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Biodiversity and geographic distribution of basidiomycetes causing esca-associated white rot in grapevine: a worldwide perspective

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Summary. An overview is provided of the biodiversity, taxonomic classification and geographic distribution of basidiomycetes isolated from white rot in esca-affected grapevine all over the world. A total of 10 species have been recovered, all belonging to the Hymenochaetales and five of them of uncertain identity. Identified taxa include *Fomitiporia mediterranea* (Europe, Asia), *F. polymorpha* (North America), *F. australiensis* (Australia), and *Inocutis jamaicensis* (South America). One species, not yet formally described, is *Fomitiporella vitis* (South America). Uncertain taxa occur in Australia and South Africa. Existence of the fungi is widely restricted to vegetative mycelium. Fruit bodies are rare in the field and molecular data are usually necessary for differentiation.

Key words: *Fomitiporia*, Hymenochaetales, molecular systematics.

In spite of some progress achieved in recent years, information is still sparse on the taxonomic classification, geographic distribution and life strategies of basidiomycetes on esca-diseased grapevine. There are several reasons for this:

i) very often the occurrence of a particular fungus is limited to the vegetative state, i.e., to the mycelium living inside the infected plant;
ii) if fruit bodies develop, they are often inconspicuous, forming crust-like layers only on the less obvious parts of infected trunks;
iii) frequently, fruit bodies are in poor condition, making proper identification time-consuming or even impossible;
iv) in several wine-producing countries basidiomycetes are not considered serious pathogens of grapevine, and they have not attracted particular attention from researchers.

It was at the 2nd workshop of the International Council on Grapevine Trunk Diseases (ICGTD), held in Lisbon, Portugal in September 2001, that white rot was presented as one of the crucial components of so-called ‘esca proper’, evident in older vines (Graniti *et al.*, 2000; Surico, 2001). In this way esca proper was separated from the dieback phenomena observed in younger vines, which are now called Petri disease. Before this it had already been shown that esca-related wood deterioration, including white rot as well as other symptoms such as brown-red wood and dark stripes, may be caused by a number of fungal organisms, including members of the mitosporic genera *Phaeomoniella* and/or *Phaeoacremonium*, but that white rot is always

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linked with the occurrence of basidiomycetes.

Esca and its possible precursor, Petri disease, have been reported from wine-growing countries worldwide (Larignon and Dubos, 1997; Ferreira *et al.*, 1999; Mugnai *et al.*, 1999; Chiarappa, 2000; Pascoe and Cottral, 2000). In several areas, mostly in Europe, white-rot fungi are considered major contributors to esca-related problems. In other regions however, these fungi play only a subordinate role, although they have been shown to occur virtually everywhere in northern and southern-hemispheric vineyards (Fischer, 2001).

Since the data are incomplete, the taxonomic classification and phylogenetic relationships of esca-related basidiomycetes are uncertain for many countries, and only a few defined names have so far been introduced (Fischer, 2002; Fischer *et al.*, 2005). It is interesting to note that the spectrum of fungi associated with precursor diseases of esca seems to be fairly uniform, while for the basidiomycetes the situation is more complex. At present, it is not known how far white rot fungi in general are an essential part of esca and whether injured wood should also be seen as an important source of nutrients attracting a wide range of more or less well-adapted fungal organisms.

This paper provides an overview of the biodiversity, taxonomic classification and geographic distribution of basidiomycetes isolated from white rot in esca-affected grapevines all over the world. Several colleagues, by supplying fungal isolates and sharing valuable information, have contributed to this paper. I gratefully acknowledge the support of Laura Mugnai, Francesca Mela, Walter Gams and Hanns-Heinz Kassemeyer (see chapter on Europe), Douglas Gubler and his working group (North America), Marta Gatica and Jaime Auger (South America), Jacky Edwards, Ian Pascoe and James Cunnington (Australia), and Francois Halleen and Paul Fourie (South Africa).

Basidiomycetes on grapevine - from past to present

Ravaz (1909) was probably the first researcher to study basidiomycete mycelium and fruit bodies on diseased vines. He identified the fruit bodies as belonging to *Phellinus igniarius* (L.) Quél. (at the time: *Fomes igniarius* (L. ex Fr.) Kickx.). Besides *P. igniarius*, *Stereum hirsutum* (Willd.: Fr.) Pers. was also reported from French vineyards (Vinet, 1909; Viala, 1926). In their 'Hyménomycètes de France', Bourdot and Galzin (1927) mentioned a resupinate basidiomycete as a strong parasite on *Vitis* in the Midi region of southern France, but this observation was not repeated in later publications.

While studies in esca had intensified by the end of the 1990's, no significant progress was made in the classification and distribution of white rot basidiomycetes. The taxa most mentioned were still *P. igniarius*, *S. hirsutum*, and also *Trametes hirsuta* (Wulf. : Fr.) Pilát (Mugnai *et al.*, 1996; Larignon and Dubos, 1997; Chiarappa, 2000). In the years following, *P. igniarius* was omitted from this list as it was shown to be a European-based species occurring only on species of *Salix* (Fischer, 1995, 2002; Fischer and Binder, 1995, 2004). However, another species, *Fomitiporia punctata* (P. Karst.) Murrill (= *P. punctatus* (P. Karst.) Pilát) now gained increasing prominence and came to be viewed as the main causal agent of white rot in both Italian and German vineyards (Mugnai *et al.*, 1999). *F. punctata* was the first esca-related basidiomycete to be thoroughly studied using both molecular and epidemiological data, though in a limited geographic area (Cortesi *et al.*, 2000; Fischer, 2000). Shortly afterwards, as a result of pairing tests of single-spore isolates, growth data of the vegetative mycelium and molecular sequence data of the nuclear encoded ribosomal ITS region, a new species, *F. mediterranea* M. Fischer (designated *Fmed* in what follows), was separated from *F. punctata* and specimens collected from decayed wood of grapevine that had originally been identified as *F. punctata* were now re-classified as the closely related *Fmed* (Fischer, 2002).

During the last few years, grapevine-inhabiting basidiomycetes have attracted increasing attention also outside Europe, and valuable information on their geographic distribution, life strategy, as well as the symptoms associated with them have been provided by several working groups (Gatica *et al.*, 2000, 2004; Pascoe and Cottral, 2000; Edwards *et al.*, 2001). At the 2nd ICGTD workshop a preliminary overview was presented illustrating the genetic diversity of esca-related basidiomycetes worldwide, without however making any statements about taxonomic classification (Fischer, 2001). Nevertheless, more detailed studies, although conducted in a limited period of time, clearly showed that a considerable number of basidiomycetes occur on grapevine.

Why only white rotters on grapevine?

To our knowledge, no brown rot species have so far been reported from *Vitis vinifera* (for central Europe, see checklists in Kreisel, 1961; Fischer and Kassemeyer, 2003). On the other hand, of the several thousand basidiomycetes living on wood, brown rot fungi probably constitute less than 10% of the total (Gilbertson, 1981; Gilbertson and Ryvarden, 1986–1987; Ryvarden and Gilbertson, 1993–1994) and as a strong tendency they occur primarily on conifer wood; those on hardwood are mostly restricted to members of the Fagaceae and Betulaceae (Nobles, 1958, 1971; Gilbertson, 1981).

Geographic distribution and taxonomic classification of basidiomycete species on grapevine

Esca-related isolates included in the present survey originated from Europe, North and South America, Australia, and South Africa. Some fruit bodies were available for *Fmed* and the newly described *F. australiensis* M. Fisch. *et al*. (Fischer *et al*., 2005); all the other isolates came from vegetative mycelium collected from infected wood. For first-hand insight into the phylogenetic relationships and taxonomic identity of the isolates, a molecular analysis was employed, based on the nuclear ribosomal ITS region (ITS1-5.8S-ITS2). After amplification and sequencing of the ribosomal ITS region, a phylogenetic tree was generated using the neighbour-joining method (Fig. 1). The methods of DNA extraction, PCR and phylogenetic analysis were those described in Fischer (2002) and in Fischer and Binder (2004).

To help clarify the identity of uncertain isolates, selected reference taxa were added to the analysis; except for *S. hirsutum* they were all members of the Hymenochaetales (Oberwinkler, 1977). It has been indicated earlier (Fischer, 2002; Fischer *et al*., 2005) that this large group of mostly lignicolous fungi comprises the great majority of esca-related basidiomycetes. The reference taxa included here belonged to five different genera, none of which were thought to be related to diseases of grapevine. Except where otherwise noted, see Wagner and Fischer (2001, 2002) for taxonomic details. The reference taxa were as follows:

Mensularia radiata (Sow. : Fr.) W.B. Cooke (= *Inonotus radiatus* (Sow. : Fr.) P. Karst.); *P. igniarius* (see Fischer, 1995; Fischer and Binder, 2004, for geographic distribution and host range); *Inonotus hispidus* (Bull. : Fr.) P. Karst. (while sometimes found on grapevine, this species has no pathogenic significance on this host; see Fischer and Kassemeyer, 2003); *I. cuticularis* (Bull. : Fr.) P. Karst.; *Inocutis rheades* (Pers.) Fiasson & Niemelä (= *I. rheades* (Pers.) Pil.); *Fomitiporia hesleri* M. Fischer (see Fischer and Binder, 2004); *F. polymorpha* M. Fischer (see Fischer and Binder, 2004); *F. robusta* (P. Karst.) Fiasson & Niemelä, and *F. punctata*.

In all, 35 isolates were included in the survey (Fig. 1), 22 of which came from grapevine (in italics in Fig. 1). Two more isolates, while collected from non-*Vitis* hosts, have been shown previously to belong to species associated with white rot of grapevine. These isolates are 99-105 (*Fmed*; Fischer, 2002) and VPRI 22409b (*F. australiensis*; Fischer *et al*., 2005).

One sample, 'CHILE.IV', formed a distant clade and was essentially identical to the European reference strain of *S. hirsutum*, indicating a low level of interspecific genetic diversity within this species. The remaining grapevine-derived isolates fell into two large groups, both within the Hymenochaetales. One group, comprising collections from Europe, Australia, South Africa, and North America, represented *Fomitiporia*. The other group contained collections from Europe, Australia, and South Africa. Generic affinities were not unequivocally revealed within the latter group; however, all unknown samples fell into the former genera *Phellinus* s.l. and *Inonotus* s.l., both now subdivided into a number of smaller subunits (Fiasson and Niemelä, 1984; Wagner and Fischer, 2001, 2002).

A total of eight taxa are formed by the uncertain isolates from grapevine, and two more esca-related taxa were represented by *Fmed* and *F. australiensis*. Within the *Fomitiporia* clade, isolate A2 from California surprisingly represented the recently described *F. polymorpha* (Fischer and Binder, 2004). Besides *F. australiensis* (Fischer *et al*., 2005), one more taxon of this genus exists in Australia, and another taxon, apparently related to *Fmed*, was found in South Africa. Within the *Phel-*

linus-*Inonotus* clade, four unidentified taxa, one each from Argentina and Chile, and two from South Africa, were somewhat close to the genus *Inocutis*, in the tree represented by *I. rheades*. Another taxon, from Australia, showed affinities to members of the genus *Inonotus* s.str., represented by the reference species *I. hispidus* and *I. cuticularis*.

In what follows, the tree topology as shown in Figure 1 is used as a basis for discussing the biodiversity of esca-associated basidiomycetes. For clar-

Fig. 1. Genetic diversity and phylogenetic relationships of white rot basidiomycetes on grapevine inferred from the nuclear ITS1-5.8S-ITS2 region. The tree is rooted with isolates belonging to *Stereum hirsutum*. Isolates in italics are derived from *Vitis vinifera*. The uncertain affinity of *Inocutis jamaicensis* and *Fomitiporella vitis* is explained in the text.

Table 1. Geographic distribution and host range of *Fomitiporia mediterranea* in Europe.

Country	Host[a]
Austria	*Vitis vinifera*
France	*V. vinifera, Quercus sp.*
Germany	*V. vinifera*
Greece	*V. vinifera, Actinidia chinensis*[a], *Olea europaea*
Hungary	*V. vinifera*
Italy	*V. vinifera, Acer negundo, Actinidia chinensis*[a], *Corylus avellana, Lagerstroemia indica*[a], *Ligustrum vulgare, Olea europaea, Quercus ilex, Robinia pseudoacacia*[a]
Portugal	*V. vinifera*
Slowenia	*V. vinifera, Laurus nobilis*
Spain	*V. vinifera*
Switzerland	*V. vinifera*

[a] Introduced to Europe

ity, the continents and their respective fungi are treated in separate chapters. Characters examined include geographic distribution, host preferences and the morphology of the fruit bodies and the vegetative mycelium. Some related information provided in earlier publications is indicated as 'Further reading'.

Europe

It is in Europe, where it all began, and over the decades a number of basidiomycetes have been discussed as being related to esca. In recent times, however, *Fmed* has been generally accepted as the main causal agent of white rot in grapevine, while *F. punctata*, with fruit body characters essentially identical, is restricted to other hosts. Two other white-rot fungi, *S. hirsutum* and *T. hirsuta*, are found more frequently on European grapevine, but they usually act as secondary invaders only and do not attack living wood. Based on personal observations, a preliminary checklist of basidiomycete wood rotters on grapevine in Germany is presented in Fischer and Kassemeyer (2003); another list, not including polypores, is provided by Kreisel (1961).

The study of *Fmed* (sometimes named *F. punctata* or *P. punctatus*) was initiated in the late 1990's, and was further enhanced by its formal description in 2002. This fungus has now been reported from most European wine-growing countries (see Larignon and Dubos, 1997; Mugnai *et al.*, 1999; Cortesi *et al.*, 2000; Fischer, 2000, 2002, unpublished data; Armengol *et al.*, 2001; Jamaux-Despréaux and Péros, 2003). As a striking phenomenon, the occurrence of *Fmed* in central Europe is restricted to *V. vinifera*, while in the Mediterranean area it is found on a considerable number of host plants, including, besides *Vitis, Actinidia*, *Corylus*, and *Olea* (for *Fmed* on this last host see also Hartig, 1893 and Plank, 1980). An overview of the distribution and host range of *Fmed* is shown in Table 1.

Fmed is able to colonize new host plants in a short period of time. For culturing reasons, *Actinidia*, the kiwifruit, was introduced to southern Europe some fifty years ago, and in this time it became severely affected with white rot caused by *Fmed* (Elena and Paplomatas, 2002; Di Marco *et al.*, 2003, 2004). Recently, the species has also been reported from grapevine in Iran (Karimi *et al.*, 2001; Fischer, Mela and Mugnai, unpublished results; Gräfenhahn, pers. comm.) and it may be speculated to what extent *Fmed* is correlated with viticulture in Europe and, partly, in Asia.

The vegetative mycelium of *Fmed* is well adapted to higher temperatures (for details on cultural requirements see Fischer, 2002), and the increasing spread of the species in central Europe may be due to a number of warmer years observed in the last decade.

Design of *Fmed*-specific primers

Compared to other taxa of *Fomitiporia*, strains of *Fmed* have diagnostic insertions in both the ITS1 region (nucleotides 201-206, AATAAT) and the ITS2 region (nucleotides 748-754, CCTTTGA; Fischer, 2002; Fischer and Binder, 2004). Based on these insertions, specific primers were designed, designated prFmed1 and prFmed2, respectively. Specific features of these primers are as follows:

prFmed1: 5´ GCA GTA GTA ATA ATA ACA ATC 3´;
GC = 28.6%, T_M = 50.1°C;
prFmed2: 5´ GGT CAA AGG AGT CAA ATG GT 3´;
GC = 45%, T_M = 55.3°C.

Primers clearly differentiated *Fmed*, *F. punctata* and *F. australiensis* using the following amplification parameters: 95°C denaturation step (1 min), 47°C annealing step (1 min), 72°C primer extension (45 s). Usually 35–40 cycles are performed, and a final incubation step at 72°C (7 min) is added after the last cycle. One μl each of the primers (25 pmol each) should be used in a 50 μl assay. Under these conditions, a product of approximately 550 bp was obtained for *Fmed*, while no band was observed for *F. punctata*, *F. australiensis*, *P. igniarius*, *S. hirsutum*, or *T. hirsuta*, the last two sometimes isolated from rotted wood of European *V. vinifera*.

Further reading: Cortesi *et al*. (2000), Fischer (2002), Jamaux-Despréaux and Péros (2003).

North America

Wood-rotting basidiomycetes are usually not seen as harmful pathogens, causing decline and premature death of grapevine in North American vineyards. By contrast, the incidence of Petri disease has increased continuously over the last decade, preferably attacking young grapevines (Scheck *et al*., 1998; Morton, 2000; Eskalen and Gubler, 2001; Gubler *et al*., 2004). Still, internal wood rot symptoms observed in Californian vineyards were shown to be in good accordance with the disease then called black measles (Chiarappa, 1959). Although Chiarappa (1959) found no fruit bodies, he succeeded in isolating fungal mycelium from infected wood and assigned it to *P. igniarius*, which at that time was considered to be a multi-host member of the Hymenochaetales (Gilbertson and Ryvarden, 1987; Chiarappa, 1997). More recent studies, however, have shown that this fungus is essentially restricted to species of *Salix* in Europe. North American counterparts of the fungus exist, but belong to different, partly undescribed taxa (Fischer, 1995; Fischer and Binder, 1995, 2004). Both the geographic distribution and the host range of these taxa, related to the European *P. igniarius*, are in large part unknown, and their occurrence on grapevine cannot be wholly excluded.

Molecular analysis showed that a hitherto unknown member of *Fomitiporia* occurred on North American grapevine. Isolate A2, kindly provided by the group of Douglas Gubler (UC Davis, CA, USA), and originating from decayed wood of Californian *V. vinifera*, was found to belong to the recently described *F. polymorpha* (Fischer and Binder, 2004). This perennial species is characterized by a broad host range, even including conifers, and a distinct variability in the shape of the fruit bodies, from resupinate to effused-reflexed, and sometimes pileate. With the available data, the taxonomic status of *F. polymorpha* rests on 10 isolates, including A2, all from the western USA. The fruit bodies of the species were originally recognized as *F. robusta* or as a taxon of uncertain affinity. In the original paper, the ITS characters of *F. polymorpha* placed it in one clade together with *F. robusta* and *F. punctata* (Fischer and Binder, 2004) and this is confirmed in the present study (Fig. 1). The length of the ITS region was 725–726 in *F. robusta*, while in *F. polymorpha* it was exceptionally large, 746–749 bp.

We do not know if what Chiarappa named *P. igniarius* was in fact *F. polymorpha*, but it seems likely that more basidiomycete species, identified or not, still await detection in North American grapevines.

Further reading: Fischer and Binder (2004). Adaskaveg and Ogawa (1990) provide a checklist of wood rot fungi occurring on fruit and nut trees in California.

South America

Hoja de malvon and chlorotic leaf roll are grapevine wood diseases on mature vines in Argentina and Chile. Distinct characters separating these diseases from esca in Europe, include symptoms on the leaves and the basidiomycete species that are isolated from rotted wood (Gatica *et al*., 1998, 2000, 2004; Auger *et al*., 2005). Both hoja de malvon and chlorotic leaf roll are associated with extensive white rot of the trunk and basidiomycete mycelium is isolated with high frequency from wood affected with these diseases. In Argentina, pileate to effused reflexed fruit bodies are evident on the trunks of some infected vines (Gatica *et al*., 2004).

In a molecular study presented at the 2nd

ICGTD workshop, three isolates each from Argentina and Chile formed a separate clade within the Hymenochaetales, and came out as closely related to *I. rheades* (Fischer, 2001). One other isolate from Chile was mostly identical with two European-based reference strains of *S. hirsutum*. One year earlier, Gatica *et al*. (2000) had already suggested on the basis of mycelial characteristics that a species of *Phellinus* s.l. was the causal agent of the white rot in Argentinian vineyards.

Recently, considerable progress has been achieved by working groups in Argentina and Chile, and at the 4th ICGTD meeting in Stellenbosch (2004) a more definite identification was presented for the fungi under study.

For Argentina, Bettuci *et al*. (2005) suggested that the basidiomycetous pathogen associated with hoja de malvon was *I. jamaicensis* (Murrill) Gottlieb, J.E. Wright & Moncalvo. The characters of the vegetative mycelium and the fruit bodies were essentially identical with those of a reference strain from *Eucalyptus globulus* in Uruguay. These findings were supported by the molecular data, including RFLPs and sequencing of the ITS region and partial LSU rDNA. It should be noted, however, that slight differences were found in some of the ITS restriction patterns, thought to be related to host specificity and geographic origin. Complete sequencing and alignment of the ITS region might help to resolve this question.

A morphological description of fruit bodies of *I. jamaicensis* on grapevine is given in Lupo *et al*. (2005). Geographic distribution covers North and South America (Gottlieb *et al*., 2002; Martinez, 2005), where the species is found both on deciduous and coniferous trees such as *Diostea*, *Eucalyptus*, *Prunus*, *Quercus*, and *Taxodium*. Host conditions include both living and dead plants.

For Chile, Auger *et al*. (2005) suggested that another member of the Hymenochaetales, *Fomitiporella vitis* Auger, Aguilera & Esterio was the causal agent of the wood decay associated with 'chlorotic leaf roll'. Identification was carried out by integrating characters of fruit bodies and vegetative mycelium with sequences of the LSU rDNA. While a formal description of *Fomitiporella vitis* is not yet available, the genus as a whole can be characterized as follows (Wagner and Fischer, 2002): occurrence on deciduous wood; fruit bodies resupinate to effused-reflexed, dimitic and perennial; setae lacking; basidiospores ellipsoid to globose, brownish, and non-dextrinoid. The coloured basidiospores of this genus combine it with *Inocutis* and *Inonotus* s.str. In a worldwide study on the natural classification of the taxa of *Phellinus* s.l. and *Inonotus* s.l. (Wagner and Fischer, 2002), *Inocutis* and *Fomitiporella* appeared as sister groups, thus refining the preliminary results reported by Fischer (2001).

Further reading: Gatica *et al*. (2004); Lupo *et al*. (2005).

Australia and New Zealand

In Australia, white heart rot similar to that of esca is common in grapevine, and mycelium referable to the Hymenochaetales can be isolated from it (Edwards *et al*., 2001). The corresponding fruit bodies are only rarely detected and are usually in poor condition, hampering correct identification.

Figure 1 includes nine isolates from Australia, two of which, 22409b and 22859, were associated with fruit bodies. Except for 22409b (from *Dodonaea*, native hop-bush) all isolates originated from *V. vinifera*. Isolates were subdivided into three clades in Fig. 1, two of them within *Fomitiporia*, the other, clearly apart, next to northern-hemispheric species of *Inonotus* s.str. and *Phellinus* s.str. Recently, one of the groups within *Fomitiporia*, including strains from *Vitis* and *Dodonaea*, has been described as a new species, *F. australiensis* (Fischer *et al*., 2005). The remaining groups, distinct by their molecular ITS data and partly by the growth characteristics of their vegetative mycelium, remain unidentified. The Australian isolates grow well between 15°C and 30°C. The fastest mycelial growth is observed in strains grouped within the *Phellinus* - *Inonotus* clade, while *F. australiensis* mycelium grows more slowly (Fischer *et al*., 2005).

A striking phenomenon is that the fruit bodies of *F. australiensis* are variable in shape, being resupinate or pileate. Microscopic characters allow no differentiation between *F. australiensis* and the European species *F. punctata* and *Fmed*. With the data currently available, no definite statements can be made about the exact distribution and host range of *F. australiensis*.

For New Zealand, only *S. hirsutum* has been reported (Fischer, 2001). However, several basidiomycetous mycelia were collected on the occasion of the 3rd ICGTD meeting at Christchurch (NZ) in 2003, all belonging to the Hymenochaetales (Fischer, unpublished results).

Further reading: Fischer *et al*. (2005). Cunningham (1965) should be consulted for an overview on polypores in New Zealand and Australia. Some information on southern hemispheric *Fomitiporia*, also proposing a key to poroid Hymenochaetales with dextrinoid basidiospores (including *Fomitiporia*) is presented by Decock *et al*. (2005).

South Africa

Until a few years ago, nothing was known about the biodiversity and taxonomic classification of basidiomycetes on grapevine in South Africa. Symptoms of white rot were previously seen only in the viticultural regions of Slanghoek and Rawsonville (Western Cape Province), especially in older vines, and were considered part of a natural process of degradation of vines, without contributing very much to the observed symptoms of decline, which was most apparent in younger plants (Marais, 1981). Although this was never confirmed, the causal organisms were believed to be *S. hirsutum* and *P. igniarius*, based on the literature of Galet (1977).

In recent years however, a considerable number of vegetative isolates, all from infected vines in the Stellenbosch region, have been collected by François Halleen, and are included in the present survey. Before that, another mycelial isolate from the same area was identified as *S. hirsutum* (Fischer, 2001).

The six South African strains, all of uncertain affinity, fall into three groups (Fig. 1). One group is closely related to the European-based *Fmed*, the others are within the *Inocutis* and relatives clade, comprising the isolates from Argentina and Chile. Several other isolates from South Africa, not included in Fig. 1, were examined by molecular means, but all were assignable to one of these three groups.

As in other wine growing regions, it is rare to find basidiomycete fruit bodies in South Africa, and the molecular-based groups revealed in Fig. 1 are not correlated with any generative structures offering a larger number of characters for identification. However, fruit bodies may occur in larger numbers than formerly thought: at the 4th ICGTD workshop a first-hand examination of a single vineyard detected three basidiomycete fruit bodies, all forming inconspicuous brownish crust-like structures on the trunk (Fischer and Halleen, unpublished data). Throughout, affected vines were without external symptoms on the leaves or berries. No detailed work on the characters of fruit bodies has been conducted so far.

Taking into account both the limited time span and the area of collection, the biodiversity of grapevine inhabiting basidiomycetes in South Africa is astounding. It is likely that more taxa will be detected in future.

Further reading: Ryvarden and Johansen (1980) studied fruit body morphology and host data and provide ample illustrations and keys of East African polypores, including both *Phellinus* s.l. and *Inonotus* s.l. Decock *et al*. (2005) examined southern-hemispheric members of *Fomitiporia*; however, no South African isolates were available for their study.

Final comments

In-depth studies on grapevine inhabiting basidiomycetes have revealed a number of facts and raised several questions. In the present survey, the main focus was on ten taxa of basidiomycetes, all isolated from esca-associated white rot. While the exact affinity of some of these was uncertain, they were all members of the Hymenochaetales. Remarkably, 50% of these taxa belonged to one small genus, *Fomitiporia*, which hardly contains a dozen species worldwide (Fischer, 1996; Dai, 1999; Decock *et al*., 2005; Fischer *et al*., 2005). No particular attention was paid to the ubiquitous *S. hirsutum* and *T. hirsuta*, which most likely play only a subordinate role as decomposers of living wood (Jahn, 1964, 1971; Gilbertson and Ryvarden, 1987; Ryvarden and Gilbertson, 1994).

The geographic distribution of relevant taxa can be described as follows: one taxon, *Fmed*, is mostly European; one taxon, *F. polymorpha*, so far has been reported only from North America; two taxa, *I. jamaicensis* and *Fomitiporella vitis*, occur in South America; *F. australiensis* and two uncertain taxa are from Australia, and three others, with uncertain affinity, occur in South Africa. It is not known if these taxa, all restricted to a particular, although large, geographic region, will also be

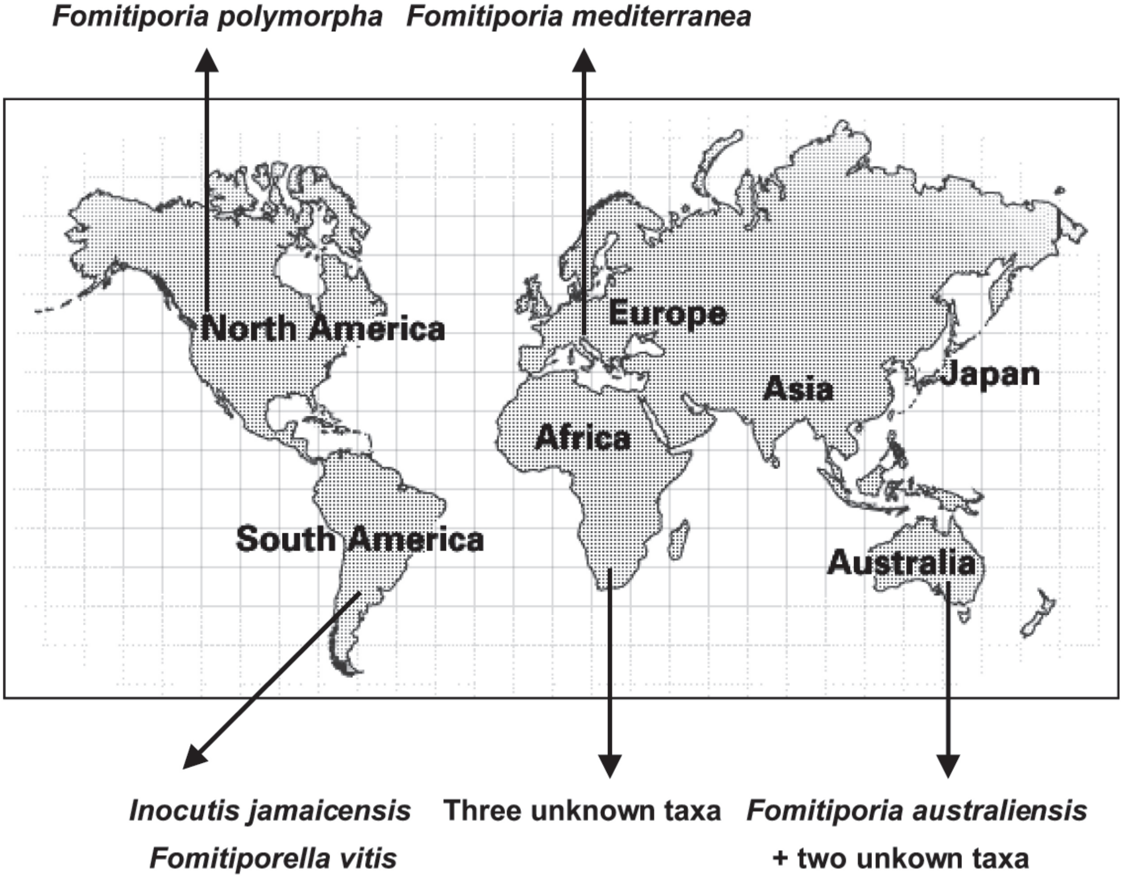

Fig. 2. Biodiversity and geographic distribution of white rot basidiomycetes associated with esca of grapevine.

found in other areas in the future. At this stage, the geographic distribution and taxonomic classification of these fungi may be summarized as in Figure 2.

Within the so-called polypores several thousand species, comprising numerous genera, occur on wood (Gilbertson and Ryvarden, 1986-1987; Ryvarden, 1991; Ryvarden and Gilbertson, 1993-1994).However, esca associated white rotters are restricted to a handful of genera, mostly within the Hymenochaetales. The predominant genus, *Fomitiporia*, is acknowledged to be a more derived group within *Phellinus* s.l., and this is supported by characters of sexuality, nuclear behaviour, and the DNA content of its nuclei (Fiasson and Niemelä, 1984; Fischer, 1996). If fruit bodies with spores are available, members of *Fomitiporia* may be readily recognized by the (sub)globose spores showing a strong dextrinoid and cyanophilic reaction.

While the taxa under study here have been isolated from affected wood of grapevine, the pathogenic significance of most of them in relation to esca remains uncertain. In several wine-growing countries, such as Australia, California, and South Africa, white rot within the trunk is not thought to be an indispensable part of grapevine decline, and a more important role is assigned to hyphomycetes like *Phaeomoniella* and *Phaeoacremonium* species. Nevertheless, it should be kept in mind that the taxa of *Fomitiporia* are perthophytes, i.e., they produce and segregate wood-degrading enzymes such as laccase, and in this way they invade and kill living wood.

White rot, as a prominent feature of the esca syndrome (Mugnai *et al*., 1999; Graniti *et al*., 2001), was reproduced by artificial inoculation with *Fmed,* and in one case foliar symptoms were also reported to appear in inoculated vines (Sparapano *et al*., 2001). While it is unusual for foliar symptoms to be closely linked to basidiomycetous infections, they sometimes occur, a well known example being the cosmopolitan *Chondrostereum purpureum* (Pers. : Fr.) Pouz., the causal agent of a disease in fruit trees called silver leaf.

For vineyards in Germany, a ratio of approximately 100:1 was estimated for the occurrence of vegetative mycelium and fruiting structures of *Fmed* (Fischer, unpublished). Using a different approach, similar results were obtained for vineyards in Tuscany surveyed for esca symptoms and fruit bodies (Cortesi *et al*., 2000). A maximum of 50 fruit bodies were found in one single vineyard where 1741 vines were sampled; at the other end of the scale, only one fruit body was detected in another vineyard with 906 vines sampled. In the Argentinian Mendoza region, 'basidiocarps .... can be found on the trunks of some infected vines' (Gatica *et al*., 2004), and in Australia, 'fruit bodies were detected in low numbers or very rarely' (Edwards *et al*., 2001; Fischer *et al*., 2005). All in all, the formation of fruit bodies, although an essential part of the fungus life cycle, seems rather exceptional when compared to the omnipresent white rot. Several reasons may account for this discrepancy:

i) in wood rotting fungi the formation of fruit bodies is often correlated with the age and/or overall condition of the host plant. Dead trunks of grapevine, probably most suitable for bearing fruit bodies, are usually removed from the vineyard.
ii) As shown for *Fmed* in the Mediterranean area (Fischer, 2002, unpublished data), a considerable number of fruit bodies may occur on non-*Vitis* hosts outside the vineyards.
iii) Even for the trained eye, fruit bodies are hard to find in the field and they may occur in greater numbers than appears.

As regards esca-related basidiomycetes, comprehensive host range data are available only for *Fmed* (Fischer, 2002), *F. polymorpha* (Fischer and Binder, 2004), and *I. jamaicensis* (Gatica *et al*., 2004; Lupo *et al*., 2005). To our knowledge, all other taxa are mainly or wholly restricted to *V. vinifera*. The occurrence of *Fmed* outside Mediterranean vineyards leads to increased infection pressure and,

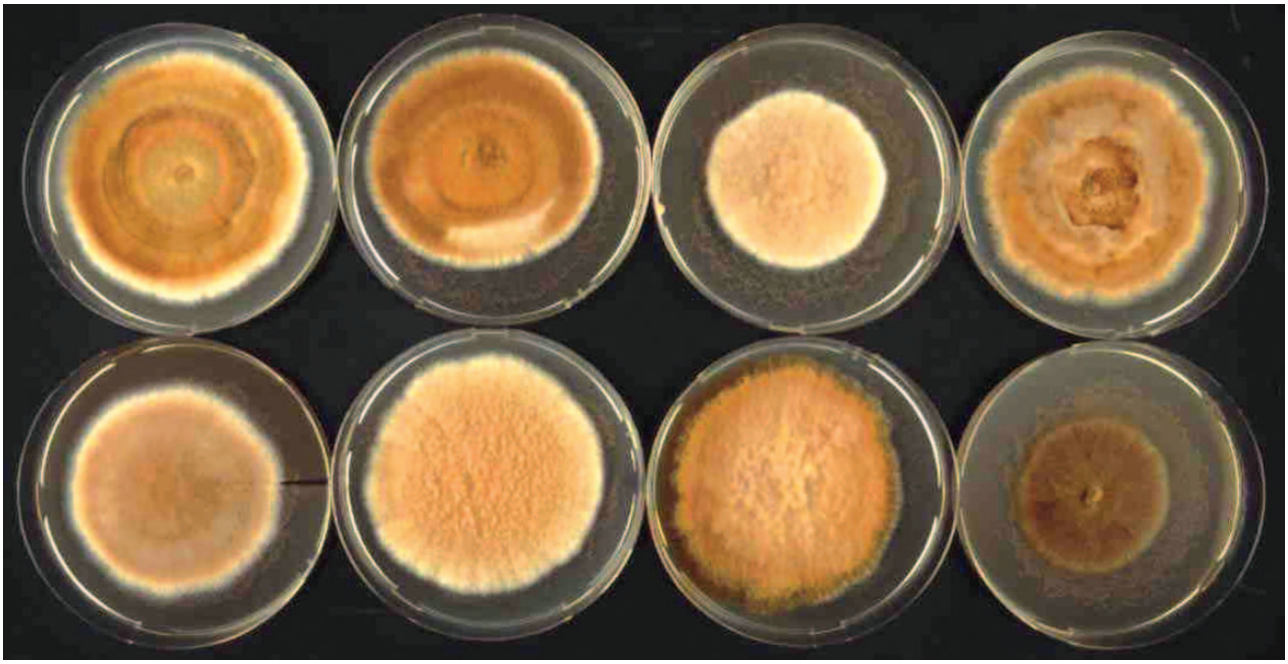

Fig. 3. *Fomitiporia mediterranea*. Isolates after three weeks cultivation at room temperature. Note differences in colour and growth rate.

consequently, an enlarged gene pool and genetic diversity (Cortesi *et al*., 2000; Péros *et al*., 2000; Jamaux-Déspreaux and Péros, 2003). Possibly the same scenario applies to *F. polymorpha* and *I. jamaicensis*. As is not unusual for lignicolous fungi, in the centre of their distribution area they occur on a variety of host plants, while at the margin of their range they are more specific. Here they often occur on a limited number of hosts, others being pre-occupied by other fungal organisms that may or may not be related to them (Jahn, 1967, 1978). White-rot fungi may be highly adaptable to new host plants, and as mentioned above, this is well illustrated by *Fmed* spreading to *Actinidia chinensis*. Within a few decades *Fmed* established itself on this new host plant on which in the meantime it has also been reported in Italy (Fischer, 2002) and Greece (Elena and Fischer, unpublished data). Whether esca symptoms also occur on non-*Vitis* hosts is still an open question, but some related symptoms such as wood discoloration or extensive white rot have recently been observed in *A. chinensis* (Elena and Paplomatas, 2002; Di Marco *et al*., 2003, 2004).

With a traditional approach, the identification of fungi requires fruit bodies providing different morphological and anatomical features ('morphological species recognition'; Taylor *et al*., 2000). The limitations of this approach are evident when attempting to distinguish between sibling species (Petersen and Hughes, 1999) such as *F. punctata*, *Fmed*, *F. australiensis,* and in part *F. polymorpha*, which are not distinguishable by traditional characters. Hymenochaetales, with few exceptions, do not form fruit bodies in culture. Without fruit bodies, identification must rely on cultured mycelium; however, this likewise is often problematic and time-consuming. While a considerable number of mycelial characters were introduced by Nobles (1958, 1965) and Stalpers (1978), resolution remains insufficient in closely related taxa. It was noted by Fischer (2002) that with *Fmed* the growth rate and overall appearance of cultured mycelium may vary with subsequent inoculations, and the same is true for other members of the Hymenochaetales (Niemelä, 1977). Possibly due to temperature differences, supply of nutrients, presence of antagonists, or for other, mostly unknown reasons, mycelium of *Fmed* may be of two types, the B- or the S-type (bleaching or staining of the culture medium). In some cases both these types may occur side by side, further complicating the task of using them as diagnostic characters. While most isolates of *Fmed* are B-type, considerable variation exists within the species (Fig. 3). The vegetative mycelium of the Hymenochaetales differs from other polypores in having a yellow to brown mat, sometimes containing setae or setal hyphae; clamps are always absent. As white rot fungi, they usually test positive for oxidase on artificial media containing tannnic or gallic acid.

In the present survey, molecular sequences were used to achieve a more accurate insight into biodiversity and taxonomic classification. With time and continuing search, increasing numbers of fruit bodies will be recovered in the field; however, vegetative isolates will remain the main basis for identification. In this way, molecular data will become increasingly important, although formal descriptions of new species will be hampered by the lack of fruiting structures.

Species-specific primers should prove useful for the rapid and reliable identification of unknown isolates, and such primers have been introduced for *Fmed*. The development and practical use of discriminating primers should be based on a large number of molecular sequences generated from the taxon in focus and its putative relatives. It is a major drawback that throughout the range of the Hymenochaetales, whether esca-related or not, taxonomic classification at species level is far from complete, particularly with respect to taxa from the southern hemisphere.

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