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 die-back of grapevines (*Vitis vinifera* L.). Internal symptoms can normally be seen in the trunk and cordon. 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 Cottrial, 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*.
(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, Pacliomyces punctatus, and Phaeoacremonium punctata (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, Phaeoacremonium (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 Phaeoacremonium parasiticum (Fig. 2C and D) in Argentina (Dupont et al., 2002), and Phaeoacremonium 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 Phaeoacremonium 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 fraxinopsylvanica (Eskalen et al., 2005; Rooney-Latham et al., 2005). DNA sequence data also confirmed the anamorph-telemorph relationship between Phaeoacremonium mortoniae and Togninia fraxinopsylvanica (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, Phaeoacremonium alvesii, Phaeoacremonium krajdenii, Phaeoacremonium parasiticum and Phaeoacremonium 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>
<thead>
<tr>
<th><em>Phaeoacremonium</em> species</th>
<th>Host</th>
<th>Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pm. aleophilum</em></td>
<td>Actinidia chinensis, Vitis vinifera, Olea europaea, Prunus pennsylvanica, Prunus sp., Salix sp.</td>
<td>Argentina, Australia, Austria, Canada, Chile, Iran, Italy, France, South Africa, Spain, Turkey, USA, Yugoslavia</td>
</tr>
<tr>
<td><em>Pm. alvesii</em></td>
<td>Dodoneae viscosa, human</td>
<td>Australia, Brazil, USA</td>
</tr>
<tr>
<td><em>Pm. amstelodamense</em></td>
<td>Human</td>
<td>Netherlands</td>
</tr>
<tr>
<td><em>Pm. angustius</em></td>
<td>Vitis vinifera</td>
<td>Portugal, USA</td>
</tr>
<tr>
<td><em>Pm. australiense</em></td>
<td>Vitis vinifera</td>
<td>Australia</td>
</tr>
<tr>
<td><em>Pm. griseorubrum</em></td>
<td>Human</td>
<td>Japan, USA</td>
</tr>
<tr>
<td><em>Pm. inflatipes</em></td>
<td>Hypoxylon truncatum, Nectandra sp., Quercus virginiana, Vitis vinifera</td>
<td>Chile, Costa Rica, USA</td>
</tr>
<tr>
<td><em>Pm. krajdenii</em></td>
<td>Human, Vitis vinifera</td>
<td>Canada, India, Japan, Norway, South Africa, USA, Zaire</td>
</tr>
<tr>
<td><em>Pm. mortoniae</em></td>
<td>Fraxinus excelsior, Fraxinus latifolia, Fraxinus pennsylvanica, Vitis vinifera</td>
<td>Sweden, USA</td>
</tr>
<tr>
<td><em>Pm. parasiticum</em></td>
<td>Actinidia chinensis, Aquilaria agallocha, Cupressus sp., human, Nectandra sp., Phoenix dactylifera, Prunus armeniaca, Quercus virginiana, Vitis vinifera</td>
<td>Argentina, Australia, Brazil, Canada, Chile, Costa Rica, Finland, Iran, Iraq, Italy, South Africa, Tunisia, USA</td>
</tr>
<tr>
<td><em>Pm. rubrigenum</em></td>
<td>Human</td>
<td>USA</td>
</tr>
<tr>
<td><em>Pm. scolyti</em></td>
<td>Vitis vinifera, larvae of Scolytus intricatus</td>
<td>France, South Africa</td>
</tr>
<tr>
<td><em>Pm. subulatum</em></td>
<td>Vitis vinifera</td>
<td>South Africa</td>
</tr>
<tr>
<td><em>Pm. tardicrescens</em></td>
<td>Human</td>
<td>USA</td>
</tr>
<tr>
<td><em>Pm. viticola</em></td>
<td>Sorbus intermedia, Vitis vinifera</td>
<td>Iran, France, Germany, South Africa, USA</td>
</tr>
<tr>
<td><em>Pm. venezuelense</em></td>
<td>Human, Vitis vinifera</td>
<td>Canada, South Africa, Venezuela</td>
</tr>
</tbody>
</table>

* 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; Croux and Gams, 2000; Dupont et al., 2000; Pascoe and Cottral, 2000; Pérès 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.

* Countries where *Phaeoacremonium* strains were isolated from human infections.
Pachytrype Berl. ex M.E. Barr and Pleurostoma were included in the Calosphaerales (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 calosphaeriaceseous genus Graphostroma. The Calosphaerales 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 Calosphaerales is uncertain (Petrak and Sydow, 1936; Barr, 1985). Fresh specimens were recently collected of types of the Calosphaerales, 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 Calosphaerales. A collection was also made of a new genus, described as Togninia 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 (Calosphaerales), and the Togniniaceae (Diaporthales). The Togniniaceae and the Gnomeniaceae (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. fraxinopennsylvania (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 ascocoma with necks (usually more prominent in vitro) uninate acsi that are oblong with a clearly truncate base and thickened apices; ascii 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 ascii is also found among the related genera, Togniniella and Pleurostoma, but these genera are distinguished from Togninia in the way the ascii 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 dieback 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.
sítica 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 *Magnaportheaceae* (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, *Pheomoniella* Crous & W. Gams, which resided within the *Chaetothryales* (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. Zanotto 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 discolorations 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 aerily 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. fraxinoparssylvaniaica* (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 cirri 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. rubiginenum* (Tegli et al., 2000). Dupont (2002) distinguished among five species of *Phaeoacremonium*, *Pm. aleophilum*, *Pm. inflatipes*, *Pm. parasiticum*, *Pm. rubiginenum* 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* (Pa1N + Pa2) (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. rubriigenum* 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.
Phytophthora 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: tetralone 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. Phaeomoniiella + 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-
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 hereewith 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; 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, Pa. aleophilum has been the most commonly isolated species from grapevines in various countries across the world, followed by Pa. parasiticum and Pa. viticola. The host range of Phaeoacremonium includes mostly woody plants, larvae of bark beetles (Pm. sapyti) as well as humans. Both Pa. aleophilum and Pa. 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 Pa. aleophilum) and the air (Pa. chlamydospora, Pa. aleophilum and Pa. mortoniae). Molecular detection protocols for Pa. chlamydospora and Pa. 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 Pa. 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, Pa. aleophilum, Pa. angustius, Pa. inflatipes, Pa. parasiticum, Pa. rubiginum and Pa. viticola), pectic enzymes (for Pa. chlamydospora, Pa. aleophilum and Pa. rubiginum) and enzymes involved in lignin degradation (for Pa. 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 Pa. aleophilum, but remain unanswered for other species of Phaeoacremonium.

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