Review

Fungi associated with Esca disease of grapevine in Germany

M. FISCHER and H.-H. KASSEMEYER

Staatliches Weinbauinstitut Freiburg, Freiburg, Deutschland

Summary

Esca disease of grapevine is gaining increasing importance in Central European wine-growing countries. Several fungi, all of which are wood-inhabiting, were found to be associated with the disease. The taxa thought to act as main causal agents are the basidiomycete, Fomitiporia mediterranea, and, less frequently, the deuteromycetes, Phaeomoniella chlamydospora and Phaeoacremonium aleophilum. In addition, the species Eutypa lata, Phomopsis viticola, Botryosphaeria obtusa, and Cylindrocarpon destructans were isolated from Esca-affected vines. These species have been described in a standardized style and information is provided on taxonomy, cultured mycelium, microscopical characters, nuclear behaviour, as well as restriction and sequence data of ribosomal DNA.

Keywords: Esca, fungi, Fomitiporia mediterranea, Phaeomoniella chlamydospora, Phaeoacremonium aleophilum.

Introduction

Esca is a widespread disease of grapevine in many countries all over the world (overview: CHIARAPPA 2000). It is generally accepted that Esca is not a new disease; in fact, it was recognized already more than 100 years ago in the Mediterranean region as well as in California (RAVAZ 1898, PETRI 1912, VIALA 1926, BOURDOE and GALZIN 1927). There is even some evidence that the disease may be as old as vine cultivation itself, since references to esca-like symptoms were found in several ancient Greek and Latin works (VIALA 1926, MUGNAI et al. 1999, SURICO 2000).

A dramatic upsurge of the disease has been reported in recent years. This is most obvious for the Mediterranean vine-growing countries, where in some areas such as Tuscany more than 50 % of the vineyards have a disease incidence ranging from 20 to 30 % (CORTESI et al. 2000 a). The average annual increase is estimated to be 4-5 % (MUGNAI et al. 1999). Similar observations have been made for France, Greece, or Portugal (MUGNAI et al. 1999, REGO et al. 2000, ARMENGOL et al. 2001, REDONDO et al. 2001, RUMBIOS and RUMBAU 2001). It is striking to note that also the geographical range of the disease has extended; for instance, the vine-growing regions of Germany and Austria, essentially not affected by the disease up to the nineties, reported increasing incidence of Esca over the last few years (KASSEMEYER 1998, REISENZEIN et al. 2000, FISCHER and KASSEMEYER 2002, KASSEMEYER et al. 2002). In Germany, Esca has been shown to exist since approximately 15 years at least (FISCHER and KASSEMEYER 2002). Presently, the most severely affected areas are located in the southwestern part.

Esca is a complex disease comprising an array of symptoms. Usually within several years (“chronic esca”), but also within several months only (“acute esca”) vines are killed by the disease. Economic losses can be considerable, and any control of the disease by means of chemicals and/or based on altered viticultural management seems unlikely for the near future. In spite of intensive studies, the etiology of the disease is still not fully understood. It is therefore a main goal of these studies to unequivocally identify the causal agents and to learn as much as possible about their biology and life strategies. These data shall be used as a basis for far-reaching strategies in order to control the disease.

Esca comprises symptoms inside the trunk and larger branches, on the shoots, on the leaves and on the berries. While the symptoms on leaves and berries can vary considerably from year to year, even for the same vines (MUGNAI et al. 1999), wood decay symptoms are relatively stable. Thus it is concluded that its appearance in the woody parts provides the most reliable information on the occurrence and, to a certain degree, the intensity of the disease.

Symptoms in the wood such as white rot or small, dark-brown or black spots in cross sections indicate wood-inhabiting fungi to act as a potential pathogenic source of the Esca disease. It may be speculated that the symptoms on leaves and/or berries are caused by extracellular fungal toxins segregated into the vessels of the plant. In fact, the chemical structure of such toxins has been partly clarified in relation to other wood affecting diseases of grapevine (SPARAPANO et al. 2000, 2001, TABACCHI et al. 2000).

In 2001, a specific project was initiated by the State Institute for Viticulture in Freiburg concentrating on several aspects of Esca; among other things, emphasis was given to the putative organisms involved in the disease. In the course of investigations a considerable number of Esca-affected vines was examined and the spectrum of wood-colonizing fungi was elucidated. Vines were different in age and geographic origin, and represented most of the cultivars grown in Germany.

In this paper, the fungi most frequently isolated from the infected vines are presented both by illustrations and text. In this way, the morphology and microscopy of cultured mycelium, the nuclear behaviour of conidia and vegetative hyphae, and restriction and sequence data of the ribosomal ITS region are provided for the species Fomitiporia mediterranea, Phaeomoniella chlamydospora,
Phaeoacremonium aleophilum, Eutypa lata, Phomopsis viticola, Botryosphaeria obtusa, and Cylindrocarpon destructans. Although far from being complete, this compilation may provide a sound basis for a reliable assignment of the organisms involved in Esca disease in Germany.

In the literature, dispersed information is found in relation to lignicolous fungi and their possible occurrence on vine. These data have been compiled in an appendix and have been supplemented with our own observations. It should be noted that none of the enlisted fungi plays a role in Esca; nevertheless, they often indicate a somewhat weakened condition of the plant. The respective fruitbodies, which in most cases are formed regularly on the vine, are noticeable also for the less trained eye.

Material and Methods

Sampling of vines: The plants studied originated from different parts of Germany, mostly Baden-Württemberg, but also Rhineland-Palatinate, Hesse, Bavaria, and Saxony. Age of plants ranged from 1 to approximately 40 years. Esca-affected plants included red wine cultivars such as Merlot, Pinot meunier, Pinot noir, and Trollinger, and white wine cultivars such as Bacchus, Chasselas, Chardonnay, Pinot blanc, Pinot gris, Kerner, Morio-Muskat, Müller-Thurgau, Muskateller, Riesling, Ruländer, Scheurebe, Silvaner, and Traminer.

Isolation and culturing of fungi associated with esca symptoms: The trunks of diseased vines were cut into 3-5 parts of equal length. Exfoliating bark was peeled off and mycelia were isolated from symptomotic wood. Each section of the trunk was surface-sterilized by submersion in 30 % H₂O₂ for 30 s, followed by flaming. Using a sterile scalpel, a thin slice of the outer wood was removed from the surface, and small wood chips were sliced along the necrotic and/or spongy wood areas across the dark-brown or discolored line between healthy and diseased wood. Three chips were placed into 9 cm diameter petri plates containing ME (malt extract medium; 2 % agar, 2 % malt extract, 0.05 % yeast extract) or PDA (potato dextrose agar; 1.5 % agar, 0.4 % potato extract, 2 % glucose). Petri plates were incubated under permanent dark conditions at 21 °C.

For determination of mycelial growth rates selected strains were incubated on ME at 21 °C under permanent dark conditions. Mycelial growth was measured by calculating the mean of two perpendicular colony diameters. Two repeats were performed for each isolate.

Comparative microscopy: Usually slide cultures (van Uden 1951) were used for comparative microscopy of vegetative cultures. Observations were made in a drop of water, Melzer’s reagent, or lactophenol-cotton blue (Meixner 1975) at 500x or 1250x using phase contrast optics. Twenty observations were recorded for measurements of hyphae and conidia. Nuclei of conidia or mycelium were stained with Giemsa (Fischer 1987) for light microscopy, and with DAPI for fluorescence microscopy (Meixner and Bresinsky 1988). Micrographs were obtained under differential interference contrast optics (ZEISS Axioshot equipped with the digital camera Axiocam and the imaging software Axiovision).

DNA isolation and PCR amplification: Whole cell DNA was isolated from fresh mycelium; isolation was essentially as described by Lee and Taylor (1990). Quantity and quality of the DNA were examined on 1 % agarose gels. Isolated DNA was diluted 1:100 in distilled water. The polymerase chain reaction (PCR) was used to amplify a portion of the nuclear encoded ribosomal DNA unit defined by the primer combination prITS5 and prITS4 (for primer sequences, see White et al. 1990). The fragment spans the entire ITS1 region, the 5.8S rRNA gene, and the ITS2 region.

The PCR reactions were set up in 50 µl volumes and were overlaid with two drops of mineral oil. Hot start PCR was applied throughout (p’Aquila et al. 1991). Forty cycles were performed on a TRIO-Thermoblock (Biometra, Germany), using the following parameters: 95 °C denaturation step (1 min), 50 °C annealing step (1 min), 72 °C primer extension (1 min). A final incubation step at 72 °C (7 min) was added after the final cycle. 5 µl of each PCR reaction were electrophoresed on 1 % agarose gels. DNA molecular weight marker VI (Roche Diagnostics, Germany) was used as standard. The amplified products were purified with the QIAquick PCR Purification Kit (Qiagen, Germany) following the manufacturer’s instructions. DNA was suspended in 50 µl Tris-HCl buffer (10 mM, pH 8.0).

Restriction analyses: For restriction analysis, PCR products were extracted with one volume of 1:1 phenol/chloroform and centrifuged at 10.000 x g for 15 min; 80 µl of the aqueous portion were removed, and DNA was precipitated by the addition of 8 µl of sodium acetate (pH 8.0) and 190 µl of 100% ethanol (> 1 h, -20° C). Precipitates were collected by centrifugation (10.000 x g, 15 min), washed with 750 µl of 70 % ethanol, and resuspended in 30-50 µl TE buffer. For restriction analysis, the restriction enzymes Hpa II and Mbo I were used according to the manufacturer’s instructions (MBI Fermentas, Vilnius, Lithuania). The restriction products were separated on 2.5 % agarose gels. Fragment lengths were calculated using the Webcuter 2.0 program.

Sequencing: Representative strains of the isolated fungi were included in the sequencing experiments. Instead of mycelium derived from diseased wood, single spore isolates were used for strains of Fomitiporia mediterranea. Fragments were sequenced with the AmpliTaq DNA Polymerase FS Dye Terminator Cycle Sequencing kit (Perkin Elmer, USA), using 2 µl of premix, 1 µl of the primers (8 pmol of prITS1 and prITS4, respectively), and 3.5 µl of the PCR products. The reactions were set up in 11 µl volumes, and were overlayed with one drop of mineral oil.

Sequences were generated in two directions and 25 amplification cycles were carried out, using the following parameters: 96 °C denaturation step (30 s), 59 °C annealing step (15 s) for prITS1, 53 °C annealing step (15 s) for prITS4, 60 °C primer extension (4 min). DNA was precipitated by addition of 2 µl of NaAc (3 M, pH 4.8) and 55 µl of EtOH 100 %, and was then washed with 150 µl of EtOH 70 %. The DNA pellet was resuspended in 1:4 EDTA (50 mM, pH 8.0): formamide.
The electrophoresis was done with an ABI 373A Automatic Sequencer (Perkin Elmer). After processing the raw data with SeqEd (version 3.0), conspecific sequences were aligned using the ClustalX (version 1.64b) program (Thompson et al. 1997) and, when possible, were compared with respective sequences deposited in GenBank.

Results and Discussion

Sampling of infected vines: All in all, 156 esca-aﬀected vines were sampled in 2001 and 2002; distribution of the isolated fungal organisms is given in Tab. 1.

The basidiomycete, Fomitiporia mediterranea (Fmed), described only recently (Fischer 2002), was found to be the predominating fungus, and was isolated out of 63 % of the sampled vines. Fmed causes a white rot, and usually was recovered from zones of wood decay. While white rot was most evident in the uppermost part of the trunk, next to the pruning wounds, it also was found to extend into the more basal parts of the plant; here, it was often limited to the area around the pith or it spread along a sector, eventually reaching the surface of the trunk. In several cases Fmed was isolated out of darkened, very hard wood, colonized by the ascomycete, Eutypa lata. Remarkably, this species was frequently isolated from Esca-diseased vines (26 %), and often occurred side-by-side with Fmed (22 %).

Table 1

<table>
<thead>
<tr>
<th>Fungal organisms in esca-aﬀected grapevines (n=156)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fomitiporia mediterranea (Fmed)</td>
<td>98 (63 %)</td>
</tr>
<tr>
<td>Phaeoconiella chlamydospora (Pch)</td>
<td></td>
</tr>
<tr>
<td>Phaeoacremonium aleophilum (Pal)</td>
<td>46 (30 %)</td>
</tr>
<tr>
<td>Eutypa lata</td>
<td>40 (26 %)</td>
</tr>
<tr>
<td>Others1</td>
<td>70 (45 %)</td>
</tr>
<tr>
<td>Fmed + Pch/Pal</td>
<td>12 (8 %)</td>
</tr>
<tr>
<td>Fmed + Eutypa lata</td>
<td>32 (22 %)</td>
</tr>
<tr>
<td>Fmed + others1</td>
<td>38 (24 %)</td>
</tr>
<tr>
<td>Pch/Pal + Eutypa lata</td>
<td>8 (5 %)</td>
</tr>
<tr>
<td>Pch/Pal + others1</td>
<td>10 (6 %)</td>
</tr>
</tbody>
</table>

1) mostly Botryosphaeria, Cylindrocarpon, Phomopsis; also unidentified mycelia.

Fungi assignable to the genera Phaeoconiella and Phaeoacremonium were isolated less frequently (30 %). Usually these organisms were associated with small, brown or black spots distributed around an annual growth ring or they were recovered from the woody tissues close to the pith. The mycelial cultures isolated from such discoloured wood were mostly identiﬁed as Phaeoconiella chlamydospora (Pch) and, sometimes, Phaeoacremonium aleophilum (Pal); distinction to related taxa was often diﬃcult in the latter case (see notes below).

Sometimes, Pch and Pal could also be isolated from wood that had been decayed by white rot, and together with Fmed they grew out from the same wood chip in culture. In general, however, joint occurrence of Fmed and Pch respectively Pal in the same vine was observed less frequently (8 %).

45 % of the sampled vines were infected by some other ascomycetous fungi such as Botryosphaeria obtusa, Cylindrocarpon destructans, or Phomopsis viticola. While identiﬁcation was readily accomplished for the two latter taxa, not all of the Botryosphaeria isolates could unequivocally be assigned to B. obtusa. None of these three taxa is thought to be closely associated with Esca, instead they have their own symptomatology (Mugnai et al. 1999). These data demonstrate that different diseases may occur side-by-side in the same vine, in this way probably promoting the diseases’ progress.

The symptoms of wood deterioration caused by Pch and species of Phaeoacremonium became visible together with or, mostly, were preceding the white rot symptoms. The systematic isolation of fungi from discoloured wood indicated a close relation between individual stages of wood deterioration and particular fungal taxa. Based on the presented data, wood decay caused by Fmed seems to be the main reason for Esca disease in the geographic area under study, but quite often is preceded by discoloration of the wood caused by Pch and Pal. In this connexion it should be stated that Pch and Pal were isolated from plants between 1 year and 32 years old, while Fmed was isolated from plants between 4 years and approximately 40 years old.

Descriptions of fungal taxa

1. Fomitiporia mediterranea (M. Fischer): Taxonomy: Member in Hymenochoetaeae, Hymenochoetaeae, Basidiomycetes.

Cultured mycelium: Variable, two types can be distinguished at 21 °C on ME; type B (“bleaching type”): cottony to woolly, aerial hyphae yellowish to brownish, pigmentation of the medium weak or lacking, growth rate 3.0-4.5 cm in 14 d; type S (“staining type”): sparse development of aerial hyphae, pigmentation of the medium modest to strong, growth rate 1.5-2.5 cm in 14 d.

Microscopy (Fig.1): Hyphae septate and branched, hyaline to yellowish brown, smooth, septa partially hardly visible, without clamps, 1.5-5.5 µm wide; conidia absent; side-branches often arising next to septum.

Nuclear behaviour: Hyphal segments oligokaryotic, 2-4 (6-8) nuclei; hyphal tips often with distinctly increased number of nuclei. ITS fragments (strain 3, 316, 425 bp). ITS sequence (strain 45/23.3): 744 bp long.

AAGGATCATTAACGAGTTGGAACGTGGAGGTTGATGCT
TAATGCTCCTTGTGAGCGAAATACAAATATACAACTTTCA
CTTTTACTTATACAAACACTTTGCTTGTTCTTGTGAATGTG
CTCTTCATCCACTCAACCCCTGTGCACTTTATCAGAGTT
TGGTGCATATATAGTGTACATGTGTGCTCGCCTTCACA
TCTTCATACCACCAACCCCTGTGACCTTATACAGAGTT
AGTAAATGTTATGTTGTCACCTGACCTTGATCTGTATCTT
TTAGAAGCGGGTTAICTTCTACTAGCATGAGTAATAT
AACAATCTGTTGTTCTACTTACTTACCTGGAACACTTTGA
CTTATTTACTAACAACACTTTGCTTCTGCTGAAATGTT
TAATGTCCTTCTTGACCGAAATACAAATACACACTTTCA
ACAACGGATCTCTTTGGCTCTGCACTGATGAAAGACCCAG
CGAAATGCCGATAAGTAATGGAATTCGAAATTCGTAAT
TCGTGACAACTTCTGAGCCATTCCGGCCCTTGGATCTCC
GAGGGCATCTTGGTATGTCATGTATATCTCAATCTCT
CTTTCCTCTTAATGGAAGAGGGGCTTGTAGGATTTTA
ATATATATATATGCTGTCTCTGCTATGCGCTCTCTCAA
AATTGATAGTCCGACGTTGACGTCTGCTTTGTGTTAGT
AAATAGTTTCTACTTATATCTACTAGCTGTTACTGACT
GTCTGCTTCTATAGGTCCGATATGCGGACAGTACTCT
GTACTCCTAACATTTGACTCTTCTTGAACATACGGTA
GCTTACCGCTGACTTAA

Notes: *Fomitiporia mediterranea* (*Fmed*) seems to be the main causal agent for Esca disease in Germany; data are somewhat less conclusive for other grape-growing countries. The species causes a white rot in the trunk; especially in the uppermost parts, less distinct in the basis. Fruit bodies are somewhat less conclusive for other grape-growing countries. More literature, providing data on morphology, microscopy, and the molecular background: CROUS et al. (1996), CROUS and GAMS (2000), and GROENEWALD et al. (2001).


Cultured mycelium: Colonies wooly, with weak development of aerial hyphae, honey to olivaceous-brown, pigmentation of the medium concolorous, growth rate 1.0-1.6 cm in 14 d.

Microscopy (Fig. 2): Hyphae septate and branched, occurring in strands of up to 7, smooth or with tiny warts, without clamp. ITS fragments (strain 3302/I): 555 bp long.

Notes: *Phaeomoniella chlamydospora* (*Pch*) is usually present in wood showing stages of discoloration such as "brown wood-streaking" or black streaks; it is often associated with black exudate that oozes from the xylem when vines are cut in cross section. However, the fungus is difficult to isolate because of slow growth of colonies, which may require several weeks before growing out from wood chips onto agar plates. *Pch* together with *Phaeoacremonium aleophilum* (*Pal*; see below) is discussed as the main causal agent of Esca ("Petri disease") in some grape-growing countries.


Cultured mycelium: Colonies wooly, with weak development of aerial hyphae, honey to olivaceous-brown, pigmentation of the medium concolorous, growth rate 1.0-1.6 cm in 14 d.
Fungi associated with Esca

Fig. 1: *Fomitiporia mediterranea* - vegetative mycelium on ME; note septa (s) and branching (b) of hyphae.

Fig. 2: *Phaeomoniella chlamydospora* - vegetative mycelium on ME; note strand of hyphae (st), conidiogenous cells (co), conidia (c), and chlamydospore (ch).

Fig. 3: *Phaeoacremonium aleophilum* - vegetative mycelium on ME; note allantoid conidia (c).

Fig. 4: *Eutypa lata* - curved conidia released from pycnidium.

Fig. 5: *Phomopsis viticola* - vegetative mycelium on ME; note ellipsoid A-conidia (a).

Fig. 6: *Botryosphaeria obtusa* - vegetative mycelium on ME; note rarely branched hyphae and hyphal swellings (sw).

Fig. 7: *Cylindrocarpon destructans* - vegetative mycelium on ME; note rarely branched hyphae.
P. mortoniae. All these taxa are difficult to distinguish and their taxonomic rank may not be fully established yet. Further literature, providing data on morphology, microscopy, and molecular background: Crous et al. (1996), Dupont et al. (2000), and Groenewald et al. (2001).


Cultured mycelium: Colonies cottony, white at first, becoming greyish to cream-coloured with age, reverse side becoming partly dark-grey to blackish, pigmentation of the medium concolorous, fast growing, 5.5-7.1 cm in 7 d; pycnidia formed after 3-4 weeks, black, scattered to slightly aggregated, up to 600 µm wide; conidial mass subglobose to globose, cream-colored to orange.

Microscopy (Fig. 4): Hyphae septate, branched, hyaline, smooth, length of hyphal segments very variable, without clamps, (1.0)1.5-4.0 (7.0) µm wide; ring-like structures rarely formed by single hyphae, 10-20 µm in diameter; A-conidia hyaline, unicellular, ellipsoidal, 6.0-7.0 x 2.0-3.0 (3.5) µm; B-conidia less common, hyaline, filiform, curved, 14.0-18.0 (22.0) x 1.0-2.0 µm.

Nuclear behaviour: Hyphal segments with 1-3 (7) nuclei; A-conidia uninucleate, B-conidia unknown. ITS fragments (strain MT.Zi.6): Hpa II: 7 fragments (10, 37, 70, 73, 86, 92, 192 bp); Mbo I: 3 fragments (38, 208, 314 bp). ITS sequence (strain MT.Zi.6): 560 bp long.

AACCCTTTGGATCATACCACTTACGGCCGACGCTTGCACGACGTCCGCCAGAGGAGGCCCTCGCGGGCCCCCCCG
GCAGGCCGGCCCCCCCCGGGCGGGGCGGCAGCGACGCCCTGGTATACCTCTTGTTGTTTAACCTCGGAAATCGTGATTTG
TTGCGCTTCGTTAGGTGTGTCAGCGTCGTTGTTGGGAGCCTATCTCCGGATAGCTCCTCAAAA
GCCTGTTCGAGCGTCATTTCGACCTTCAAGCCCTAGCTG
GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCT
ACTTAATAAGTTAAAACTTTCAACAACGGATCTCTTGG
CTTAAACTCTTGTTTTTTAGTGATTATCTGAGTGTTTAT
CCCTGTAGCCCGCTGCAGGCCTACCCGCCGGTGGACA
GTGAACTTACTATGTTGCCTTGGGCGGGGAAGCTTAC

Notes: Phomopsis viticola is another fungus detected quite regularly in Esca-affected vine. However, the diseases caused by P. viticola, are distinct from Esca, and are known as "black arm disease" or "cane and leaf spot disease". It should be noted that taxonomy and pathogenicity within P. viticola has not been fully clarified yet. A comprehensive overview is provided by Mostert et al. (2001).

5. Phomopsis viticola (Sacc.) Sacc.: Taxonomy: Teleomorph not formed in artificial culture; member in Botryosphaeriaceae, Pleosporales, Ascomycetes; anamorph within Diplodia Fr. apud Mont.

Cultured mycelium: Colonies wooly, with dense aerial mycelium, grey-brown to olivaceous-brown, pigmentation of the medium concolorous, fast growing, 4.8-6.2 cm in 4 d, no pycnidia formed on ME (see “Notes” below).

Microscopy (Fig. 6): Hyphae septate, rarely branched, hyaline to light brown, smooth, without clamps, (1.0) 1.5-5.0 µm wide; ring-like structures formed by single hyphae, 10-20 µm in diameter; A-conidia hyaline, unicellular, ellipsoidal, 6.0-7.0 x 2.0-3.0 (3.5) µm; B-conidia less common, hyaline, filiform, curved, 14.0-18.0 (22.0) x 1.0-2.0 µm.

Nuclear behaviour: Hyphal segments with 1-3 (7) nuclei; A-conidia uninucleate, B-conidia unknown. ITS fragments (strain We.Oc.2): Hpa I: 4 fragments (8, 18, 197, 312 bp). ITS sequence (strain We.Oc.2): 535 bp long.

AAACCTTTGGATCATACCACTTACGGCCGACGCTTGCACGACGTCCGCCAGAGGAGGCCCTCGCGGGCCCCCCCG
GCAGGCCGGCCCCCCCCGGGCGGGGCGGCAGCGACGCCCTGGTATACCTCTTGTTGTTTAACCTCGGAAATCGTGATTTG
TTGCGCTTCGTTAGGTGTGTCAGCGTCGTTGTTGGGAGCCTATCTCCGGATAGCTCCTCAAAA
GCCTGTTCGAGCGTCATTTCGACCTTCAAGCCCTAGCTG
GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCT
ACTTAATAAGTTAAAACTTTCAACAACGGATCTCTTGG
CTTAAACTCTTGTTTTTTAGTGATTATCTGAGTGTTTAT
CCCTGTAGCCCGCTGCAGGCCTACCCGCCGGTGGACA
GTGAACTTACTATGTTGCCTTGGGCGGGGAAGCTTAC

Notes: The species forms perithecia on diseased trunk, and molecular background: Crous et al. (2000), and Groenewald et al. (2001).
AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATG
AAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGC
AGAATTCAGTGAATCATCGAATCTTTGAACGCACATTG
CGCCCCCTGGCATTCCGGGGGGCATGCCTGTTCGAGCG
TCATTACAACCCTCAAGCTCTGCTTGGTATTGGGCGCC
GTCCCTCTGGGAGCCGCCTTAAAGACCTCGGCGGTG
GCTGTTCAGGCCCTCAAGCGTAGTAGAATACACCTCGCT
TTGGAGCGGTTGGCGTCGCCCGCCGGACGAACCTTCTG
AACTTTTCTCAAGGTTGACCTCGGATCAGGTAGGGATA
CCC
Notes: Species of Botryosphaeria cause canker and dieback of pomaceous fruits and grapevine. In Bordeaux vineyards, B. obtusa was found to be associated with black dead arm of grapevine (LARIGNON and DUBOS 2001), showing similar foliar symptoms to those caused by Esca. While B. obtusa is not considered to be associated with Esca, it can be sometimes isolated from affected vines. According to Phillips (2002), pycnidia are formed after several days on oatmeal agar; they are scattered over the agar surface, up to 1 mm in diameter; conidia are cylindric, hyaline when young, becoming dark brown when old, smooth, (10.0) 15.0-25.0 x (7.0) 10.0-13.0 µm.


Cultured mycelium: Colonies cottony to fluffy, appressed at the margin, white to buff, sometimes with prominent growth rings, these with appressed mycelium, reverse side concolorous, growth rate 2.8-3.7 cm in 14 d.

Microscopy (Fig. 7): Hyphae septate, rarely branched, sometimes forming strands of up to 10, mostly straight, hyaline, smooth, without clamps, (1.5) 2.0-3.0 (6.0) µm wide; hyphal swellings present in most cultures, intercalar, in chains of up to 20, up to 10.0 µm in diameter; conidia absent.

Notes: In Portugal, C. destructans along with species of Phaeoacremonium is the fungus most frequently isolated out of discoloured wood (REGO et al. 2000) and is thought to be involved in young vine decline or Petri disease. Cylindrocarpon destructans has only rarely been observed in our study.

Table 2
Lignicolous fungi on grapevine

<table>
<thead>
<tr>
<th>Species</th>
<th>Literature</th>
<th>Life strategy</th>
<th>Type of rot</th>
<th>Spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Armillaria mellea</td>
<td>(KREISEL 1961)</td>
<td>saprophytic, parasitic</td>
<td>white rot</td>
<td>rhizomorphs, spores</td>
</tr>
<tr>
<td>Clitopilus hobsonii</td>
<td>(FISCHER unpubl.)</td>
<td>saprophytic</td>
<td>unknown</td>
<td>spores</td>
</tr>
<tr>
<td>Flammulina velutipes</td>
<td>(KREISEL 1961)</td>
<td>parasitic</td>
<td>white rot</td>
<td>spores</td>
</tr>
<tr>
<td>Pleurotus pulmonarius</td>
<td>(FISCHER unpubl.)</td>
<td>saprophytic, parasitic</td>
<td>white rot</td>
<td>spores</td>
</tr>
<tr>
<td>Inonotus hispidus</td>
<td>(RYVARDEN and GILBERTSON 1993)</td>
<td>parasitic</td>
<td>white rot</td>
<td>spores</td>
</tr>
<tr>
<td>Stereum hirsutum</td>
<td>(MUGNAI et al. 1996)</td>
<td>saprophytic, parasitic</td>
<td>white rot</td>
<td>spores</td>
</tr>
<tr>
<td>Trametes hirsuta</td>
<td>(FISCHER unpubl.)</td>
<td>saprophytic, parasitic</td>
<td>white rot</td>
<td>spores</td>
</tr>
<tr>
<td>Trametes versicolor</td>
<td>(FISCHER unpubl.)</td>
<td>saprophytic, parasitic</td>
<td>white rot</td>
<td>spores</td>
</tr>
<tr>
<td>Peniophora incarnata</td>
<td>(FISCHER unpubl.)</td>
<td>saprophytic</td>
<td>white rot?</td>
<td>spores</td>
</tr>
<tr>
<td>Hirsneola auriculariae-juda</td>
<td>(FISCHER unpubl.)</td>
<td>saprophytic</td>
<td>white rot</td>
<td>spores</td>
</tr>
</tbody>
</table>

Fungi associated with Esca 115
Appendix - occurrence of basidiomycetous fruitbodies on grapevine: Wood-inhabiting fungi are able to utilize components of wood cell walls as their main source of energy for growth and reproduction. They can be grouped into two categories depending on the enzyme system they produce to decay wood. Grapevine, as most other hardwoods, is susceptible to white rot fungi, decomposing both lignin and polysaccharides. Therefore, it is not surprising that a considerable number of white rot basidiomycetes can be found on grapevine. The fruitbodies of basidiomycetous taxa that have been detected on vine in the geographic area under study in 2001 and 2002 were identified (Tab. 2). For all taxa, some basal information is provided with respect to life strategy, i.e. saprophytes and/or parasites, type of rot, and mode of spread. While Armillaria mellea, Flammulina velutipes, Inonotus hispidus, and Stereum hirsutum have been known before as occurring on Vitis vinifera, several taxa, namely Clitopilus hohsonii, Pleurotus pulmonarius, Trametes hirsuta, T. versicolor, Peniophora incarnata, and Hirneola auriculo-laeuda are demonstrated here for the first time as living on grapevine. All species in Tab. 2 are not restricted to Vitis, and can be found on a considerable number of other hard-wood genera as well. Certainly, additional vine-inhabiting fungi will be detected in the future.

Acknowledgements

We are indebted to all those persons who have provided grapevine material. This study was financially supported by the Bundesanstalt für Landwirtschaft und Ernährung (BLE).

References


Received April 28, 2003