

REVIEW - 9TH SPECIAL ISSUE ON GRAPEVINE TRUNK DISEASES

Hymenochaetales associated with esca-related wood rots on grapevine with a special emphasis on the status of esca in South African vineyards

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Summary. Esca disease is a problem on grapevines worldwide. This disease complex is characterised by several external and internal symptoms including foliar tiger-stripe chlorosis and necrosis, dieback, wood necrosis and white rot. The causal organisms of esca are primarily *Phaeoacremonium chlamydospora*, several *Phaeoacremonium* species and basidiomycete species from the order Hymenochaetales, the latter ones responsible for causing the white rot symptom. Basidiomycete species causing the wood rot symptom of esca differ among grapevine-growing areas worldwide. South African vineyards are unique in having a minimum of ten different basidiomycete taxa from five different genera associated with the esca complex. In general, Hymenochaetales species are associated with white rot on woody plants and there are several species that are economically important to the agricultural and forestry industries. Few Hymenochaetales species have been described from the African continent, though this review is an indication of the previously unknown diversity of these fungi in Southern Africa.

Key words: esca, grapevine, basidiomycetes, Hymenochaetales, *Fomitiporia*.

A brief introduction to grapevine trunk diseases

Grapevine trunk diseases include Phomopsis, Botryosphaeria and Eutypa dieback, black foot, Petri disease, and esca complex (*sensu* Surico, 2009), which affect young and mature vineyards in several ways causing an overall loss of longevity. They affect the longevity of individual *Vitis vinifera* L. vines by causing the deterioration of structural wood, leading to gradual dieback of the arms and trunk and the eventual decline and death of the entire plant (Edwards *et al.*, 2001; Rumbos and Rumbou, 2001; Petit *et al.*, 2006; Calzarano *et al.*, 2009). This leads to a gradual loss in

productivity per plant. In the grapevine leaf stripe disease, within the esca complex, grape quality may be compromised due to uneven ripening (Mugnai *et al.*, 1999) and losses in grape quality will affect the alcohol content and the flavour components of wine (Mugnai *et al.*, 1999; Calzarano *et al.*, 2001, 2009; Pasquier *et al.*, 2013). In table grapes, where the appearance of clusters is its most important characteristic, yield losses may be due to cosmetic damage caused by uneven colouration (Mugnai *et al.*, 1999).

Petri disease, also thought of as one of esca related syndromes, is a major problem in South Africa, and is an important disease in nurseries (Halleen *et al.*, 2003). It was previously known as Black Goo or young grapevine decline, and affects nursery plants and young vines in the field (Fourie and Halleen, 2004). Petri disease has been mainly associated with *Phaeoacremonium chlamydospora* (W. Gams, Crous, M.J.

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Wingf. & Mugnai) Crous and W. Gams and *Phaeoacremonium* W. Gams, Crous & M.J. Wingf. species (Crous and Gams, 2000; Mostert *et al.*, 2006). To a lesser extent species of *Cadophora* have also been associated with Petri diseased vines (Halleen *et al.*, 2007; Gramaje *et al.*, 2011). Symptoms include wilting, decline and dieback in young plants and graft-failure in nursery grafted cuttings caused by blockage of xylem vessels by fungal colonisation or plant response to colonisation (Edwards *et al.*, 2001; Edwards *et al.*, 2007; Mutawila *et al.*, 2011).

Esca complex is far more common in South Africa than previously thought (White *et al.*, 2011b). Although certain external symptoms of esca such as dead spurs overlap with *Eutypa* and *Botryosphaeria* dieback and in some cases its foliar symptoms (grapevine leaf stripe disease) were even regarded, even if not accepted by all authors, as related to *Botryosphaeria* dieback (Larignon *et al.*, 2001; Surico, 2006; Lecomte *et al.*, 2012), the disease has a distinct and complicated array of external and internal symptoms.

The importance of esca

Esca was first described in detail by Ravaz (1898) and after by Viala (1926) in France. Finally the disease was described as a complex of different diseases (mainly white rot and grapevine leaf stripe disease) (Surico *et al.*, 2006, Surico, 2009). The disease (here referred to as esca in general terms), has been the subject of extensive study in most grapevine growing regions of the world since the 1990's, when it became a prominent problem in Europe, made worse by the banning of sodium-arsenite as fungicide treatment in the EU (Mugnai *et al.*, 1999; Surico, 2000). Reizenzein *et al.* (2000) estimated a 2.7% annual increase in vineyards showing foliar symptoms in Austria over several years. The disease affected between 11 and 19% of vines in affected vineyards throughout Italy (Surico *et al.*, 2000). A marked increase occurred between results published in 2000 and 2006, where increases between 30% and 51% were found in surveyed vineyards (Surico *et al.*, 2006). A three year survey of vineyards in Spain, revealed that 38% of vineyards had vines showing external symptoms of esca (Armengol *et al.*, 2001). A survey of vineyards in Catalonia (Spain) showed that 19% of the 192 vines showing decline had external symptoms of esca (Luque *et al.*, 2009). Kuntzmann *et al.* (2010) estimat-

ed that up to 10% of plant material replacements in the Alsace region of France may be due to esca and Bruez *et al.* (2012) found esca and *Botryosphaeria* dieback symptoms on 0.9 and 8.2% of French vines, respectively, recorded in a survey of five different grapevine growing regions. They found an overall incidence of esca/*Botryosphaeria* dieback affecting between 54 and 95% of vineyards, depending on the region (Bruez *et al.*, 2012). Replacement costs, yield loss, the costs of preventative control measures and increased labour and material costs linked to corrective measures make up the total cost associated with trunk disease infection (Siebert, 2001). Many studies have been conducted on effective preventative strategies since treatment mainly consists of removing infected material. Preventative strategies are generally focused on wound protection, as wounds caused by viticultural practices such as pruning and suckering are the main ports of entry for the grapevine trunk disease pathogens, including the esca fungi (Chapuis *et al.*, 1998; Epstein *et al.*, 2008; Fischer, 2009b; Makatini *et al.*, 2012; Luque *et al.*, 2014). In South Africa, esca-affected vines have been found in all the major wine-, table-, and raisin production areas (White *et al.*, 2011b). The exact cost as a result of these infections has not been determined. However, the effect of esca and other grapevine trunk diseases on the productive lifespan of South African vineyards is substantial. At the moment only 38% of all planted wine grapes in South Africa (28% of red cultivars) are older than 16 years (Anonymous, 2014).

Symptomatology associated with *Hymenochaetales* species of grapevine

Esca includes an array of symptoms which have been observed and studied on grapevines in most grape-growing regions of the world (Chiarappa, 1959; Larignon and Dubos, 1997; Mugnai *et al.*, 1999; Auger *et al.*, 2005; Fischer *et al.*, 2005; White *et al.*, 2011b). The definition of esca and the related symptoms have been an issue of debate during the past two decades.

After an extensive survey of esca-infected vineyards in South Africa, White *et al.* (2011b) described several types of symptoms associated with the disease under South African conditions (Figure 1). Externally, dieback was common. Apoplexy, the sudden death of an entire vine during hot weather was also observed, though not frequently. Leaf stripe



Figure 1. Symptoms on esca diseased grapevines in South Africa. a. Tiger stripe leaf symptoms on cv. Sauvignon blanc. b. "Black measles" on berries of cv. Hanepoot. c, d. White rot and internal wood symptoms on cv. Pinotage (c.) and Chardonnay (d.). e. Leaf symptoms and decline on cv. Hanepoot. f. Apoplexy of a Cabernet Sauvignon vine (f. from White *et al.*, 2011b).



Figure 2. Symptoms associated with esca in Europe, chlorotic leafroll in Chile and hoja de malvón in Argentina. Esca (grapevine leaf stripe disease) symptoms on a white wine grape cultivar in a. Siebeldingen, Germany; b. Tuscany, Italy; c. Mosel, Germany; d. leaf and berry symptoms on white cultivars in Siebeldingen, Germany; e. vine showing apoplexy in Siebeldingen, Germany; Zig-zag shoots typical of chlorotic leaf roll f. and g. in Santiago, Chile and h. Casablanca, Chile; i. chlorotic leafroll affected leaf (left) vs healthy leaf (right) of Malbec in Talca, Chile; j. trunk of 10-year-old chlorotic leafroll affected vine, Casablanca, Chile; k. and l. typical symptoms of hoja de malvón on grapevines in Argentina (both photos supplied by Cecilia Césari from INTA, Mendoza, Argentina).

symptoms were sometimes observed during the period between January and March. Berry symptoms were observed as discolouration and shrivelling, though black spots similar to Californian black measles were observed on a single occasion in one vineyard, although it is known to occur from time to time on certain cultivars. Five internal symptom types were recorded, namely white rot, black and brown streaking, brown necrosis within white rot, V-shaped necrosis and a brown/red black margin surrounding the other symptom types (White, 2010; White *et al.*, 2011b; Surico, 2009). The external symptoms corresponded to Marais (1981), who reported dieback, decline, apoplexy, and leaf stripe symptoms appearing on affected vines. In general, these internal and external symptoms correspond to esca proper as described in Europe (Figure 2 a-e) (Mugnai *et al.*, 1999; White *et al.*, 2011b) and not to chlorotic leafroll (Figure 2 f-j) and “Hoja de malvón” (Figure 2 k-l), as described from Chile and Argentina, respectively. “Hoja de malvón”-affected vines are characterized by leaves that are smaller than normal, chlorotic and the edges rolled downward, resembling a geranium leaf. The shoots are reduced in growth, and the clusters are smaller and sparser with berries of uneven size (Gatica *et al.*, 2000). Internal symptoms in the trunk or cordon of “Hoja de malvón”-affected vines are characterized by a yellowish necrosis of soft consistency surrounded by a black line and a brownish area, and a sectorial light brown necrosis of hard consistency surrounded by a brown zone. Black spots can sometimes also be observed at the margins of these necrotic areas (Gatica *et al.*, 2000).

Basidiomycetes associated with esca

During early studies on esca, Ravaz (1909) identified *Fomes igniarius* (L.) Fr. (later renamed *Phellinus igniarius* (L.) Quél.) based on fruit bodies found on vines in France, but was unable to prove the pathogenicity of this organism. Vinet (1909) found fruit bodies of *Stereum hirsutum* (Willd.) Pers. on vines in France. Viala (1926) also found *S. hirsutum* associated with diseased vines in France, but was unable to subject the organism to conclusive pathogenicity trials. Chiarappa (1997) performed pathogenicity trials in California that proved that *P. igniarius* and not *S. hirsutum* was the cause of wood rot associated with esca. The extensive and seminal study by Larignon and Dubos (1997) connected *Phellinus punctatus* (P. Karst.)

Pilát with wood rot in esca through isolation studies conducted in French vineyards. Today, it is generally accepted that this *P. punctatus* is synonymous to *Fomitiporia punctata* [P. Karst] Murrill; (see Fiasson and Niemelä, 1984; Fischer, 1996). During an extensive survey of esca-infected vineyards in Italy, Cortesi *et al.* (2000) found only *F. punctata* on infected vines and concluded that it must be the main source of wood decay in esca. Fischer (2002) found that strains collected from *Vitis* and some other hosts were different from the boreal *F. punctata* strains and, based on molecular data, mycelial growth and pairing tests introduced the new species *Fomitiporia mediterranea* M. Fischer.

Today, *F. mediterranea* is the main wood rotting basidiomycete associated with esca in Europe and the Mediterranean regions. In Australia, *Fomitiporia australiensis* M. Fisch., J. Edwards, Cunningt. and Pascoe has been associated with esca (Fischer *et al.*, 2005). In South America, the main wood rotting organism associated with local trunk diseases “hoja de malvón” (Argentina) and chlorotic leaf roll (Chile) are *Inocutis jamaicensis* (Murrill) A.M. Gottlieb, J.E. Wright & Moncalvo and an unidentified species of *Fomitiporella* Murrill, respectively (Gatica *et al.*, 2004; Auger *et al.*, 2005; Lupo *et al.*, 2006). In North America, Chiarappa’s “*P. igniarius*” was widely associated with esca-related rot in the San Joaquin Valley of California (Chiarappa, 1959); however, *P. igniarius sensu stricto* has never been reported from North America (Fischer and Binder, 2004). *Fomitiporia polymorpha* M. Fischer has been associated with esca-related rot in California, though only on a single occasion (Fischer and Binder, 2004; Fischer, 2006). No further work has been published on the occurrence and cause of the white rot symptom of esca in the United States.

Esca has also been reported in South Africa in the past (Marais, 1981), and in recent years several pure fungal cultures have been isolated from wood decay, a symptom which occurs often, though fruit bodies are seldom found (White *et al.*, 2011a). Fischer (2006) placed several of the South African isolates within *Fomitiporia* based on ITS phylogeny, but fruit bodies were not available at that time and no formal descriptions were made. White *et al.* (2011a) attempted to further identify some of the South African mycelial isolates through ITS phylogeny and found ten discrete taxa falling under the order Hymenochaetales (Figure 3). These taxa included single *Fomitiporia* and *Phellinus* species, two *Fomitiporella* species, two *Inocutis* species and four *Inonotus* P. Karst. species.

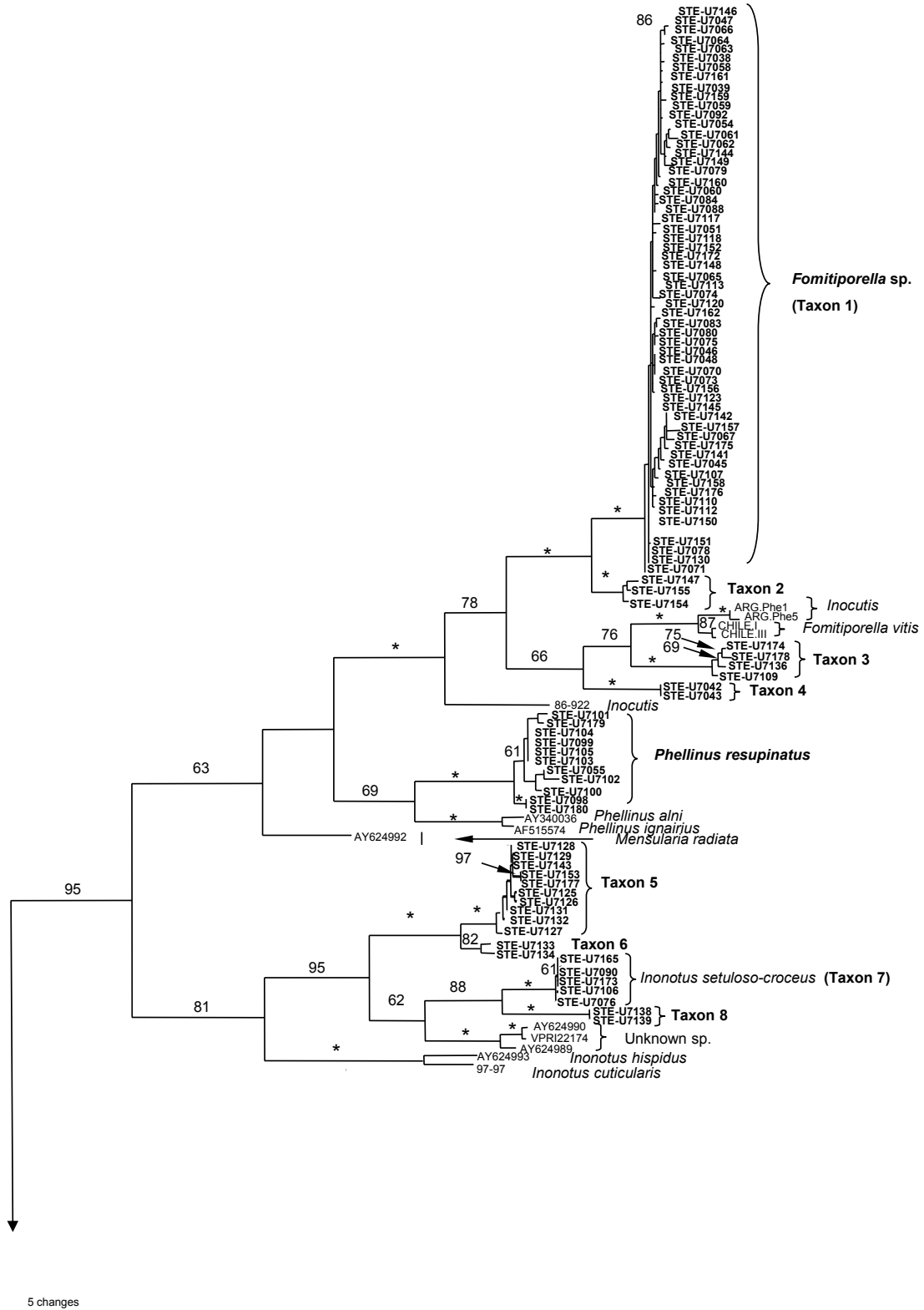


Figure 3. (continued).

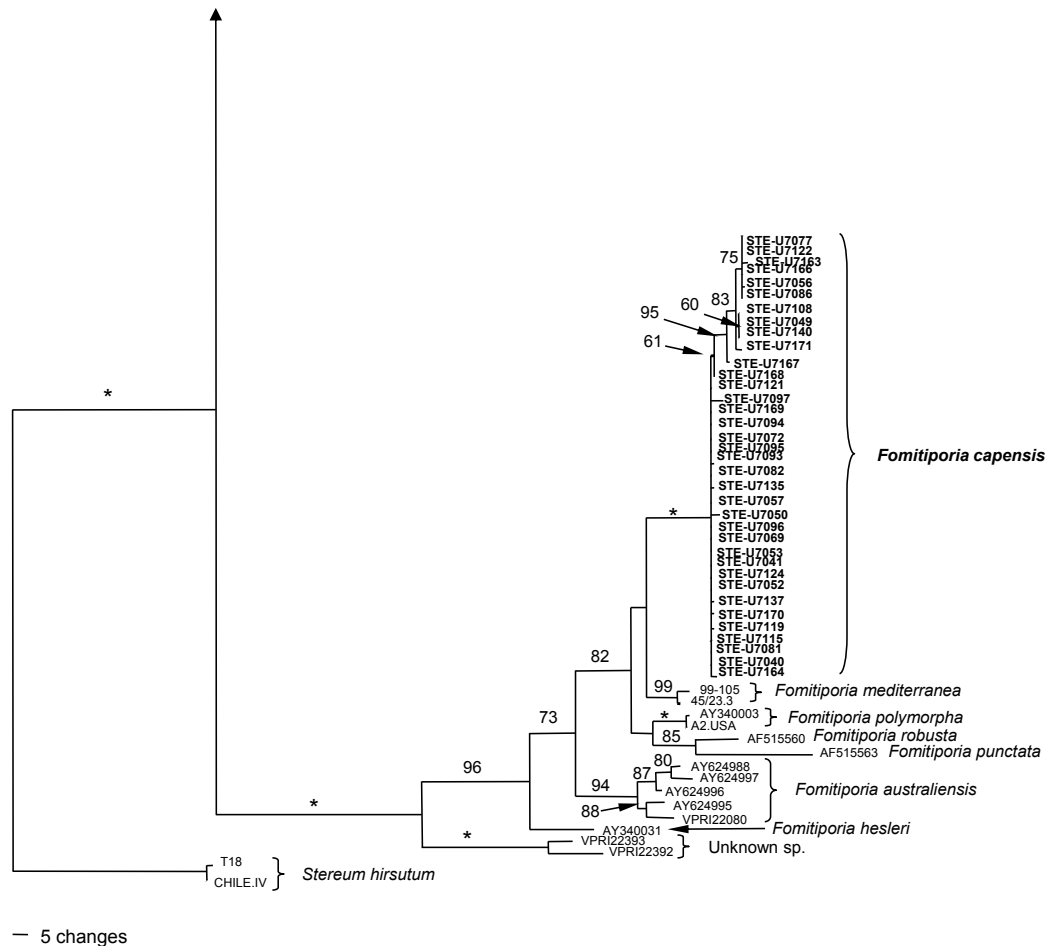


Figure 3. One of 10 most parsimonious trees obtained from heuristic searches of the ITS sequences (length: 2050 steps; CI: 0.560; RI: 0.938; RC: 0.526) of the Basidiomycete isolates. Bootstrap support values (1000 replicates) are shown above the nodes and bootstrap values of 100 % are indicated by an asterisk (*). The outgroups used were *Stereum hirsutum* isolates T18 and Chile IV. From White *et al.* (2011a). (continued on next page)

One of the *Fomitiporella* species and the *Fomitiporia* species were isolated most frequently in the Western Cape Province. The *Phellinus* species was isolated exclusively in the Northern Cape and Limpopo provinces (White *et al.*, 2011a). One of these species has been described as *Fomitiporia capensis* M. Fisch. *et al.* (Cloete *et al.*, 2014), another as *Phellinus resupinatus* M. Fisch. *et al.* (Cloete, 2016) (Figure 4a-b). Fruiting bodies of a third species, *Fomitiporella* sp. (previously designated Taxon 1) were also found on grapevine (Figure 4c). The other taxa have yet to be described, due to the scarcity of fruit bodies. These have been the first significant descriptions of novel Hymenochaetales species in South Africa.

The discrepancy between the amount of white rot found in vineyards and the amount of fruit bodies found is well documented in Italy (Cortesi *et al.*, 2000), Germany (Fischer, 2006), Argentina (Gatica *et al.*, 2004) and Australia (Edwards *et al.*, 2001; Fischer *et al.*, 2005). According to Fischer (2006), a ratio of more or less 100:1 can be expected for vegetative mycelium to fruit bodies in Germany. Fischer (2006) gives the following three possible reasons for the discrepancy between the occurrence of white rot and the occurrence of fruit bodies. First, badly rotted grapevines are often removed from the vineyard in accordance with good viticultural practices, possibly before fruit bodies have the opportunity to form.



Figure 4. Fruit bodies of Hymenochaetales species associated with esca diseased grapevines in South Africa. a. Fruit body of *Fomitiporia capensis* on *Vitis vinifera* cv. Chenin blanc. b. Fruit body of *Phellinus resupinatus* on cv. Sultana. c. Fruit body of *Fomitiporella* sp. on cv. Pinotage. d. Fruit body of *Inonotus setulosus-croceus* which was found on *Salix* sp.

Secondly, fruit bodies are difficult to spot and may simply be missed in surveys. Finally, fruit bodies may occur primarily on hosts other than grapevine. The spread of *F. mediterranea* is due to basidiospores within outcrossing populations (Fischer, 2002; Jamaux-Despreaux and Péros, 2003). This points to the last two possibilities, as fruit bodies must be present in some form in order for basidiospores to be available as an inoculum source.

Host range

Fischer (2006) states that lignicolous basidiomycetes occupy a wider host range within their centre

of distribution, and that most of these are often quite cosmopolitan. The occurrence of *F. mediterranea* on *Actinidia* in Greece and Italy (Elena and Paplomatis, 2002; Di Marco *et al.*, 2004), *Citrus* in Greece (Elena *et al.*, 2006) and *Inocutis jamaicensis* on *Eucalyptus* (Martinez, 2005) are examples of how esca-related lignicolous basidiomycetes are no exception. The diversity of native and introduced flora in the Western Cape is such that there are countless opportunities for examining potential alternative hosts for the occurrence of fruit bodies still unaccounted for on grapevine. The fruit bodies morphologically identified as *Inonotus setulosus-croceus* (Cleland & Rodway) P.K. Buchanan & Ryvarden were found in wood-

pecker holes on *Salix* soon after starting the search for fruit bodies on alternative hosts (Figure 4d). The DNA was isolated from the fruit bodies and the ITS sequences were similar to Taxon 7 isolated from esca diseased grapevines (Cloete, 2015). *Fomitiporia capensis* has since been found on *Quercus* and *Psidium*, and *Fomitiporella* sp. on *Psidium* in the Western Cape (unpublished data). The alternative host hypothesis would seem to be the most promising avenue to find and describe the remaining six taxa.

Pathogenicity of basidiomycetes on grapevines

Studies involving the pathogenicity of white rot basidiomycetes on grapevine and other hosts are rarely undertaken and the etiology of the Hymenochaetales is poorly understood. To date, there have been six trials of varying sizes and complexity involving esca and white rot on mature and young grapevines.

Chiarappa (1997) successfully performed inoculations with the basidiomycetes he commonly found on grapevines, which he, on the base of the knowledge at that time available, reported as *P. igniarius*, on 7-year-old commercial vines and established *P. igniarius* as the main causal organism of the spongy decay symptom of the disease known as black measles in California.

In France, Larignon and Dubos (1997) inoculated a mycelial suspension of *P. punctatus* (probably representing *F. mediterranea*) on Cabernet Sauvignon cane segments, which were rooted for two months and grown in the glasshouse and the field for four months and a year, respectively. Larignon and Dubos (1997) also inoculated wooden blocks taken from healthy Cabernet Sauvignon vines by placing them in a culture tube containing *F. mediterranea*. Blocks were incubated for a year. The cane inoculations of *F. mediterranea* showed brown vascular streaking, but the researchers were unable to re-isolate the basidiomycete from the inoculated plants. The wood blocks inoculated with *F. mediterranea* showed soft white rot after twelve months.

Sparapano *et al.* (2000) obtained white rot symptoms two years after inoculating *F. punctata* (probably *F. mediterranea*) on 13-year-old Sangiovese vines in Italy. During further inoculations made by the authors on six- and nine-year-old Italia and Matilde grapevines, the first signs of white rot could be

detected after six months. Inoculations were made by inserting colonised wooden toothpicks in holes drilled in grapevine arms and covered in cotton wool and paper tape.

Sparapano *et al.* (2001) included *F. punctata* (probably *F. mediterranea*) in a cross-inoculation trial with *Phaeoacremonium aleophilum* and *P. chlamydospora* on mature grapevines and found that *F. punctata* was able to cause limited, localised white rot within three years after inoculation.

Researchers in Argentina performed a limited experiment with an undescribed *Phellinus* sp. associated with the trunk disease, “hoja de malvón” (Gatica *et al.*, 2004). Five mature plants were inoculated with the “*Phellinus*” sp. by inserting mycelial plugs into 5 mm holes drilled into various points on the grapevine trunks. White rot symptoms could only be detected after six to seven years. This species was later identified as *Inocutis jamaicensis* (Lupo *et al.*, 2006).

In a pathogenicity trial in Chile, Díaz *et al.* (2013) inoculated a local *Inocutis* sp. on axenic plantlets incubated for 28 days, rooted 2 year old grapevines incubated for 15 months, grapevine shoots incubated for 60 days and detached grapevine shoots incubated for 14 days. All inoculations were via mycelial plugs inserted into holes of varying diameters bored in plant material. The *Inocutis* sp. was associated with brown vascular discolouration in all the inoculations, but no white rot symptoms were observed in that study.

White rot in wood is caused by the degradation of lignin and cellulose within the cell-walls of woody plants. Lignin and cellulose degradation are effected by extracellular enzymes released by wood rotting fungi, which break up the complex components of the cell wall (Manion, 1981). Lignin is a complex compound that is difficult to degrade, and only white rot basidiomycetes have been found to do it efficiently (Songulashvili *et al.*, 2006). Three enzymes have been found to be essential for lignin degradation, namely a copper containing phenoloxidase, laccase and two heme-containing peroxidases, lignin peroxidase (LiP) and manganese-dependent lignin peroxidase (MnP) (Overton *et al.*, 2006; Songulashvili, 2006). According to Morgenstern *et al.* (2010), it is unlikely that ligninolytic processes would be possible without production of either lignin peroxidase or manganese peroxidase. Past trials involving enzymatic assays and basidiomycetes involved with esca have shown that *Fomes* (*Phel-*

linus) *igniarius* produces laccase and peroxidases and *F. mediterranea* produces laccase and peroxidase (Chiarappa, 1997; Mugnai *et al.*, 1999).

An introduction to the Hymenochaetales

The “Série des Igniaires” was first recognised as an entity by Patouillard in 1900 and was characterised by him and his successors as having brown hyphae and brown basidiomata with modified brown cystidia known as setae in the hymenium, simple-septate hyphae and a xanthochroic reaction when mounted in KOH (Patouillard, 1900). Many of the species were also associated with white rot in woody plants (Patouillard, 1900; Kühner, 1950; Donk, 1964; Oberwinkler, 1977).

Oberwinkler (1977) raised the Hymenochaetales to the rank of order based on the same set of characteristics described by Patouillard and his successors, but it was only with the emergence of genetic studies that there was an indication that the Hymenochaetales might have to be expanded to include other polyporoid and even corticioid genera that lacked one or more of the abovementioned characteristics. Poroid *Oxyporus* (Bourdot and Galzin) Donk and *Trichaptum* Murrill and corticioid *Hyphodontia* J. Erikss. spp., *Basidioradulum radula* (Fr.) Nobles and *Schizopora paradoxa* (Schrad.) Donk were found to be closely related to the Hymenochaetales *sensu* Oberwinkler (Hibbett and Donaghue, 1995; Hibbett *et al.*, 1997). Further groups were also included later, including species from the agaricoid genera *Cantharellopsis* Kuypers, *Omphalina* Qué. and *Rickenella* Raithel. (Redhead *et al.*, 2002). The morphological characteristics of genera now considered part of the Hymenochaetales are currently highly varied (Larsson *et al.*, 2006).

The poroid Hymenochaetales as described in Oberwinkler (1977), called Hymenochaetales in Binder *et al.* (2005) and Larsson *et al.* (2006), are characterised by imperforate parenthosomes and include the two large, morphologically diverse and economically important genera *Phellinus sensu lato* and *Inonotus sensu lato*, among others. All *Phellinus* and *Inonotus s.l.* species cause white rot on a variety of woody perennials (Wagner and Fischer, 2002). The division of species between *Phellinus* and *Inonotus* was initially based on hyphal mitosis (dimitic vs. monomitic) and fruit body consistency, but many in-

termediate morphological forms have been reported over the years (Fiasson and Niemelä, 1984; Ryvarden and Gilbertson, 1994; Wagner and Fischer, 2001).

Fiasson and Niemelä (1984) did a multivariate analysis based on morphological and chemical characteristics of European poroid taxa and placed *Phellinus* and *Inonotus* into two families, the *Inonotaceae* consisting of *Inonotus sensu stricto*, *Inocutis* Fiasson and Niemelä and *Phylloporia* Murrill and the *Phellinaceae* consisting of *Phellinus s.s.*, *Fomitiporia* Murrill, *Porodaedalea* Murrill, *Fuscoporia* Murrill, *Fulvifomes* Murrill, *Onnia* P. Karst., *Inonotopsis* Parmasto, *Ochroporus* J. Schroet. and *Phellinidium* (Kotlába) Fiasson and Niemelä. The subdivision of *Phellinus s.l.* and *Inonotus s.l.* was supported by the nuclear large subunit (nuLSU) study of Wagner and Fischer (2001). The Wagner and Fischer (2002) study of *Phellinus s.l.* and *Inonotus s.l.* showed that the two genera are polyphyletic in origin and confirmed the status of all of the above, with the exception of *Phellinidium* which remained uncertain. Larsson *et al.* (2006), also working with the nuLSU, were still unable to find a satisfactory resolution in terms of related subclades within the Hymenochaetales.

Ecology and epidemiology of Hymenochaetales

Despite the economic impact of some members of the Hymenochaetales, little is known about their ecology and epidemiology. Certain species, such as *Fuscoporia weirii* (Murrill) Aoshima spread via an asexual state by root to root contact in infected forests (Hansen and Goheen, 2000). Many species spread via basidiospores. Cortesi *et al.* (2000) and Fischer (2002) used the high diversity of somatic incompatibility to demonstrate that *F. mediterranea* infects grapevine via basidiospores.

Infection by members of the Hymenochaetales can be through naturally occurring wounds, such as in the case of *Phellinus torulosus* (Pers.) Bourdot & Galzin infecting trees via fire or frost scars (Panconesi *et al.*, 1994). Infection can also occur through man-made pruning wounds, which has been hypothesized for *F. mediterranea* on grapevine (Cortesi *et al.*, 2000; Graniti *et al.*, 2000). In a casual study presented at a conference, Fischer (2009a, b) found fruit bodies of *F. mediterranea* sporulating between 190 to 250 days in a year under Central European conditions. This kind of life-strategy, also observed in common polypores

such as *Ganoderma applanatum* (Pers.) Pat., is thought to increase the likelihood of a spore finding a suitable substrate, according to Rockett and Kramer (1974).

There have been relatively few studies on sporulation of the Hymenochaetales. Yohe (1982), studying *Inonotus weirianus* (= *Phellinus weirianus*), a causal agent of destructive heart rot in walnut trees, reported the use of spore traps. In that study, spore traps consisting of microscope slides were set up underneath fruit bodies between mid-January and mid-February in Arizona, USA. The author reported spores adhering to the slide-surfaces during this period, though no other information was reported and the aim of the study remains unclear.

Fischer (2009a, b) measured sporulation by affixing slides to fruit bodies of *F. mediterranea* in the field. The author reported that sporulation of *F. mediterranea* in Germany was largely dependent on average daily temperatures and relative humidity, requiring conditions with temperatures higher than 10°C and a relative humidity higher than 80%. He also reported increased spore deposit after periods of rain.

Spore traps using microscope slides covered with a sticky substance are commonly used in grapevine trunk disease research in France, the United States and South Africa (Larignon and Dubos, 1997; Eskalen and Gubler, 2001; Úrbez-Torres *et al.*, 2008; Kuntzmann *et al.*, 2009; Van Niekerk *et al.*, 2010). Slides are covered with petroleum jelly and left in the field for a set period of time, after which traps are removed and processed. Spores are collected by washing traps with water, which can either be filtered and plated out or processed through PCR-based techniques. Identification through colony growth has been used more often and is largely dependent on the ability of spores to germinate quickly under laboratory conditions, an ability often absent from members of *Fomitiporia* (unpublished data). Rockett and Kramer (1974) noted that basidiospores have a lower rate of viability than spores of other types of fungi and presumably there are still more factors involved in basidiospore viability that need to be studied.

The sporulation of *Phaeoacremonium inflatipes* and *P. chlamydospora* in California was found to be directly correlated to rainfall events (Eskalen and Gubler, 2001; Rooney-Latham *et al.*, 2005). Úrbez-Torres *et al.*, (2011) found higher levels of sporulation of the Botryosphaeriaceae to be directly related to rainfall and overhead irrigation in various parts of California. Under South African conditions, Van Niekerk *et al.*

(2010) found rainfall, relative humidity and temperature to be the most important weather variables involved in the sporulation of the Botryosphaeriaceae and *Phomopsis* spp. Several studies investigating the relation between South African conditions and the sporulation of esca disease pathogens are currently being conducted and will contribute to the further understanding of this subject.

Conclusion

South African vineyards are subject to an unprecedented variety of Hymenochaetales species that are associated with esca symptoms. Despite extensive searches, fruit bodies representing several species are yet to be found in the field. Further studies on native flora and other hosts may yet deliver the missing fruit bodies. The diversity of species initially associated with disease symptoms, as well as the discovery of several fruit bodies on alternative hosts gives an insight to the potential of future studies on the ecology of esca, as well as the position of wood-rotting basidiomycetes in the disease development cycle.

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