tul. (Barr, 1985). In a later study only the genera *Calosphaeria*, *Enchnoa*, *Jattaea*,

Table 1. List of *Phaeoacremonium* species, host range and world-wide distribution[a].

Phaeoacremonium species	Host	Countries
Pm. aleophilum	*Actinidia chinensis*, *Vitis vinifera*, *Olea europaea*, *Prunus pennsylvanica*, *Prunus* sp., *Salix* sp.	Argentina, Australia, Austria, Canada, Chile, Iran, Italy, France, South Africa, Spain, Turkey, USA, Yugoslavia
Pm. alvesii	*Dodoneae viscosa*, human	Australia, Brazil[b], USA[b]
Pm. amstelodamense	Human	Netherlands[b]
Pm. angustius	*Vitis vinifera*	Portugal, USA
Pm. australiense	*Vitis vinifera*	Australia
Pm. griseorubrum	Human	Japan[b], USA[b]
Pm. inflatipes	*Hypoxylon truncatum*, *Nectandra sp.*, *Quercus virginiana*, *Vitis vinifera*	Chile, Costa Rica, USA
Pm. krajdenii	Human, *Vitis vinifera*	Canada, India[b], Japan[b], Norway[b], South Africa, USA[b], Zaire[b]
Pm. mortoniae	*Fraxinus excelsior*, *Fraxinus latifolia*, *Fraxinus pennsylvanica*, *Vitis vinifera*	Sweden, USA
Pm. parasiticum	*Actinidia chinensis*, *Aquilaria agallocha*, *Cupressus* sp., human, *Nectandra* sp., *Phoenix dactylifera*, *Prunus armeniaca*, *Quercus virginiana*, *Vitis vinifera*	Argentina, Australia, Brazil[b], Canada[b], Chile, Costa Rica, Finland[b], Iran, Iraq, Italy, South Africa, Tunisia, USA[b]
Pm. rubrigenum	Human	USA[b]
Pm. scolyti	*Vitis vinifera*, larvae of *Scolytus intricatus*	France, South Africa
Pm. subulatum	*Vitis vinifera*	South Africa
Pm. tardicrescens	Human	USA[b]
Pm. viticola	*Sorbus intermedia*, *Vitis vinifera*	Iran, France, Germany, South Africa, USA
Pm. venezuelense	Human, *Vitis vinifera*	Canada[b], South Africa, Venezuela[b]

[a] From references Hawksworth and Gibson, 1976a; Hausner *et al.*, 1992; Crous *et al.*, 1996; Dupont *et al.*, 1998; Larignon and Dubos, 1997; Ari, 2000; Chicau *et al.*, 2000; Crous and Gams, 2000; Dupont *et al.*, 2000; Pascoe and Cottral, 2000; Péros *et al.*, 2000; Armengol *et al.*, 2001; Groenewald *et al.*, 2001; Dupont *et al.*, 2002; Auger *et al.*, 2005; Damm *et al.*, 2005; Eskalen *et al.*, 2005; Mostert *et al.*, 2005b and Overton *et al.*, 2005.

[b] Countries where *Phaeoacremonium* strains were isolated from human infections.

Pachytrype Berl. ex M.E. Barr and *Pleurostoma* were included in the *Calosphaeriales* (Barr, 1993). *Romellia*, *Togninia* and *Erostella* were synonymised with *Pleurostoma* and *Wegelina* Berl. with *Calosphaeria* (Barr, 1993). It was clear that the octosporous *Togninia* was not a synonym of the multisporous *Pleurostoma* (Mostert *et al.*, 2003), which was also confirmed by DNA phylogenetic studies (Vijaykrishna *et al.*, 2004). In the case of *Erostella*, there was uncertainty as to whether the name *Erostella* or *Togninia* should be used since both had *Calosphaeria minima* Tul. & Tul. as lectotype. This issue was resolved by Clements and Shear (1931), who designated *T. minima* as lectotype of *Togninia*. Arguments around the interpretation of the Latin used by Berlese (1900) supports the lectotypification by Clements and Shear (1931) (Hausner *et al.*, 1992; Holm, 1992; Mostert *et al.*, 2003). *Graphostroma* with its *Nodulosporium*-like anamorph clearly showed that it should be classified in the *Xylariales* (Pirozynski, 1974). Barr (1993) erected the family *Graphostromataceae* to accommodate the stromatic calosphaeriaceous genus *Graphostroma*. The *Calosphaeriales* currently includes six nonstromatic genera, i.e. *Calosphaeria*, *Jattaea*, *Pleurostoma*, *Romellia*, *Wegelina,* and *Togninia*, and the stromatic *Pachytrype* (Réblová *et al.*, 2004). Whether *Enchnoa* Fr. should remain within the *Calosphaeriales* is uncertain (Petrak and Sydow, 1936; Barr, 1985). Fresh specimens were recently collected of types of the *Calosphaeriales*, namely, *Calosphaeria pulchella* (Réblová *et al.*, 2004) and *Pleurostoma ootheca* (Vijaykrishna *et al.*, 2004). Cultures of these fungi made DNA phylogenetic studies possible, thereby shedding new light upon the phylogenetic relationship amongst the fungi found within the *Calosphaeriales*. A collection was also made of a new genus, described as *Togniniella* Réblová, L. Mostert, W. Gams & Crous (Réblová *et al.*, 2004). The phylogenetic analysis of the nuclear large subunit and small subunit ribosomal DNA showed that *Togninia* formed a unique cluster that fell within the *Diaporthales* (Réblová *et al.*, 2004). Two new families were also erected, namely the *Pleurostomataceae* (*Calosphaeriales*), and the *Togniniaceae* (*Diaporthales*). The *Togniniaceae* and the *Gnomoniaceae* (*Diaporthales*) have dark, globose, long-beaked and non-stromatic perithecia; asci with a rounded base, floating freely within the centrum, and a phialidic anamorph with phytopathogenic life style in common (Réblová *et al.*, 2004).

Twelve species and one variety of *Togninia* were described by Berlese (1900). Barr (1985) only included *T. minima* in the genus *Togninia* and commented only on the species that Berlese (1900) illustrated. An additional three new species were accepted in *Togninia*, *T. inconspicua* (Rehm) Yue & Eriksson (Eriksson and Yue, 1990), *T. fraxinopennsylvanica* (Hinds) Hausner, Eyjólfsdóttir & J. Reid and *T. novae-zealandiae* Hausner, Eyjólfsdóttir & J. Reid (Hausner *et al.*, 1992). The teleomorphs of *Pm. parasiticum*, *Pm. viticola*, *Pm. krajdenii*, *Pm. rubrigenum* and two unnamed *Phaeoacremonium* species have been found by means of *in vitro* mating studies (Mostert *et al.*, 2005a). The genus *Togninia* is distinguished by having ascomata with necks (usually more prominent *in vitro*) unitunicate asci that are oblong with a clearly truncate base and thickened apices; asci are arranged in a spicate formation on the ascogenous hyphae; having hyaline, septate paraphyses and ascospores that are hyaline, allantoid or ellipsoidal to oblong-ellipsoidal and one-celled (Barr, 1985; Hausner *et al.*, 1992). The spicate arrangement of asci is also found among the related genera, *Togniniella* and *Pleurostoma*, but these genera are distinguished from *Togninia* in the way the asci are attached to the ascogenous hyphae (Réblová *et al.*, 2004).

Phaeoacremonium

Petri (1912) found two *Cephalosporium* and one *Acremonium* species associated with black streaking of the wood in declining grapevines. Chiarappa (1959) also reported a *Cephalosporium* species (CBS 239.74) associated with grapevines affected by black measles. Consequently, Hawksworth *et al.* (1976b) examined the strain of Chiarappa (1959) and found that it was morphologically different to *Phialophora parasitica* strains associated with die-back symptoms of woody hosts. *Phialophora parasitica* and morphologically similar strains were examined by Crous *et al.* (1996) who established the genus *Phaeoacremonium* for these strains that originated from humans and various woody hosts including grapevines. The genus *Phaeoacremonium* is intermediate between *Acremonium* Link: Fr. and *Phialophora* Medlar. *Phaeoacremonium parasiticum*, under its original name *Phialophora para-*

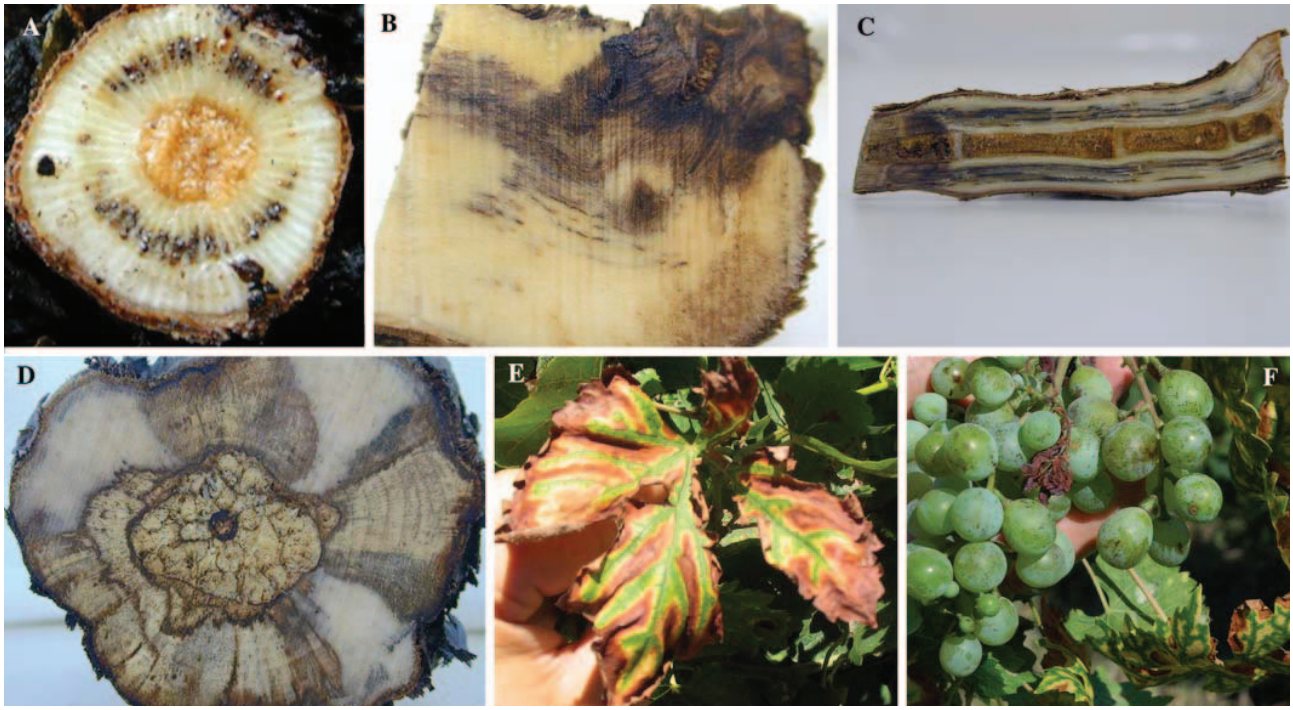

Fig. 1. Symptoms associated with Petri disease (A–C) and esca (D–F). A. Black spots visible on the rootstock, '101-14 Mgt' of a one-year-old vine. B. Black streaking associated with natural pruning wound infection of a 9-year-old 'Shiraz' vine. C. Spur showing typical brown to black streaking 14 months after inoculated with spore suspension of *Pa. chlamydospora* on the pruning wound. D and E. Cross section showing different wood discoloration and 'tiger stripes' on the leaves of an 18-year-old 'Chenin blanc' vine. F. Brown spots or 'black measles' symptoms on berries of a 'Chenin blanc' vine.

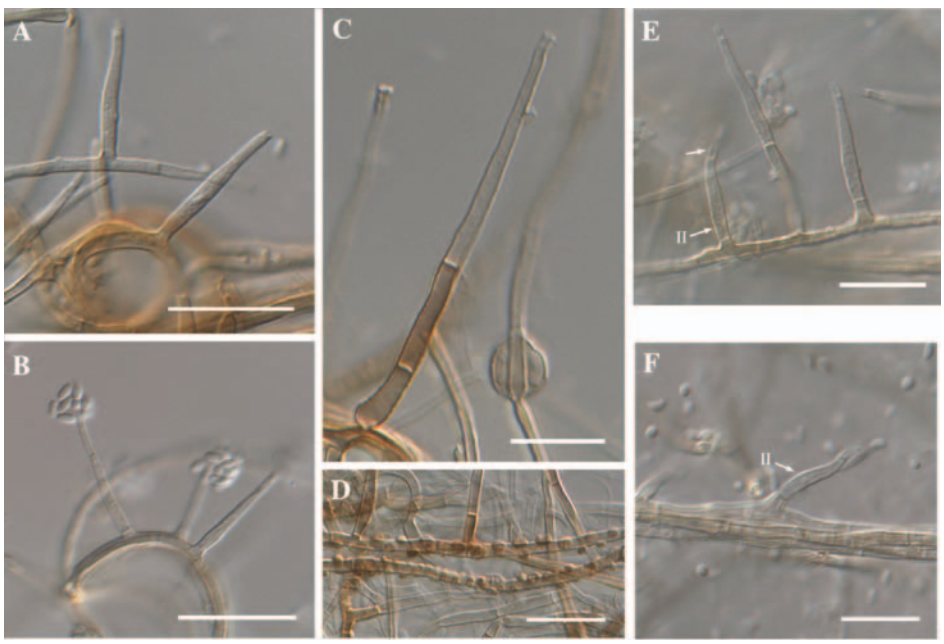

Fig. 2. Micromorphology of three *Phaeoacremonium* species. A and B. Type III phialides of *Pm. aleophilum*. C. Conidiophore of *Pm. parasiticum* with the basal cells being distinctly darker pigmented. D. Mycelium showing prominent warts associated with *Pm. parasiticum*. E and F. Conidiophores with type III phialides and type II phialides of *Pm. viticola*. Of the three species illustrated here, *Phaeoacremonium viticola* has the longest collarettes (indicated by an arrow at the apex of a phialide) of up to 2.5 mm. Scale bars = 10 μm.

sitica Ajello, Georg & C.J.K. Wang is the type species for the genus. *Phaeoacremonium* can be distinguished from *Phialophora* by its aculeate phialides and inconspicuous, non-flaring collarettes, and from *Acremonium* by its pigmented vegetative hyphae (Crous *et al.*, 1996). DNA phylogeny of the 26S showed that *Phaeoacremonium* lies close to the *Magnaporthaceae* (Dupont *et al.*, 1998). This affinity was due to the fact that only a few taxa were used for this phylogeny and it also did not include the *Diaporthales*. Further analyses including more taxa have shown that *Phaeoacremonium* lies closer to the *Diaporthales* (Mostert *et al.*, 2003; Réblová *et al.*, 2004). Six species of *Phaeoacremonium* were originally identified based on morphology and cultural characters (Crous *et al.*, 1996). It soon became apparent that the taxon once referred to as '*Cephalosporium*' species or *Pm. chlamydosporum* represented a new genus, *Phaeomoniella* Crous & W. Gams, which resided within the *Chaetothyriales* (Crous and Gams, 2000). Two additional *Phaeoacremonium* species were identified based on their morphology as well as DNA phylogeny of the transcribed spacers (ITS 1 and 2) and 5.8 S rRNA gene region for *Pm. mortoniae* (Groenewald *et al.*, 2001) and combined with the β-tubulin gene region for *Pm. viticola* (Dupont *et al.*, 2000). A further nine species were identified with morphological, cultural and a combination of β-tubulin, actin and calmodulin sequence data (Mostert *et al.*, 2005b). Morphological characters that were useful in distinguishing species included conidiophore morphology, phialide type and morphology, the size of hyphal warts, and to a lesser extent conidial size and shape (Mostert *et al.*, 2005b). Cultural characters that were useful included colony colour on 2% malt extract agar (MEA), yellow pigment production on potato-dextrose agar, growth rate at 25°C and maximum growth temperature (Mostert *et al.*, 2005b). Yellow pigment production on oatmeal agar was used by Dupont *et al*. (2000) and has also proven to be a better medium to test yellow pigment production in our studies.

The genus *Phaeoacremonium* is characterised by its mycelial bundles, conidiophores that can be branched or not, slender phialides occurring in three size classes, narrowly funnel-shaped collarettes at the apex of the phialides, conidia aggregated into slimy heads and conidial shape ranging from mostly oblong-ellipsoidal to allantoid. Generic descriptions of *Phaeoacremonium* have been published by Crous (1996) and Mostert (2005b).

Distribution and host range

Phaeoacremonium species have been isolated from grapevines from across the world, including Argentina (Dupont *et al.*, 2002), Australia (Pascoe and Cottral, 2000; Mostert *et al.*, 2005b), Austria (Reisenzein *et al.*, 2000), Chile (Auger *et al.*, 2005), France (Larignon and Dubos, 1997; Dupont *et al.*, 2000; Péros *et al.*, 2000; Mostert *et al.*, 2005b), Greece (Rumbos and Rumbou, 2001), Iran (T. Gräfenhaen, personal communication), Italy (Mugnai *et al.*, 1996, 1999; Groenewald *et al.*, 2001), Portugal (Chicau *et al.*, 2000), South Africa (Crous *et al.*, 1996; Groenewald *et al.*, 2001; Mostert *et al.*, 2005b), Spain (Armengol *et al.*, 2001), Turkey (Ari, 2000) and the USA (Dupont *et al.*, 1998; Groenewald *et al.*, 2001; Eskalen *et al.*, 2005; Overton *et al.*, 2005). *Phaeoacremonium aleophilum* is the most commonly isolated species from grapevines occurring in Argentina (Dupont *et al.*, 2002), Australia (Pascoe and Cottral, 2000), Chile (Auger *et al.*, 2005), Iran (T. Gräfenhaen, personal communication), Italy (Mugnai *et al.*, 1996; 1999), France (Larignon and Dubos, 1997), South Africa (Groenewald *et al.*, 2001), Spain (Armengol *et al.*, 2001), Turkey (Ari, 2000), Yugoslavia (Crous *et al.*, 1996) and the USA (Scheck *et al.*, 1998; Overton *et al.*, 2005). *Phaeoacremonium parasiticum* and *Pm. viticola* have also often been found on diseased grapevines. *Phaeoacremonium parasiticum* has been found on grapevines in Argentina (Dupont *et al.*, 2002), Australia (Mostert *et al.*, 2005b), Chile (Auger *et al.*, 2005), Iran (T. Gräfenhaen, personal communication), South Africa (Mostert *et al.*, 2005b) and the USA (Eskalen *et al.*, 2005). *Phaeoacremonium viticola* has been isolated from grapevines in Iran (T. Gräfenhaen, personal communication), France (Dupont *et al.*, 2000), South Africa (L. Mostert, unpubl. data) and the USA (Overton *et al.*, 2005).

Recent molecular studies have clarified certain doubtful/wrongful records. In the cases where *Pm. rubrigenum* was identified from grapevines, it could represent one of the pink coloured species such as *Pm. griseorubrum*, *Pm. scolyti* or pink to brown coloured *Pm. alvesii*. Of these, *Pm. scolyti*

and *Pm. alvesii* have been isolated from grapevines. These species are phylogenetically closely related, and the strains analysed by Groenewald *et al.* (2001) were all referred to as *Pm. rubrigenum*. However, when more strains were included, these strains were revealed to be phylogenetically different species, being also supported by morphological and cultural characters (Mostert *et al.*, 2005b). Various reports on finding *Pm. angustius* have been based on comparative studies with the ex-type strain of *Pm. angustius*, CBS 249.95, which was contaminated with *Pm. aleophilum* (Dupont *et al.*, 2000; Alves *et al.*, 2004). Dupont (2000) therefore concluded that *Pm. aleophilum* and *Pm. angustius* were conspecific. However, ITS and β-tubulin sequence data from the original isolate clearly showed that *Pm. angustius* was different from *Pm. aleophilum* (Groenewald *et al.*, 2001). Although various reports have been made of *Pm. inflatipes* occurring in grapevines, this species has thus far only been confirmed on grapevines from Chile and *Hypoxylon truncatum* and *Quercus virginiana* from the USA and a *Nectandra* sp. from Costa Rica (Dupont *et al.*, 2002). Other grapevine strains identified as '*Pm. inflatipes*' proved to be *Pm. aleophilum* (Groenewald *et al.*, 2001; Dupont *et al.*, 2002; Rooney-Latham *et al.*, 2005a).

Phaeoacremonium species have been isolated from a range of woody hosts (Table 1). The role of alternative host plants in the vicinity of vineyards could be a potential source of inoculum and needs to be examined. Other substrates from which *Phaeoacremonium* has been isolated include insect larvae in Czechia (Kubátová *et al.*, 2004), soil in Argentina (Crous and Gams, 2000) and Tahiti (Dupont *et al.*, 2002). *Phaeoacremonium rubrigenum* has been isolated from the galleries and larvae of *Scolytus intricatus* (on *Quercus robur*) and adults of *Leperisinus fraxini* (on *Fraxinus excelsior*) (Kubátová *et al.*, 2004). These strains proved to be representative of a new species, *Pm. scolyti*, which produces medium pink colonies on 2% MEA and is phylogenetically closely related to *Pm. rubrigenum* (Mostert *et al.*, 2005b). *Phaeoacremonium scolyti* has also been found on grapevines in South Africa and France, indicating that bark beetles might play a role in the dispersal of this species from other woody hosts to grapevines. The presence of larval galleries on trees where species of *Phaeoacremonium* have been isolated has also been reported for *Pm. parasiticum* and *Pm. mortoniae*. Boring beetles were present in *Nectandra* sp. trees in Costa Rica from which *Pm. parasiticum* was isolated from vascular discolouration (Hawksworth *et al.*, 1976b). *Togninia fraxinopennsylvanica* (teleomorph of *Phaeoacremonium mortoniae*) was isolated from a brown stain of *Fraxinus pennsylvanica* in North Dakota, which also had larval galleries of *Leperisinus californicus* (Hausner *et al.*, 1992).

Epidemiology

Various aspects pertaining to the source of inoculum, the port of entry and the spread of *Pa. chlamydospora* and *Phaeoacremonium* species are known. The main sources of inoculum of these fungi in vineyards include infected propagation material, infected soils and aerial inoculum. Infected mother vines have proven to be a source of infected propagation material (Mugnai *et al.*, 1999; Pascoe and Cottral, 2000; Rego *et al.*, 2000; Halleen *et al.*, 2003; Edwards *et al.*, 2004, Fourie and Halleen, 2004a). The presence of *Pa. chlamydospora* in naturally infected rootstock mother vines has also been confirmed by means of polymerase chain reaction (PCR) detection (Ridgway *et al.*, 2003; Retief *et al.*, 2005a). Fourie and Halleen (2002) found *Pa. chlamydospora* and *Phaeoacremonium* species in symptomless canes sampled from rootstock mother vines, although the incidence thereof was very low (< 0.2%). Propagation material can also get infected during the grafting process. Zanzotto *et al.* (2001) found very little infection in rootstock and scion cuttings made from mother plants. However, he did find *Pa. chlamydospora* and *Phaeoacremonium* species in certified, grafted plants and 1-year-old plants. Higher infection rates had been recorded by Bertelli *et al.* (1998). These results support the fact that infections take place during nursery operations. Investigation of nursery operations with primers using polymerase chain reactions (PCR), have shown that *Pa. chlamydospora* is present in hydration tanks, grafting tools and callusing media in nurseries in New Zealand (Whiteman *et al.*, 2003) and in hydration water and callusing media in nurseries in South Africa (Retief *et al.*, 2005a). Both *Pa. chlamydospora* and *Phaeoacremonium* species were frequently isolated from rootstock and graft unions of vine cuttings before and after planting in nurseries,

indicating that these infections were derived from infected mother material and/or nursery operations (Halleen *et al.*, 2003). It is interesting to note that in two cases where isolations were made from grafted plants, *Phaeoacremonium* species were more often isolated than *Pa. chlamydospora* (Zanzotto *et al.*, 2001; Halleen *et al.*, 2003).

The infection of field grapevines can be through the roots or pruning wounds. *Phaeomoniella chlamydospora* has been detected in soil from mother vines with PCR (Whiteman *et al.*, 2003; Retief *et al.*, 2005a). *Phaeoacremonium aleophilum* has also been detected in soil and puddles of water under grapevines by using a filtering system and Rose Bengal Chloramphenicol selective medium (Rooney *et al.*, 2001). Pathogenicity studies have shown that *Pa. chlamydospora* and *Pm. aleophilum* can infect and colonise grapevine roots (Adalat *et al.*, 2000). It was also shown that *Pm. aleophilum* was much more successful to infect via the roots than *Pa. chlamydospora* (Adalat *et al.*, 2000). However, root symptoms are not always present in diseased vines (Morton, 2000). Black streaking throughout the entire length of an infected root is rarely found and in most cases these discolourations are found in roots close to the base of the cutting. The extent to which root infections take place in the field remains unclear. Pruning wounds are the most obvious port of entry for aerial inoculum. Various pathogenicity studies have shown that *Pa. chlamydospora* and *Pm. aleophilum* can readily infect pruning wounds following inoculation with conidia (Adalat *et al.*, 2000; Larignon and Dubos, 2000). Inoculation of grapevine spurs (cv. Chardonnay and Pinot Noir) revealed that *Pa. chlamydospora* is much more aggressive than *Pm. aleophilum* as a pruning wound invader (Adalat *et al.*, 2000).

Conidia of *Pa. chlamydospora* and *Phaeoacremonium* species can be aerially dispersed. The presence of aerial inoculum of *Pa. chlamydospora*, *Pm. aleophilum* and *Pm. mortoniae* have been detected in the field with vaseline-covered glass slides (Larignon and Dubos, 1997; Eskalen and Gubler, 2001; Eskalen *et al.*, 2005). The extent to which the aerial inoculum is a source of pruning wound infection was assessed by Larignon and Dubos (2000), who found that in the case of *Pa. chlamydospora*, there was a marked increase of the fungus in pruned canes versus unpruned canes. In contrast, *Pm. aleophilum* occurred with the same frequency on pruned and unpruned canes. Conidia of *Pm. aleophilum* were not trapped in the winter, but were found during the vegetative period, indicating that this fungus might enter the plant via some other route than pruning wounds (Larignon and Dubos, 1997). Despite its ability to penetrate pruning wounds, Larignon and Dubos (2000) suggested that this might not be the way *Pm. aleophilum* invades grapevines in France, mainly because of the absence of spores in the air during winter pruning. These inoculation studies also revealed that infections were more serious and of a longer duration with early pruning. Pruning wounds were susceptible for 7–9 weeks during early pruning, whereas this decreased to 1–2 weeks when the vines were pruned during the period of bleeding shortly before budbreak.

Eskalen and Gubler (2001) found that airborne inoculum of *Pm. aleophilum* was present during the winter and spring, but also found conidia of *Pm. aleophilum* more frequently in early to midsummer. *Phaeoacremonium aleophilum* was also found in symptomatic berries (Eskalen *et al.*, 2001a), indicating that berries can become infected during the time when aerial conidia are present. The correlation of rainfall with the presence of aerial conidia showed that conidia of *Pa. chlamydospora* are released during and following rainfall in late winter and early spring in Californian vineyards (Eskalen and Gubler, 2001). Van Niekerk *et al.* (2005) correlated the occurrence of *Pa. chlamydospora* and *Phaeoacremonium* spp. in cordons of mature grapevines with rainfall patterns and found that *Pa. chlamydospora* predominantly occurred in winter rainfall regions, whereas *Phaeoacremonium* spp. had a similar distribution pattern, although with higher incidences in summer rainfall regions.

The presence of perithecia of *T. minima* (Pascoe *et al.*, 2004, Rooney-Latham *et al.*, 2005b) and *T. fraxinopennsylvanica* (Eskalen *et al.*, 2005) on moist incubated grapevine wood and grapevines in the field indicates that under the right environmental conditions ensuring enough moisture, ascospore dispersal could also be a source of inoculum. *In vitro* studies showed that forcible discharge of ascospores can take place in rehydrated perithecia, and led Rooney-Latham *et al.* (2004) to conclude that ascospores of *T. minima* may be an important inoculum source in the field. Aerial spore

catch data also showed that spores of *Pm. aleophilum/T. minima* were indeed present in the air after rainfall (Rooney-Latham *et al.*, 2004). Asexual and sexual spores could occur simultaneously in the field since conidial sporulation can occur on mycelium present on and around perithecia (from wood in moist chambers) as illustrated by Pascoe *et al.* (2004) and also seen by our own observations.

Diseased vines could release aerial inoculum from freshly cut pruning wounds or across the vine in places that favour anamorph or teleomorph sporulation. *Phaeomoniella chlamydospora* has been detected in wound sap and bark at soil level (Rooney *et al.*, 2001). The sporulation of the hyphomycete and the pycnidial synanamorphs of *Pa. chlamydospora* have been observed on protected wood surfaces inside deep cracks, 2- to 4-year-old pruning wounds and beneath the bark where injury resulted in exposed vascular tissue of grapevines (Edwards *et al.*, 2001a, Eskalen *et al.*, 2003). Mycophagous insects and mites could also disperse conidia when coming into contact with the phialidic conidial heads and pycnidial cirrhi of *Pa. chlamydospora* (Edwards *et al.*, 2001a).

Molecular identification and detection

The molecular identification of *Pa. chlamydospora* and *Phaeoacremonium* species has been done with RFLP (restriction fragment length polymorphisms) or phylogenetic analysis of the internal transcribed spacers (ITS 1 and 2) and 5.8 S rRNA gene, β-tubulin, actin and calmodulin gene regions. RFLP patterns of the ITS region were used to distinguish *Pa. chlamydospora*, *Pm. aleophilum*, *Pm. inflatipes* and *Pm. rubrigenum* (Tegli *et al.*, 2000). Dupont (2002) distinguished among five species of *Phaeoacremonium*, *Pm. aleophilum*, *Pm. inflatipes*, *Pm. parasiticum*, *Pm. rubrigenum* and *Pm. viticola* using PCR-RFLP markers from the ITS regions and partial β-tubulin gene. DNA phylogenies have been used in various studies to aid in the determination of new species of *Phaeoacremonium* (Dupont *et al.*, 2000; Groenewald *et al.*, 2001; Mostert *et al.*, 2003; Mostert *et al.*, 2005b). Groenewald *et al.* (2001) reported that the ITS region was not able to distinguish all species of *Phaeoacremonium*. Recently Mostert *et al.* (2005b) developed a polyphasic identification tool including morphological and cultural characters as well as β-tubulin sequences generated with primers T1 (O'Donnel and Ligelnik, 1997) and Bt2b (Glass and Donaldson, 1995). This *Phaeoacremonium* database can be accessed from the website of the Centraalbureau voor Schimmelcultures (www.cbs.knaw.nl/phaeoacremonium.htm).

Several primer sets have been developed to facilitate species identification. Species-specific primers have been developed for the detection of *Phaeomoniella chlamydospora* (PCL1 + PCL2 and Pch1 + Pch2) (Groenewald *et al.*, 2000; Tegli *et al.*, 2000) and *Phaeoacremonium aleophilum* (Pal1N + Pal2) (Tegli *et al.*, 2000) from the internal transcribed spacers ITS1 and ITS2 of the rRNA gene. Genus-specific primers for *Phaeoacremonium* (Pac1f + Pac2r) and species-specific primers for *Pa. chlamydospora* (Pmo1f + Pmo2r) were developed from the ITS1 and ITS2 regions for use in real-time PCR detection with SYBR® Green (Overton *et al.*, 2004).

These primers have been used to detect Petri disease fungi in soils, vines and in the different media used during the grafting process, and have shed more light on the epidemiology of these fungi. A nested PCR has been developed for the detection of *Pa. chlamydospora* and *Phaeoacremonium* spp. from soil and host tissue using the universal primers ITS4 and ITS5 as external primers, and two sets of internal primers (Eskalen *et al.*, 2001b). Ridgway *et al.* (2002) developed a DNA extraction protocol and used species-specific primers, Pch1 and Pch2, for the detection of *Pa. chlamydospora* in grapevine wood. Whiteman *et al.* (2002) adjusted this protocol to include a nested PCR using universal primers NS1 and ITS4 as well as the species-specific primers, Pch1 and Pch2, to detect *Pa. chlamydospora* in artificially infected and naturally infected soils (Whiteman *et al.*, 2003). *Phaeomoniella chlamydospora* DNA was also detected in nursery and vineyard soils with a PCR protocol developed by Damm and Fourie (2005), using the primers Pch1 and Pch2. Retief *et al.* (2005b) modified the DNA extraction protocol of Ridgway *et al.* (2002) and using the primers of Tegli *et al.* (2000) detected *Pa. chlamydospora* in grapevine wood. Overton *et al.* (2004) detected *Pa. chlamydospora* in roots, shoots and young trunks of drill-inoculated vines and *Pm. aleophilum* from trunks of naturally infected vines by making use of real-time PCR. PCR detection of *Pa. chlamydospora* also showed that the fungus is present during the propagation

process in the hydration tanks, grafting tools and callus media (Whiteman *et al.*, 2003; Retief *et al.* 2005a).

PCR detection is more reliable, sensitive and faster than traditional plating methods. As little as 1 pg of DNA could be detected (Ridgway *et al.*, 2002; Retief *et al.*, 2005b) from spiked wood material. In spiked soil samples, 500 pg DNA was detected with a non-nested approach and 50 fg of DNA with a nested PCR approach (Whiteman *et al.*, 2002). When traditional plating methods were compared with PCR detection, Retief *et al.* (2005b) found on average four times less positive detections with traditional plating methods in comparison with PCR detection in naturally infected grapevine material. By comparing molecular detection and traditional plating from hot water treated and non-treated dormant nursery vines, Retief *et al.* (2005b) demonstrated the inability of PCR detection to distinguish between dead and viable fungal material of *Pa. chlamydospora*.

Pathogenesis

Inoculation studies

Petri (1912) was the first scientist who could reproduce internal symptoms associated with esca. However, the role of the different fungi in the esca complex was largely unknown until the study of Larignon and Dubos (1997), especially since it became clear that the composition of the fungi in the complex might vary depending on the geographic region. Larignon and Dubos (1997) concluded that *Pm. aleophilum* and *Pm. chlamydosporum* (= *Pa. chlamydospora*) were pioneering fungi that colonised living wood, thus 'preparing' the wood for further colonization by the basidiomycete fungi, which were responsible for the typical decay associated with esca. Mugnai *et al.* (1996, 1999) reported in detail on the occurrence of the different fungal species at different stages of wood decay and decay progression. The lack of foliar symptoms after artificial inoculations with esca fungi could, however, not be explained, although various hypotheses have been proposed (Mugnai *et al.*, 1999). Sparapano *et al.* (2001a) induced foliar symptoms of esca in a study that was evaluated after three years either by individual inoculation with *Pm. aleophilum, Pa. chlamydospora, F. mediterranea* or by co-inoculations in various combinations on cv. Italia. Foliar symptom expression on cv. Matilde did not occur in any of the combinations, which might be attributed to cultivar susceptibility. An interesting observation was the non-synergistic, competitive association of *Pa. chlamydospora* and *Pm. aleophilum* and a marked antagonistic effect of *Pm. aleophilum* against *F. mediterranea* (*in planta*). This antagonistic effect was previously shown to occur when the interaction between the three fungi were investigated *in vitro* (Sparapano *et al.*, 2000b). Sparapano *et al.* (2001b) also investigated these interactions in the presence of callus tissue and found that when *F. mediterranea* was located between *Pm. aleophilum* and *Pa. chlamydospora* or near *Pm. aleophilum* with the *Pa. chlamydospora* colony on the outside, the growth rate of *F. mediterranea* decreased and its effect on callus growth was lower. Bruno and Sparapano (2005) also showed that colonies of *Pa. chlamydospora*, *Pm. angustius*, *Pm. inflatipes*, *Pm. parasiticum*, *Pm. rubrigenum* and *Pm. viticola* had an antagonistic effect on the colonies of *F. mediterranea* with *in vitro* malt extract assays. Pathogenicity studies were recently conducted with several newly described *Phaeoacremonium* species as well as *Pm. parasiticum* and *Pm. viticola* to determine their potential as decline pathogens (Halleen *et al.*, 2005). Grapevine spurs and trunks of cv. Periquita were inoculated with *Pm. krajdenii*, *Pm. venezuelense* and *Pm. subulatum*. Results obtained from this field trial (evaluated after 14 months) confirmed *Pa. chlamydospora* as the most aggressive pathogen since it produced the largest lesions in the trunks, as well as from the pruning wound inoculation. Furthermore, it was re-isolated more frequently than any of the other fungi, especially from the pruning wounds. However, all the *Phaeoacremonium* species were able to infect, colonise and produce lesions statistically different to those caused by the water control and the non-pathogen confirming their status as possible decline pathogens.

Mugnai *et al.* (1999) speculated that foliar and berry symptoms were mainly caused by substances that originate in the discoloured woody tissues of the trunk and branches that are then translocated to the leaves in the transpiration stream. Evidence to support this theory was given by Sparapano *et al.* (2001a) when they observed black measles (spotting on berries) on cv. Italia after *Pa.*

chlamydospora was inoculated through wounds on spurs and trunks of standing vines and on cv. Matilde after inoculation of branches and spurs with *Pm. aleophilum*. Furthermore, inoculation of pruning wounds with *Pa. chlamydospora* or *Pm. aleophilum* caused esca symptoms on leaves and berries on cv. Thompson Seedless, on one of the Grenache vines and no symptoms developed on cv. Cabernet Sauvignon (Feliciano *et al*., 2004). Significantly reduced shoot growth was also observed in shoots from infected spurs (Gubler *et al*., 2001b). One study was conducted thus far to reproduce esca symptoms by inoculating grape berries with *Pa. chlamydospora* and *Pm. aleophilum* (Gubler *et al*., 2004a), suggesting that lesions could be caused by airborne inoculum. Lesions on berries were larger when inoculated earlier in the season indicating that young, immature berries were more susceptible to infection than mature berries.

Scheck *et al*. (1998) successfully completed Koch's postulates by dipping roots of 2-month-old 'Carignane' grape seedlings in spore suspensions of *Pm. aleophilum, Pm. inflatipes*, *Pa. chlamydospora,* the fungi believed to be the causal organisms of young grapevine decline in California. Adalat *et al*. (2000) conducted various pathogenicity studies to shed some light on the epidemiology of young vine decline. Single bud cuttings (cv. Chardonnay) planted in sand inoculated with *Pm. aleophilum* and *Pa. chlamydospora* revealed that *Pm. aleophilum* was more readily re-isolated after three weeks and inhibited callus formation more than *Pa. chlamydospora*. Inoculation with these fungi significantly reduced number of roots, plant height, number of internodes, root elongation and dry weight of above-ground parts (Adalat *et al*., 2000). Wallace *et al*. (2004) inoculated the bases of seven rootstock and five scion varieties with *Pm. aleophilum* and *Pa. chlamydospora*. *Phaeomoniella chlamydospora* inhibited callus formation on all cultivars, but *Pm. aleophilum* did not, in contrast with previous findings of Adalat *et al*. (2000). Root initiation was not affected by either fungus. Light microscopy observations of tissue-cultured grapevines cv. Cabernet Sauvignon inoculated with *Pm. aleophilum* showed that rapid spread of these fungi in roots was through the vascular tissues and intercellular spaces of the cortex (Feliciano and Gubler, 2001). In inoculated shoots, spread of the fungus was initially through the intercellular spaces of the epidermis, cortex and pith. Rapid spread occurred in the intercellular spaces of the pith. Conidia were also seen in the pith area as well as in the xylem (Feliciano and Gubler, 2001).

Toxins and enzymes

Phytotoxic metabolites extracted from culture filtrates of *Pm. aleophilum* were identified as α-glucans (pullulans) and two naphthalenone pentaketides (scytalone and isosclerone). These metabolites caused foliar symptoms similar to those shown by esca-affected vines when absorbed by detached leaves or injected into woody tissue of shoots and branches of standing grapevines (Sparapano *et al*., 2000c). Scytalone caused pale green to chlorotic, rounded to irregular, interveinal or marginal spots when assayed on detached leaves of cv. Italia. Isosclerone caused large, coalescent chlorotic and necrotic spots followed by distortion of the lamina and withering (Evidente *et al*., 2000). Tabacchi *et al*. (2000) isolated p-hydroxybenzaldehyde and scytalone from culture filtrates of *Pm. aleophilum*. P-hydroxybenzaldehyde was also isolated from culture filtrates of *Pa. chlamydospora* and *F. mediterranea*. According to Tabacchi *et al*. (2000), the presence of molecules carrying the aldehyde function seems to play an important role in the toxicity of the fungi implicated in esca. Abou-Mansour *et al*. (2004) isolated seven compounds from liquid cultures of *Pm. aleophilum*; scytalone, isosclerone, 4-hydroxy scytalone, 2,4,8-trihydroxytetralone, 3,4,8-trihydroxytetralone, 1,3,8-trihydroxynaphthalene and flaviolin. Abou-Mansour *et al*. (2004) subjected grapevine callus to these compounds and found that scytalone and isosclerone hardly inhibited growth as reported by Evidente *et al*. (2000), and in fact increased growth. According to the results of Abou-Mansour *et al*. (2004), these metabolites should be divided into two classes: tetralones such as scytalone, isosclerone, 2,4,8-trihydroxytetralone and 3,4,8-trihydroxytetralone, which promote callus growth; and naphthoquinones like 2-hydroxyjuglone and flaviolin, which inhibit growth. Culture filtrates of *Pm. angustius*, *Pm. inflatipes*, *Pm. parasiticum*, *Pm. rubrigenum* and *Pm. viticola* caused phytotoxic reactions on detached leaves of 'Italia' or 'Sangiovese' grapevines and could be linked to the presence of isosclerone, scytalone and pullulan (Bruno and Sparapano, 2005).

As regard to enzyme activities Marchi *et al*.

(2001b) detected pectic enzyme production (polygalacturonase and polymethylgalacturonase) in *Pa. chlamydospora*, *Pm. aleophilum* and *Pm. rubrigenum*. Pectic enzymes would greatly aid the spread of a fungus inside its host. Analyses of the enzymes involved in lignin degradation showed that *Pm. aleophilum* expressed low specific activity for manganese peroxidase and high specific activity for both lignin peroxidase and laccase in contrast with *Pa. chlamydospora* that showed no activity for these enzymes (Mugnai *et al*., 1997; Del Río *et al*., 2004).

Management

Few studies have reported the direct effect of treatments or management strategies on *Phaeoacremonium* spp. In fact, most published research on the management of Petri and/or esca disease focused either on *Pa. chlamydospora*, or the effect of treatments on symptom expression in naturally infected grapevines or its effect on the complex of Petri disease pathogens (i.e. *Phaeomoniella* + *Phaeoacremonium* spp.). In this section we review research on the management of Petri and esca diseases with specific reference to effects on *Phaeoacremonium* species. Aspects that will be treated are *in vitro* studies, host resistance, curative and preventive management in nurseries, and preventative, ameliorative and curative management strategies in vineyards.

In vitro studies

Following the elucidation of the complexity of pathogens and/or diseases involved in esca (Mugnai *et al*., 1999), management studies have focused on the individual pathogens involved. Several demethylation inhibitor (triazole, pyrimidine, imidazole), benzimidazole, quinone-outside inhibitor fungicides that are effective inhibitors of mycelium growth and/or conidium germination of *Pa. chlamydospora* were identified through *in vitro* growth studies (Bisiach *et al*., 1996; Groenewald *et al*., 2000; Jaspers, 2001). *In vitro* sensitivity studies of fungicide groups other than triazole fungicides (Di Marco *et al*., 2000) were not reported for the *Phaeoacremonium* spp. involved in Petri and esca disease. However, Di Marco *et al*. (1999) demonstrated a synergistic effect between phosphorous acid and the phytoalexin resveratrol, on *Pm. aleophilum*: a mixture of these compounds inhibited *in vitro* mycelium growth, whereas the compounds alone demonstrated poor efficacy.

Host resistance

Several studies have been conducted to determine the difference in susceptibility of grapevine and rootstock cultivars to *Phaeoacremonium* species. Eskalen *et al*. (2001a) inoculated 20 rootstock cultivars with *Pm. aleophilum* and despite a wide range of rootstock susceptibility, did not observe any resistant cultivars, and concluded that rootstock susceptibility might not be an important factor in disease expression under natural conditions. Conversely, Feliciano *et al*. (2004) demonstrated that 'Thompson Seedless' was significantly more susceptible to *Pm. aleophilum* than 'Grenache' and 'Cabernet Sauvignon'. Santos *et al*. (2005) also reported that two *Vitis vinifera* cultivars ('Baga' and 'Maria Gomes') were more susceptible to *Pm. angustius* than a rootstock cultivar (R3309), and also noted differences between 'Baga' and 'Maria Gomes'. Marchi (2001a) studied the disease incidence and progression of esca in a mixed cultivar vineyard and found four susceptibility groups among the 17 cultivars, with 'Semillon' the most, and 'Roussanne' the least susceptible. An *in vitro* system making use of callus cultures and micropropagated shoot cultures have been used to assess host/pathogen interactions and could be used to select grapevines for resistance towards esca fungi (Sparapano *et al*., 2001b). However, none of these studies have shown complete or high levels of resistance in any rootstock or scion cultivar tested.

Control

Nurseries

In a survey of pathogens occurring in pruning wounds in rootstock mother blocks, Fourie and Halleen (2004a) found that *Phaeoacremonium* spp. occurred at very low levels (average incidence in 34 mother blocks of 4 cultivars was 0.12%). Nonetheless, due to the relatively high occurrence of *Pa. chlamydospora*, *Botryosphaeria* and *Phomopsis* spp., they recommended that sanitation and pruning wound protection should be practiced in rootstock mother blocks in order to limit infection by trunk disease pathogens. From such in-

fections, *Phaeoacremonium* spp. can disseminate via the vascular tissue into the rootstock canes, and rootstock canes and cuttings should be considered as a potential inoculum source (Mugnai *et al.*, 1999; Zanzotto *et al.*, 2001; Fourie and Halleen, 2002; Ridgway *et al.*, 2002; Halleen *et al.*, 2003; Edwards *et al.*, 2004b; Whiteman *et al.*, 2004; Retief *et al.*, 2005). Hot water treatment of rootstock cuttings prior to grafting for 30 min at 50°C proved to be the most effective means of reducing the levels of these infections (Crous *et al.*, 2001; Edwards *et al.*, 2004a, Fourie and Halleen, 2004b). In order to protect wounds from infection during the grafting processes, Fourie and Halleen (2004b, 2005) recommended the addition of quaternary ammonium sterilants (Sporekill®), fungicides (benomyl) or biological control agents (*Trichoderma harzianum*) to hydration and drench water. *Trichoderma*-treatments during grafting (Messina, 1999; Di Marco *et al.*, 2004) and soil amendments in field nurseries (Fourie *et al.*, 2001) resulted in nursery grapevines with stronger graft unions, root systems and with lower levels of pathogen infection. Good quality nursery grapevines would most likely reduce failure rate in vineyards (Morton, 2000; Stamp, 2001; Surico *et al.*, 2004) and herewith also the reduction of stress factors that would predispose plants to these diseases (Ferreira *et al.*, 1999; Gubler *et al.*, 2004b; Edwards and Pascoe, 2005). A final resort to lowering the levels of *Phaeoacremonium* species before planting in vineyards, is hot water treatment of dormant nursery grapevines (Fourie and Halleen, 2004b), a practice that would also reduce or eradicate infection by other pathogens, such as *Phytophthora cinnamomi* (Von Broembsen and Marais, 1978), *Cylindrocarpon* spp. (Halleen *et al.*, 2004) and *Meloidogyne javanica* (Barbercheck, 1986).

Vineyards

Given the stress-predisposition of grapevines to Petri and, most likely, to esca disease, vineyard establishment should be aimed at limiting stress factors that might adversely affect optimal and balanced root and vegetative growth, such as potted root development, J-rooting, nutrient deficiencies, water stress, and heavy crop loads during the first 3 years of establishment (Ferreira *et al.*, 1999; Gubler *et al.*, 2004b; Edwards and Pascoe, 2005).

In Europe, sodium arsenite applications to the trunk and arms of grapevines in the period between pruning and bud burst have been used to combat esca since the beginning of the 20th century (Mugnai *et al.*, 1999; Di Marco *et al.*, 2000; Larignon, 2004; Surico *et al.*, 2004). However, given the high toxicity of sodium arsenite, it was either banned or its use restricted. Later studies demonstrated the *in vivo* efficacy of fosetyl-Al used as trunk injections of mature grapevines, or as foliar sprays of potted grapevines and esca-diseased vineyards (Di Marco *et al.*, 2000; Di Marco and Osti, 2005). Trunk injections resulted in moderate disease incidence and a preservation of vine productivity, whereas the foliar sprays resulted, often even if not always, in significant reductions in the necrotic areas following inoculation with *Pm. aleophilum* or *Pa. chlamydospora* in potted plants and a reduction in esca disease incidence in vineyards. Root zone application with triazoles and trunk injections with triazoles or fosetyl-Al in esca diseased vineyards resulted in significant reductions in foliar symptom development, provided that the treatments were made in vineyards with a low disease incidence and with plants at an early stage of infection (Di Marco *et al.*, 2000). Collectively, these studies indicate that the attempts at curing grapevines of Petri and/or esca disease would largely be ineffective and will at most be ameliorative by limiting symptom expression and disease progress. Moreover, Edwards and Pascoe (2005) screened ameliorative treatments, which included composts, nutrient fertilizers, extra water, phosphonates and Brotomax, and found no single treatment to be effective.

Disease prevention therefore seems to be the most effective means in managing these diseases. This can firstly be done through the limitation or prevention of stress factors in young vines that might predispose it to these diseases (Ferreira *et al.*, 1999; Gubler *et al.*, 2004b; Surico *et al.*, 2004; Edwards and Pascoe, 2005). Sanitation practices, such as the removal of infected plants, plant parts and/or pruning debris, will lead to lower inoculum loads in vineyards. Additionally, pruning wound protection in vineyards will limit infection by these pathogens. Most research in this regard was aimed at prevention of pruning wound infection by *Eutypa lata*. However, Halleen and Fourie (2005) did demonstrate that benomyl and flusilazole reduced natural *Pa. chlamydospora* infections of pruning

wounds by *circa* 80%. The ability of *Trichoderma* spp. to colonise pruning wounds and reduce infection by pruning wound pathogens was also demonstrated in this study. Di Marco *et al*. (2004) also demonstrated pruning wound protection by *T. harzianum* and *T. longibrachiatum* against artificial infection by *Pa. chlamydospora*. Furthermore, wound management, in terms of trellising systems needing smaller or less severe wounds, will also affect infection by these pathogens and incidence of esca disease (Surico *et al*., 2004).

Conclusions

Eleven species of *Phaeoacremonium* have been isolated from grapevines. Of these, *Pm. aleophilum* has been the most commonly isolated species from grapevines in various countries across the world, followed by *Pm. parasiticum* and *Pm. viticola*. The host range of *Phaeoacremonium* includes mostly woody plants, larvae of bark beetles (*Pm. scolyti*) as well as humans. Both *Pm. aleophilum* and *Pm. parasiticum* have been isolated from a wide range of woody hosts, showing that these woody hosts can be a source of inoculum for grapevine infections. The perithecia of *T. minima* and *T. fraxinopennsylvanica* have been observed from moist incubated grapevine wood and on grapevines in the field, however, the occurrence of perithecia and influence of ascospore inoculum in the field still needs to be determined. Nevertheless the most ubiquitous species on declining or esca affected grapevines around the world surely remains *Pa. chlamydospora* (a species previously belonging, and, as a pathogen, still closely related to *Phaeoacremonium*).

Infections of propagation material can originate from infected mother plants or contamination during the nursery processes. Other sources of inoculum include soil (*Pa. chlamydospora* and *Pm. aleophilum*) and the air (*Pa. chlamydospora*, *Pm. aleophilum* and *Pm. mortoniae*). Molecular detection protocols for *Pa. chlamydospora* and *Pm. aleophilum* has made it possible to study the epidemiology of these organisms and might also in future be optimised to determine and certify the phytosanitary quality of propagation material.

The pathogenicity of *Pa. chlamydospora* and *Pm. aleophilum* has been extensively tested with root, shoot and pruning wound inoculation studies. These fungi can cause black streaking of xylem tissue, reduce plant growth, cause esca leaf symptoms and black lesions on grape berries. Several substances involved in pathogenesis have been identified including phytotoxic compounds (for *Pa. chlamydospora*, *Pm. aleophilum*, *Pm. angustius*, *Pm. inflatipes*, *Pm. parasiticum*, *Pm. rubrigenum* and *Pm. viticola*), pectic enzymes (for *Pa. chlamydospora*, *Pm. aleophilum* and *Pm. rubrigenum*) and enzymes involved in lignin degradation (for *Pm. aleophilum*).

Preventative control, including stress reduction, sanitation and pruning wound protection remain important measures in managing Petri disease and esca. Various fungicides have been tested on vineyards with esca symptoms of which fosetyl-Al and triazoles showed promising results. However, little is known about the effect of these and other fungicides on *Phaeoacremonium* species. Clean nursery practices are essential since the presence of *Pa. chlamydospora* has been detected throughout the grafting process. Hot water treatment of rootstocks prior to grafting has been shown to be effective in reducing infection levels of *Phaeoacremonium* species.

Various questions still remain unanswered about the different *Phaeoacremonium* species involved in Petri disease and esca, such as their involvement in disease development, symptom expression, epidemiology and response to fungicides. Several of these aspects are known for *Pm. aleophilum*, but remain unanswered for other species of *Phaeoacremonium*.

Acknowledgement

The authors would like to thank Lucie Morton for kindly providing photos of esca symptoms on grapevine berries.

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Accepted for publication: November 11, 2005