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

Pedro W. Crous[1] and Walter Gams[2]

[1] Department of Plant Pathology, University of Stellenbosch, P. Bag X1, Matieland 7602, South Africa
[2] Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, Netherlands

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.) Quél.] and *Stereum hirsutum* (Willd. : 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

Corresponding author: P.W. Crous
Fax: +27 21 8084956
E-mail: pwc@maties.sun.ac.za

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* Link : Fr.; 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, *Pm. 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 *Pm. chlamydosporum* and other species of *Phaeoacremonium*. In *Pm. chlamydosporum*, conidiophores are green-brown, with light green to almost hyaline conidiogenous cells. Isolates also produce chlamydospore-like cells in culture (2% malt-extract agar; Biolab), and microsclerotia on 1.5% water agar. Furthermore, conidia are not dimorphic and hyaline as in other species of *Phaeoacremonium*, but are consistently straight, 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 *Pm. 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*.

Species typica: *Pa. chlamydospora* (W. Gams, Crous, M.J. Wingf. et L. Mugnai) Crous et W. Gams.

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 chlamydospore-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. *Chlamydospore*-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, monophialidic, 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. *Teleomorph* unknown. *Synanamorph* Phoma-like, induced in culture and on infected canes.

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

Etymology: resembling a smaller conidial form of *Phaeoacremonium*.

Phaeomoniella chlamydospora (W. Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams, comb. nov. Fig. 1-8

Basionym: *Phaeoacremonium chlamydosporum* W. Gams, Crous, M.J. Wingf. & L. Mugnai, Mycologia 88, 792. 1996.

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 isotype 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. Chlamydospore-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-)3.0-4.0(-4.5)x1.0-1.5(-2.0) µm. *Synanamorph*: conidiomata 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 *Pa. 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

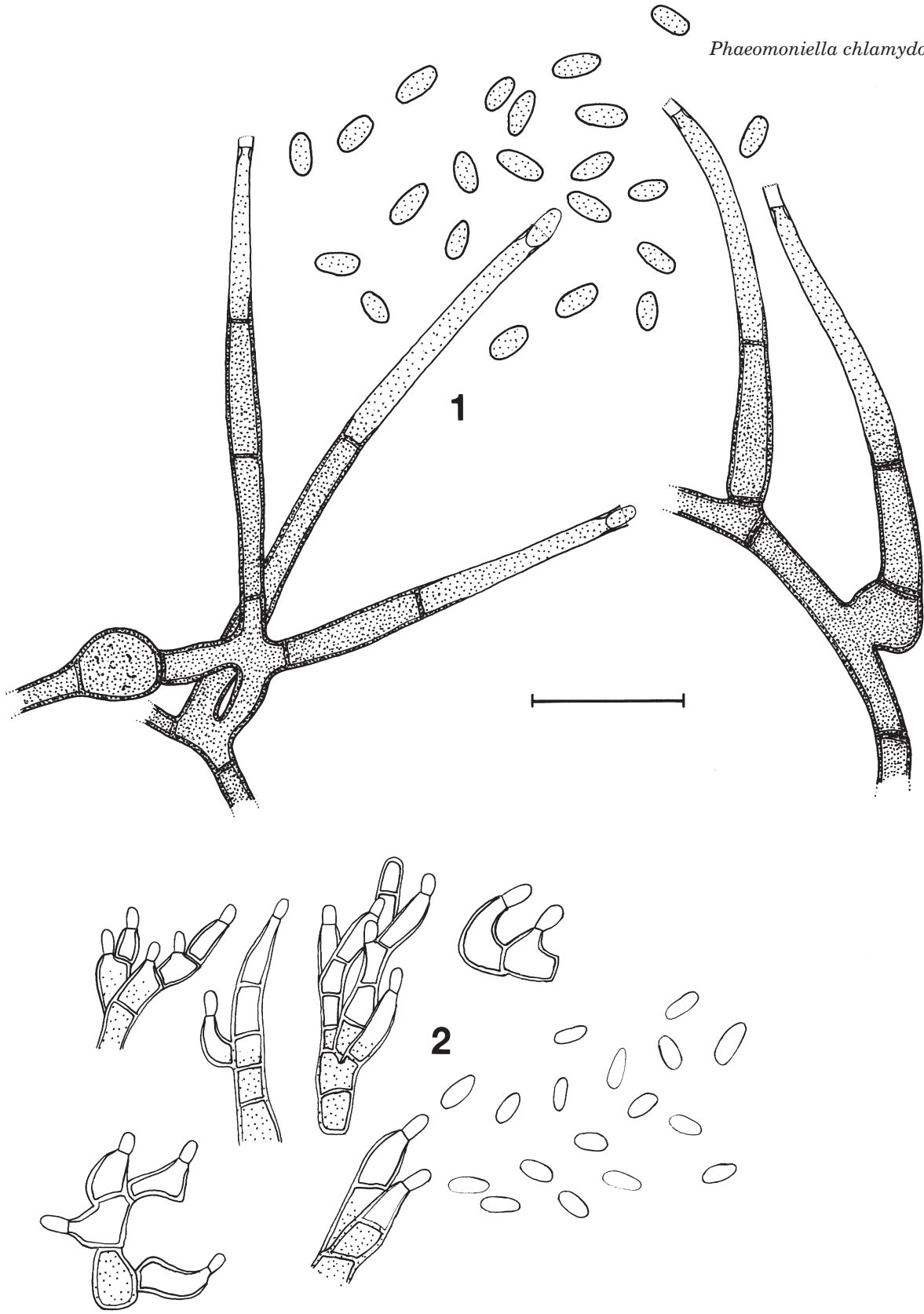

Fig. 1, 2. *Phaeomoniella chlamydospora* and its Phoma-like synanamorph on carnation leaf agar. Fig. 1. Conidiophores and conidia of *Pa. chlamydospora*. Fig. 2. Conidiophores and conidia of the Phoma-like synanamorph. Bar = 10 μm.

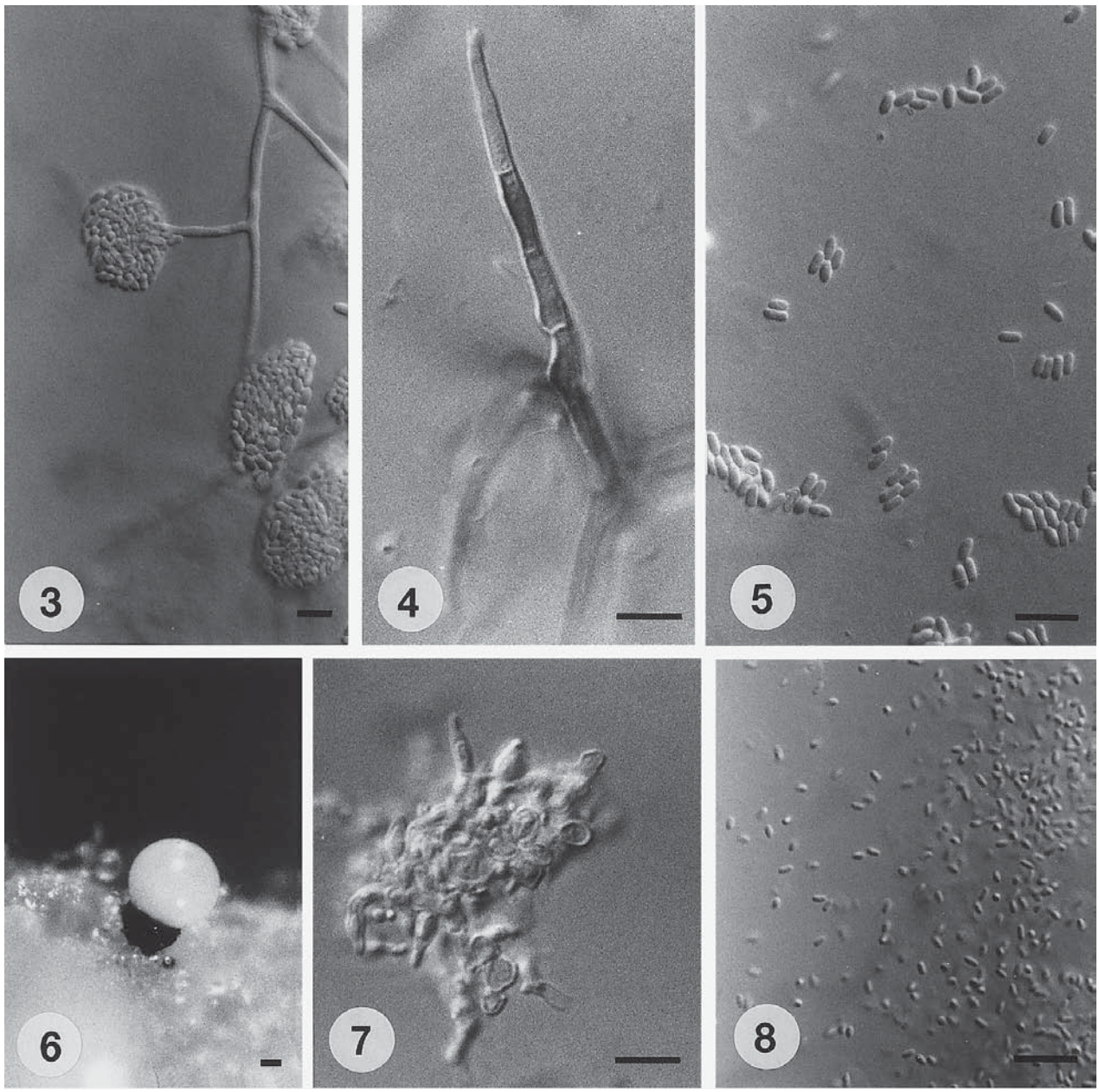

Fig. 3-8. *Phaeomoniella chlamydospora* and its Phoma-like synanamorph on carnation leaf agar. Fig. 3-5. *Pa. chlamydospora*. Fig. 3. Conidiophores and conidia forming on aerial mycelium. Fig. 4. Solitary conidiophore with terminal, subhyaline conidiogenous cell. Fig. 5. Conidia. Fig. 6-8. Phoma-like 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).

Medical isolates	Other substrates
Phaeomoniella chlamydospora	
	Vitis vinifera, Argentina, Australia, Chile, Europe, S. Afr., California USA, New Zealand
Phaeoacremonium aleophilum	
	Actinidia sinensis, Italy (CBS)
	Bark, trop. rain forest, Papua New Guinea (CBS)
	Olea europaea, Italy (CBS)
	Vitis vinifera, California, Italy, S. Afr., Yugoslavia
Phaeoacremonium angustius	
	Olea europaea, Italy (CBS), Soil, Argentina (CBS)
	Vitis vinifera, California, Italy (CBS)
Phaeoacremonium inflatipes	
Foot abscess, USA (CBS)	*Actinidia sinensis*, Italy
Mycetoma on foot, Venezuela	*Nectandra* sp., Costa Rica
Synovial fluid, California	*Olea europaea*, Italy
Subcutan. cyst, Hawaii (CBS)	*Quercus virginiana*, Texas
Toenail, Finland	Soil, Tahiti
Subcutan. granulom. lesion, South Carolina	*Sorbus intermedia*, Germany
	Vitis vinifera, California, Italy (CBS)
Phaeoacremonium parasiticum	
Subcut. infection, California	*Actinidia sinensis*, Italy (CBS)
Synovial fluid in lesion, USA (CBS)	*Prunus armeniaca*, Tunisia
	Quercus virginiana, USA (CBS)
Phaeoacremonium rubrigenum	
Periton. fluid in peritonitis, Texas (CBS)	? *Fraxinus excelsior*, Sweden (CBS)
Pneumonia patient, USA	? *Phoenix dactylifera*, Iraq (CBS)
Subcutan. lesion, South Carolina (CBS)	
Subcutan. phaeohyphomycosis, Japan (CBS)	

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.

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