Phaeomoniella chlamydospora gen. et comb. nov., a causal organism of Petri grapevine decline and esca

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Summary. Phaeomoniella is proposed as a new hyphomycete genus to accommodate Phaeoacremonium chlamydosporum, the most important fungal organism associated to Petri grapevine decline. Morphologically the genus is similar to Phaeoacremonium, but is distinguished from the latter based on its cultural characteristics, conidiophore morphology, and its uniformly straight, and slightly pigmented conidia. Petri grapevine decline is seen as an important component of the esca disease complex of grapevines.

Key words: Phaeomoniella, Phaeoacremonium, hyphomycetes, Petri grapevine decline, systematics.

Introduction

The esca disease complex is a well-known problem of grapevines worldwide. Among isolations from esca-diseased vines usually two basidiomycetous fungi are found to be present, namely Fomitiporia (Phellinus) punctata (Fr. ex Karsten) Murrill [often misidentified as Phellinus igniarius (L.: Fr.) Quel.] and Stereum hirsutum (Wild. : Fr.) Fr. Several other fungi have also been isolated from this disease complex. Petri (1912) reported having isolated fungi belonging to the genera Cephalosporium and Acremonium from brown-black streaks in the wood of declining vines and also preceding decay in esca diseased vines. These fungi could upon inoculation produce the same "brown wood-streaking" symptoms. These fungi have subsequently, on the basis of Petri’s description, been referred to Phaeoacremonium chlamydosporum and P. aleophilum respectively (Mugnai et al., 1999), two new species described by Crous et al. (1996) (see below).

Chiarappa (1959) consistently isolated a ‘Cephalosporium’ species (now Pm. chlamydosporum) from grapevines with black measles, and also demonstrated that it could cause wood discoloration. A strain of this fungus was fortunately deposited at CBS, and could thus be included in later studies aimed at revising Cephalosporium-like hyphomycetes (Gams, 1971). A similar fungus, Phialophora parasitica Ajello, Georg & C.J.K. Wang, was published by Ajello et al. (1974) as a new species isolated from a subcutaneous phaeohyphomycotic infection of a patient in the Stanford University Hospital in California, U.S.A. Hawksworth et al. (1976) reported that the latter fungus had been associated with various woody hosts, but noted that Chiarappa’s Vitis isolate (CBS 239.74), though overall similar, had shorter conidiophores with dark basal cells, almost hyaline conidiogenous cells and...
consistently straight, smaller conidia. In South Africa, Ferreira et al. (1994) conducted pathogenicity tests with similar grapevine isolates that induced a discoloration of wood, as well as extensive plugging of xylem tissue of plants in pot trials.

An accumulation of more isolates from grapevines, as well as from other woody hosts, made it quite obvious that these strains represented a natural, well-defined group distinct from Phialophora Medlar. They were distinct from Phialophora in having aculeate phialides with inconspicuous, non-flaring collarettes, resembling a pigmented form of Acremonium. Hence the name Phaeoacremonium. W. Gams et al. was introduced. The separation of Phaeoacremonium from Phialophora (in the strict sense a member of the Herpotrichiellaceae, Chaetothyriales, de Hoog et al., 1999) was also supported by molecular data published by Yan et al. (1995). In a phylogenetic study, Dupont et al. (1998) presented additional ITS sequence data to support the separation of Phaeoacremonium from Phialophora-like fungi, placing the genus in the Magnaporthaceae. Furthermore, Phm. chlamydosporum was shown to be more closely related to Phialophora verrucosa Medlar (Herpotrichiellaceae) than to Phaeoacremonium. We have subsequently confirmed these findings in a phylogenetic study incorporating most of the Phaeoacremonium strains preserved at CBS. Two respective data sets, namely of the ITS1, 5.8S and ITS2 region, as well as the beta-tubulin gene (M. Theron et al., in prep.) supported the findings of Dupont et al. (1998).

Morphologically, several differences have subsequently also been observed between Phm. chlamydosporum and other species of Phaeoacremonium. In Phm. chlamydosporum, conidiophores are green-brown, with light green to almost hyaline conidiogenous cells. Isolates also produce chlamydospor-like cells in culture (2% malt-extract agar; Biolab), and microsclerotia on 1.5% water agar. Furthermore, conidia are not dimorphic and hyaline, with heads at the apices of conidiogenous cells, pigmented, aseptate, smooth-walled, oblong-ellipsoidal to obovate and pale brown. In culture, fresh isolates have a white, yeast-like growth, which later forms dark green colonies, once again being distinct from other species of Phaeoacremonium. Finally, on carnation leaf agar (Fisher et al., 1982), and on infected canes incubated at 10°C under near-ultraviolet light in moist chambers, a Phoma-like synanamorph was observed to develop (Crous et al., 1996). Although yeast-like growth phases and pleomorphism have been noted for the Phialophora complex (Wang, 1979), this has never been observed for species of Phaeoacremonium sensu stricto.

Taxonomic part

Based on the molecular, morphological and cultural differences discussed above, a new genus is introduced below to accommodate Phm. chlamydosporum.

Phaeomoniella Crous et W. Gams, gen. nov.

Genus hyphomycetum Phaeoacremonii simile, sed conidiis rectis, pigmentatis, conidiophoris deorsum obscure viridi-brunneis et phialidibus subhyalinis, crescentia juveni levadiniformi, synanamorphosi Phomae simili, et propagulis chlamydosporalibus differens.


A hyphomycete genus morphologically similar to Phaeoacremonium, but distinct in having straight, pigmented conidia, dark green-brown conidiophores with light green to hyaline conidiogenous cells, a yeast-like growth in young colonies, a Phoma-like synanamorph, and producing chlamydospor-like structures in culture. Colonies on MEA (reverse) grey-olivaceous to olivaceous-black, with sparse aerial mycelium. Mycelium consisting of branched, septate hyphae; hyphae simple, or occurring in strands, verruculose to tuberculate, green-brown, becoming lighter to hyaline towards the conidiogenous region. Chlamydospor-like structures present, forming microsclerotia on water agar. Conidiophores micronematous, arising from aerial or submerged hyphae, erect, simple, subcylindrical, green-brown, becoming lighter toward the tip, verruculose to smooth, septate. Conidiogenous cells terminal, monopodial, elongate-ampulliform to lageniform or subcylindrical, with a terminal, narrowly funnel-shaped collarette. Conidia becoming aggregated into round, slimy heads at the apices of conidiogenous cells, pigmented, aseptate, smooth-walled, oblong-ellipsoidal to obovate, straight. Téléomorph unknown. Synanamorph Phoma-like, induced in culture and on infected canes.

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Type species: *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams

**Phaeomoniella chlamydospora** (W. Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams, comb. nov.  


_Synanamorph: Phoma-like sp._

**Type:** Italy, on stems and roots of *Vitis vinifera*, 25 Jan. 1995, L. Mugnai (CBS 229.95, dried holotype specimen and ex-type culture, dried isolate lodged at PREM).

**Mycelium** consisting of branched, septate hyphae occurring singly or in strands of up to 10, tuberculate (warts to 1 μm) to verruculose, green-brown walls and septa darker, becoming lighter towards the conidiogenous region, 2-4 μm wide. Chlamydospor-like structures abundant in the type strain, but sparse in others; globose to subglobose, mostly singular, rarely in chains of up to 5, olivaceous and smooth to green-brown and tuberculate, 7-15 μm long, 5-17 μm diam. **Conidiophores** macronematous, arising from aerial or submerged hyphae, erect, simple, cylindrical with an elongate-ampulliform to lageniform apical cell, green-brown, thick-walled at the base, becoming thinner-walled and lighter green-brown towards the apex, verrucose to smooth, 1-3-septate, 12-70 μm tall, 1.5-4.0 μm wide. **Conidiogenous cells** solitary, terminal, monophialidic, light green to subhyaline, smooth, elongate-ampulliform to lageniform or subcylindrical, 8-20 μm long, 1.5-4.0 μm wide at the swollen part, 1.0-1.5 μm wide at the apex, with a terminal, narrowly funnel-shaped collarette, 0.5-2.0 μm long and wide. **Conidia** becoming aggregated in fascicles, then forming round, slimy heads at the apices of the conidiogenous cells, subhyaline, oblong-ellipsoidal to obovate, permanently straight, (1.5-) 2.0-2.5x1.0-1.5 μm.

_Synanamorph:_ conidionata brown, pycnidial, globose, up to 70 μm diam. **Conidiophores** pale brown, subcylindrical, smooth, 1-multiseptate, 5-18x2-3 μm. **Conidiogenous cells** monophialidic, terminal and intercalary, variable in shape, but frequently subcylindrical to oblong-ellipsoidal, 3-9x2-3 μm. Conidia exuding from pycnidia in a cirrus, hyaline, oblong-ellipsoidal to obovate, permanently straight, (1.5-) 2.0-2.5x1.0-1.5 μm.

**Cultural characteristics.** Colonies on MEA (reverse) grey-olivaceous to olivaceous-black (23°i—23°k according to Rayner, 1970), reaching a radius of 5-6 mm at 25°C in the dark after 8 days. Cardinal temperatures for growth: 15°C (min.), 25°C (opt.), below 35°C (max.).

**Hosts.** *Vitis vinifera*.

**Distribution.** Occurring in most countries where grapevines are grown.

**Additional cultures examined.** South Africa, Cape Province, Stellenbosch, on stems and roots of *Vitis vinifera*, 1991, E. Venter (CMW 2255, STE-U 774); *V. vinifera*, 1994, S. Ferreira (STE-U 809); on stems of *V. vinifera*, 1990, M.J. Wingfield (CBS 161.90); on stems of *V. vinifera*, 1991, M.J. Wingfield (CBS 103.92-105.92). United States, California, on stems and roots of *V. vinifera*, 31 Aug. 1966, L. Chiarappa (CBS 239.74, IMI 192881).

**Discussion**

*Phaeomoniella* is obviously an anamorphic member of the **Herpotrichiellaceae**. We regard it as sufficiently distinct from *Phialophora*, which has short, swollen, darkly pigmented phialides with a flaring collarette (de Hoog et al., 1999).

Of the remaining five species of *Phaeoacremonium*, _Pm. aleophilum_ W. Gams, Crous, M.J. Wingf. & L. Mugnai, _Pm. angustius_ W. Gams, Crous & M.J. Wingf. and _Pm. inflatipes_ W. Gams, Crous & M.J. Wingf. have been reported from grapevines (Crous et al., 1996; Table 1). Furthermore, in separate pathogenicity studies _Phaeomoniella chlamydospora, Pm. aleophilum_ and _Pm. inflatipes_ have been shown to induce a decline of young grapevines (Scheck et al., 1998). _Pa. chlamydospora_, however, is consistently isolated from mature vines showing “brown wood-streaking”, from rooted grafted cuttings (Bertelli et al., 1998), from black streaks and brown-red wood in esca diseased plants and in the rootstock of declining vines reported as suffering from Petri grapevine decline or black goo (Morton, 1999; Mugnai et al., 1999). Internal symptoms of the decline consist in a black exudate that oozes from the xylem when vines are cut in cross section, and are frequently arranged in groups of spots close to the pith, or around annual growth rings. It is
Fig. 1, 2. *Phaeomoniella chlamydospora* and its Phoma-like synanamorph on carnation leaf agar. Fig. 1. Conidiophores and conidia of *Pa. chlamydospora*. Fig. 2. Conidiophores and conidia of the Phoma-like synanamorph. Bar = 10 μm.
Fig. 3-8. *Phaeomoniella chlamydospora* and its Phoma-like synanamorph on carnation leaf agar. Fig. 3-5. *Pa. chlamydospora*. Fig. 3. Conidiophores and conidia forming on aerial mycelium. Fig. 4. Solitary conidiophore with terminal, subhyaline conidiogenous cell. Fig. 5. Conidia. Fig. 6-8. Phoma-like synanamorph. Fig. 6. Pycnidium on carnation leaf. Fig. 7. Dense cluster of conidiophores with conidiogenous cells. Fig. 8. Conidia. All bars = 10 μm, except 6 = 20 μm.
Table 1. Origin of species and isolates of *Phaeoacremonium* and *Phaeomoniella* (from Crous et al., 1996 or CBS database).

<table>
<thead>
<tr>
<th>Medical isolates</th>
<th>Other substrates</th>
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<tbody>
<tr>
<td><em>Phaeomoniella chlamydospora</em></td>
<td><em>Vitis vinifera</em>, Argentina, Australia, Chile, Europe, S. Afr., California USA, New Zealand</td>
</tr>
<tr>
<td><em>Phaeoacremonium aleophilum</em></td>
<td><em>Actinidia sinensis</em>, Italy (CBS) Bark, trop. rain forest, Papua New Guinea (CBS) <em>Olea europaea</em>, Italy (CBS) <em>Vitis vinifera</em>, California, Italy, S. Afr., Yugoslavia</td>
</tr>
<tr>
<td><em>Phaeoacremonium angustius</em></td>
<td><em>Olea europaea</em>, Italy (CBS), Soil, Argentina (CBS) <em>Vitis vinifera</em>, California, Italy (CBS)</td>
</tr>
<tr>
<td><em>Phaeoacremonium inflatipes</em></td>
<td><em>Actinidia sinensis</em>, Italy <em>Nectandra</em> sp., Costa Rica <em>Olea europaea</em>, Italy <em>Quercus virginiana</em>, Texas Soil, Tahiti <em>Sorbus intermedia</em>, Germany <em>Vitis vinifera</em>, California, Italy (CBS)</td>
</tr>
<tr>
<td><em>Phaeoacremonium parasiticum</em></td>
<td><em>Actinidia sinensis</em>, Italy (CBS) <em>Prunus armeniaca</em>, Tunisia <em>Quercus virginiana</em>, USA (CBS)</td>
</tr>
<tr>
<td><em>Phaeoacremonium rubrigenum</em></td>
<td><em>Fraxinus excelsior</em>, Sweden (CBS) <em>Phoenix dactylifera</em>, Iraq (CBS)</td>
</tr>
</tbody>
</table>

our opinion, however, that esca is an interaction of the basidiomycete wood-rotting fungi with those species causing Petri grapevine decline (*Pa. chlamydospora*). Further research is required, however, to unravel the complicated etiology and interactions of these various pathogens in the esca disease complex of grapevines.

**Acknowledgement**

Although only a few strains are cited above, we have received numerous isolates from many pathologists worldwide, for which we are eternally grateful. The discussion group at the grapevine trunk disease meeting in Siena also singled out the distinct pathogenicity of *Pa. chlamydospora*. We gratefully acknowledge all participants at this meeting for freely sharing their information, and thus contributing to this paper. Laura Mugnai and Lucie Morton are especially thanked for keeping us focused and involved on this disease complex.

**Literature cited**


Bertelli E., L. Mugnai and G. Surico, 1998. Presence of *Phaeoacremonium chlamydosporum* in apparently


