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What if esca disease of grapevine were not a fungal disease?

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Abstract Esca disease, which attacks the wood of grapevine, has become increasingly devastating during the past three decades and represents today a major concern in all wine-producing countries. This disease is attributed to a group of systematically diverse fungi that are considered to be latent pathogens, however, this has not been conclusively established. This study presents the first in-depth comparison between the mycota of healthy and diseased plants taken from the same vineyard to determine which fungi become invasive when foliar symptoms of esca appear. An unprecedented high fungal diversity, 158 species, is here reported exclusively from grapevine wood in a single Swiss vineyard plot. An identical mycota inhabits wood of healthy and diseased adult plants and presumed esca pathogens were widespread and occurred in similar frequencies in both plant types. Pioneer esca-associated fungi are not transmitted from adult to nursery plants through the grafting process. Consequently the presumed esca-associated fungal pathogens are most likely saprobes decaying already senescent or dead wood resulting from intensive pruning, frost or other mecanical injuries as grafting. The cause of esca disease therefore remains elusive and requires well executive scientific study. These results question the assumed pathogenicity of fungi in other diseases of plants or animals where identical mycota are retrieved from both diseased and healthy individuals.

Keywords Fungal community · *Vitis vinifera* · Esca disease · ITS based fungal identification · Fungal pathogens

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Introduction

Grapevine trunk diseases are considered to be the most destructive diseases of grapevine of the past three decades and are of rapidly growing concern in all wine producing countries (Bertsch et al. 2009). The worldwide economical cost for the replacement of dead grapevine plants alone is here roughly estimated to be in excess of 1.5 billion dollars per year (Box 1). In the literature, the term 'grapevine trunk diseases' refers to a number of different diseases that are inflicted by pathogenic fungi that deteriorate the perennial organs of grapevine. The most destructive among these diseases are esca and young vine decline ('young esca') that develop respectively in established and newly planted vineyards (Halleen et al. 2003; Larignon and Dubos 1997; Martin and Cobos 2007; Mugnai et al. 1999). Esca occurs in adult plants aged 10 years or more and can manifest itself

in two ways: a slow evolving form that is recognizable by visible foliar symptoms or an apoplectic form that kills the plants within a few days (Mugnai et al. 1999). A plant may express foliar symptoms over a few years, consecutively or not, but will then generally die from apoplexy. Known since antiquity, esca was long considered as an almost negligible weakness disease that could be controlled with fungicides (Graniti et al. 2000). During the past three decades however, and coinciding with the recent ban on the use of sodium arsenite, the incidence of esca increased drastically infecting as many as 50 % of vines in some Italian vineyards (Bertsch et al. 2009; Surico et al. 2006). At the same time, the broad establishment of new vineyards globally has been accompanied by a dramatic increase of young vine decline, a disease expressing similar foliar symptoms as esca, but occurring in grapevine plants 1 to 9 years old (Edwards et al. 2001; Eskalen et al. 2007; Ferreira et al. 1999; Gramaje and Armengol 2011).

Box 1. Estimate of the yearly economic cost of worldwide grapevine (*Vitis vinifera*) replacement due to fungal trunk diseases.

Esca (including black dead arm [BDA] after Surico et al. [2006] or also called black measles), young vine decline (= Petri disease, young esca, including black foot disease), and eutypa dieback are considered fungal diseases of grapevine wood that lead generally to the death of the plant. If these diseases are present in all vineyards worldwide (Bertsch et al. 2009), their incidence is highly variable depending on the geographical area, the year, the grapevine cultivar, the rootstock used for grafting and environmental factors (Surico et al. 2006; Gramaje and Armengol 2011; Sosnowski et al. 2007). Esca diseased plants can exhibit foliar symptoms during several years, consecutively or not, before dying, but in all cases part of the yield will be lost (Marchi 2001, Surico et al. 2000). Precise information concerning fungal diseases on grapevine is sparse and the data are usually restricted to a particular wine-producing region or country, or may apply only to a single specific fungal disease or to a particular grapevine cultivar. For some Italian vineyards, the incidence of cumulated esca diseases (up to 50 %) values has been estimated (Surico et al. 2006). A six-year study of esca in Austria revealed an annual increase of 2.7 % for the appearance of the foliar symptoms in vineyards (Reisenzein et al. 2000). In the region of Alsace (France), esca and Eutypa dieback together have been reported to result in up to 10 % of plant replacement yearly (Kuntzmann et al. 2010). Young vine decline has been reported as widespread in California but is responsible for the replacement of only 1 to 5 % of the plants in newly established vineyards (Eskalen et al. 2007). Eutypa dieback alone has been estimated to cause production losses in Australia equivalent of 20 million Australian dollars (US$ 20.5 millions) for the sole Shiraz cultivar (Sosnowski et al. 2005), while in California (USA) the cost to wine grape production alone by this same disease has been estimated to be in excess of 260 million dollars per year (Rolshausen and Kiyomoto 2011).

It is clear that grapevine trunk diseases constitute a major concern in many wine-producing regions and countries of the world, but the lack of precise and comparable data makes it impossible to calculate the approximate economic impact of these diseases. Nevertheless, considering solely the replacement of dead plants it is possible to estimate the rough minimal cost due to grapevine trunk diseases. The International Organisation of Vine and Wine (OIV report 2011), estimates the actual surface of vineyards worldwide to amount to 7.550.000 ha. On the other hand, the overall cost for planting a single hectare of vineyard has been evaluated to be equivalent to 15.000 euros (Brugali 2009). Considering now a replacement of only 1 % of the plants per year - a considerable underestimate in view of the individual regional data found in the literature - the worldwide annual financial cost of the replacement of death plants due to grapevine trunk diseases is without doubt in excess of 1.132 billion euros (US$ 1.502 billion).

Studies on trunk diseases of grapevine have mainly focused on the description of the disease symptoms and on the isolation and identification of the fungi present in necrotic wood of symptomatic plants. The principal pathogenic taxa associated with esca are *Eutypa lata, Phaeomoniella chlamydospora*, and various species of the genera *Botryosphaeria, Cylindrocarpon, Fomitiporia, Phaeoacremonium, Phellinus, Phomopsis,* and *Stereum* (Armengol et al. 2001; Larignon and Dubos 1997; Mugnai et al. 1999; Surico et al. 2006). With the exception of basidiomycetous *Fomitiporia, Stereum,* and *Phellinus* species, all these pathogens have also been isolated from necrotic wood of plants suffering from young vine decline, although with a higher incidence for *Cylindrocarpon* species, *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum*, and one additional genus, *Cadophora* (Edwards and Pascoe 2004; Giménez-Jaime et al. 2006; Gramaje and Armengol 2011; Halleen et al. 2003; Martin and Cobos 2007; Scheck et al. 1998). The fungi that are held responsible for esca or young vine decline have also been associated individually with other grapevine diseases. As such, *Eutypa lata* is considered to be responsible for eutypa dieback (Kuntzmann et al. 2010), *Phomopsis viticola* for excoriosis, *Botryosphaeria dothidea* for cane blight (Phillips 2000), various *Cylindrocarpon* species for black foot disease (Halleen et al. 2006) and *Botryosphaeria* species for cankers (Urbez-Torres et al. 2006). It is unclear whether esca and young vine decline are due to these different fungi acting jointly or in succession (Graniti et al. 2000). These disease-associated fungi have also been isolated with variable incidence from nursery plants (Casieri et al. 2009), rootstock mother vines (Gramaje and Armengol 2011; Aroca et al. 2010) as well as from apparently healthy young and adult grapevines (Gonzáles and Tello 2010), leading to the view that these fungi are latent pathogens (Verhoeff 1974). Climatic and edaphic factors as well as host genotype (i.e. grapevine cultivar) have been reported to influence the incidence of these trunk diseases (Bertsch et al. 2009; Surico et al. 2006; Graniti et al. 2000), thereby suggesting that these fungal pathogens are a prerequisite for the expression of the disease symptoms, but are themselves not always responsible for their appearance. In spite of an impressive number of phytopathological studies over the past years, the epidemiology and etiology of grapevine wood diseases remain poorly understood (Bertsch et al. 2009).

The assumption that these fungi are latent pathogens implies that they may live asymptomatically for at least part of their life in a plant, but should then, at some point, modify their behavior and become invasive, thereby leading to the expression of the disease symptoms (Verhoeff 1974). A first objective of the present study was to determine which fungal species modified their latent behavior and became invasive when esca symptoms appear. Secondly, as the contamination of nursery plants is presently one of the major concerns of the wine industry, we also wanted to determine whether the esca-associated fungi were transmitted to nursery plants through grafting material. In order to achieve these objectives, we analyzed the cultivable part of the fungal community that inhabits the wood of both healthy and esca-symptomatic grapevine plants, as well as the cultivable part of the fungal community that is associated with the wood of nursery plants. In this respect, it is important that the latter were not hot water treated and were grafted on identical rootstock as adult plants using shoots of apparently healthy material sampled from the same experimental adult vineyard.

Materials and methods

Grapevine plant selection and isolation of fungal strains from Vitis vinifera wood

The Agroscope Changins-Wädenswil (Federal Research Station in Agronomy, Switzerland) has surveyed a number of vineyards for the presence of esca foliar symptoms and occurrence of apoplexy since 2002. Among these vineyards, we chose a plot of 1134 grapevine plants of a Chasselas cultivar grafted on rootstock 3309 in Perroy (Lavaux) suffering a 5.5 % incidence of esca foliar symptoms in 2009, the year of the experiment (Online Resource 1). Interested by the transition from asymptomatic to symptomatic plants, we sampled only plants expressing the esca foliar symptoms for the first time since the beginning of the vineyard survey, 38 adult plants (15–30 years old), and 69 plants that had not expressed any signs of esca disease since 2002. Interested in the transmission of esca-related fungi during the grafting process, we also isolated fungi from 73 nursery plants made by the vineyard grower himself, who cultivates his own rootstock. We considered that by sampling nursery plants grafted on the identical rootstock as that of the adult plants sampled, and by using grafting material of apparently healthy plants from the same adult vineyard, the bias of sampling location should be considerably reduced (Arnold et al. 2003). These nursery plants were not hot water treated; commercial dormant nursery plants are usually treated with hot water (50°C, 30 min) to obtain plants free from pathogenic fungi, bacteria, nematodes and *Plasmopara* (Gramaje and Armengol 2011; Crous et al. 2001).

Wood of adult plants was sampled in the field via a non-destructive method. Using a power drill with a surface-sterilized (EtOH 90 %) drill (Ø 2.5 mm), a hole was made to remove the bark and access to the deeper part of the wood. The sampling was then performed by running the drill gently in the same hole to allow coiled wood pieces (2–3 cm long) to stick to the drill bit without breaking. The wood fragments were immediately placed in an Eppendorf tube containing 1.5 ml of sterile Potato Dextrose Broth (PDB, Difco) with alcohol surface sterilized tweezers. Such wood samples were taken from three different parts of each trunk (base, middle and upper part). We sampled a maximum of 20 plants per day to be able to plate wood pieces from the PDB Eppendorfs on to 15 cm diameter Petri dishes containing potato dextrose agar (PDA, Difco) amended with aureomycin (12.5 mg L^{-1}) the same day. Very small, 2–3 mm wood pieces were placed on agar (15 wood pieces per plant, 5 from each part of the trunk) in order to maximize the chance to retrieve slow growing species. For nursery plants, the sampling method was destructive. The plants were first stripped of their bark and surface sterilized with 3.5 % NaOCl for 20 min after removal of the roots, soil and residual waxes. Fifteen small sections (1 mm) were aseptically cut regularly from the basal end to the grafting end of the plant and 2–3 mm of each wood sections transferred on PDA. Consequently fungi associated with nursery plants have been isolated from 15 independent wood samples while fungi associated with adult plants have been isolated from only three independent wood samples, each split in five pieces.

Plates were inspected daily for the emergence of fungi over 4 weeks. Emerging fungi were isolated in pure culture and grown on PDA+aureomycin at room temperature. Pieces of wood from which no fungus had grown were eventually transferred onto a new plate to avoid contamination by fast growing species developing from closely plated wood pieces. We isolated in pure culture 2595 fungi from 180 grapevine plants (934 fungal isolates from 69 asymptomatic plants, 531 fungal isolates from 38 esca symptomatic plants, and 1130 fungal isolates from 73 nursery plants). A single culture medium, PDA, was used to isolate and grow our isolates from the grapevine wood pieces, although several studies have shown that some fungi need particular media to grow (Guo et al. 2001; Van Wyk et al. 2007). However, since our study focused essentially on fungi associated with wood diseases of grapevine that all grow on PDA medium, we limited ourselves to this commonly used culture medium for our experiment even if involved the risk of missing a few wood-associated fungi that are difficult or unable to grow on this medium. We use the term fungal community or mycota aware that we isolated only part of the culturable fungi and missed uncultivable fungal species.

Amplification and sequencing of the fungal isolates ITS1-5.8S-ITS2 rDNA (ITS) region

Amplification and sequencing of the ITS of the fungal isolates was performed with the primers ITS1F (or ITS1) and ITS4 (the sequences of these primers are available at: http://www.biology.duke.edu/fungi/mycolab/primers.htm). Direct PCR was performed using a sterile pipetor tip (10 μl) to transfer aseptically a very small amount of mycelium in a PCR tube and to squash it manually with the tip in the PCR mix (25 μl mix, reagents and conditions of the *Taq* PCR core kit (QIAGEN, Inc., Valencia, California, USA). Sequencing used the amplification primers, reagents and conditions of the *BigDye®Terminator v3.1 Cycle sequencing Kit* and an automated capillary sequencer ABI 3700 DNA analyzer (Perkin Elmer, Applied Biosystems, Foster City, CA, USA).

Fungal diversity and species accumulation curves

Nomenclatural issues follow Mycobank. We estimated the species diversity in asymptomatic, esca-symptomatic, and nursery plants by calculating the Simpson index of the fungal community identified in each plant sample. The community composition was assessed based on the relative abundance of species in the culturable part of the fungal community. The expected total species diversity in the different plant categories was estimated by resampling the available plant samples. Based on 1000 replicates without replacement, we calculated the total recovered diversity within each plant category. Species accumulation curves were estimated using the vegan package implemented in the R statistical software (R Development Core Team 2006).

Principal component analyses (PCA)

A principal component analysis was performed in order to eventually identify differentiated fungal communities between symptomatic, asymptomatic and nursery plants. Each plant was considered as an independent replicate and the isolated fungal community on each plant sample was recoded as presence-absence data. We assessed the fungal community based on incidence data rather than on relative frequencies to reduce the bias introduced by species that may be more easily brought into culture than others. The R package vegan was used to calculate the main ordination axes 1 and 2 based on Euclidean distances (R Development Core Team 2006). Biplots were produced based on the PCA to show both the relationship of the fungal species and the plant samples in respect to the main axes.

Results

Delimitation and classification of the operational taxonomic units (OTUs) based on ITS sequences of the fungal isolates

The isolates were grouped based on their vegetative macromorphology. Some OTUs were readily identified based on their well-documented and singular anamorphic features (e.g. *Phaeomoniella chlamydospora*, *Aureobasidium pullulans*, *Truncatella angustata*, *Botrytis cinerea* or *Phaeoacremonium viticola*). Other species, especially closely related species within a single genus (e.g. *Cladosporium*, *Phoma*, *Alternaria* or the anamorphs of Botryosphaeriaceae and Nectriaceae), as well as some species exhibiting a variable morphology on Petri dishes (e.g. *Epicoccum nigrum*), could not be delimitated based on their vegetative morphology. We first amplified and sequenced the ITS region of a few fungal isolates for all morphotypes. For more plastic morphotypes, we sequenced more isolates. When the sequences obtained for the different isolates of plastic morphotypes were identical, we did not sequence the rest of the isolates grouped in this morphotype. When the sequences of the different isolates of a given morphotype were different we adopted two strategies depending on their similarity BLAST top score in GenBank: either the top score indicated that the isolates belong to the same species and we did not sequence the other isolates, or the BLAST top score indicated that they belonged to different species and we sequenced the ITS region for all isolates, except in the case of *Alternaria* for which we recovered ITS rDNA genotypes for 216 out of the 523 strains isolated (Online Resource 2) that differed only in the length of a T-repeat at the end of the ITS2 (see the Discussion section).

Having sequenced 907 out of a total of 2595 fungal isolates, we obtained 197 ITS genotypes. The GenBank accession numbers and the GenBank BLAST top score similarity of these ITS genotypes, excluding uncultured and environmental sequences, are listed in Online Ressource 2. We used a 99 % sequence BLAST similarity threshold for species delimitation (Gazis et al. 2011) even though previous fungal endophyte-related studies have used a lower threshold (≤98 %; Higgins et al. 2011; Neubert et al. 2006; O'Brien et al. 2005; Sánchez et al. 2007; Sánchez et al. 2008; U'Ren et al. 2010). The ITS sequence of the fungal isolate acwVHB69/4 (Online Resource 2) was 100 % similar with the ITS GenBank sequences of six different species of *Cladosporium*, including *C. subtilissimum*. In those cases where ITS rDNA sequences data discriminated more than one taxa, we used the prefix 'cf' in the fungal name (e.g. *Cladosporium cf subtilissimum*, Online Resource 2, Table 1). On the other hand, we also recovered variable ITS genotypes that corresponded to the same species under the blast results. In these cases we used the name derived from

Table 1 Classification of the fungal isolates and abundance/incidence of the OTUs in the different types of plants (asymptomatic, esca-symptomatic and nursery plants).

Taxon anamorph[a]	Class, Order	Family	Asymptomatic	Esca-symptomatic	Nursery
Acaromyces ingoldii (B)[b]	Exobasidiomycetes	?	2 iso/2 pl[c]	2 iso/1 pl	0 iso/0 pl
Acremonium alternatum (A)	Sordariomycetes, Hypocreales	?	8 iso/4 pl	6 iso/3 pl	19 iso/15 pl
Acremonium fusidioides (A)	?	?	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Alternaria alternata species complex (A)	Dothideomycetes, Pleosporales	Pleosporaceae	153 iso/51 pl	96 iso/32 pl	274 iso/68 pl
Alternaria infectoria (A)	Dothideomycetes, Pleosporales	Pleosporaceae	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Aspergillus iizukae (A)	Eurotiomycetes, Eurotiales	Trichocomaceae	4 iso/2 pl	2 iso/1 pl	0 iso/0 pl
Atheliaceae sp. (B)	Agaricomycetes, Atheliales	Atheliaceae	0 iso/0 pl	0 iso/0 pl	15 iso/9 pl
Aureobasidium pullulans (A)	Dothideomycetes, Dothideales	Dothioraceae	147 iso/50 pl	80 iso/28 pl	19 iso/16 pl
Bjerkandera adusta (B)	Agaricomycetes, Russulales	Meruliaceae	3 iso/3 pl	0 iso/0 pl	0 iso/0 pl
Boeremia telephii (A)	Dothideomycetes, Pleosporales	Didymellaceae	6 iso/3 pl	2 iso/1 pl	1 iso/1 pl
Botrytis cinerea (A)	Leotiomycetes, Helotiales	Sclerotiniaceae	37 iso/17 pl	17 iso/10 pl	28 iso/12pl
Botrytis sp. (A)	Leotiomycetes, Helotiales	Sclerotiniaceae	0 iso/0 pl	0 iso/0 pl	3 iso/1 pl
Cadophora luteo-olivacea (A)	Leotiomycetes, Helotiales	?	10 iso/7 pl	7 iso/4 pl	80 iso/32 pl
Cadophora melinii (A)	Leotiomycetes, Helotiales	?	3 iso/1 pl	1 iso/1 pl	0 iso/0 pl
Cadophora sp. (A)	Leotiomycetes, Helotiales	?	3 iso/3 pl	0 iso/0 pl	0 iso/0 pl
Candida railenensis (A)	Saccharomycetes, Saccharomycetales	?	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Candida sake (A)	Saccharomycetes, Saccharomycetales	?	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Cantharellales sp. (B)	Agaricomycetes, Cantharellales	?	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Capronia sp. (A)	Eurotiomycetes, Chaetothyriales	Herpotrichiellaceae	3 iso/3 pl	0 iso/0 pl	0 iso/0 pl
Ceratobasidium sp. (B)	Agaricomycetes, Cantharellales	Ceratobasidiaceae	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Chaetomium globosum (A)	Sordariomycetes, Sordariales	Chaetomiaceae	0 iso/0 pl	1 iso/1 pl	2 iso/1 pl
Chaetomium sp. (A)	Sordariomycetes, Sordariales	Chaetomiaceae	0 iso/0 pl	0 iso/0 pl	4 iso/3 pl
Chalara sp. (A)	Leotiomycetes, Helotiales	?	2 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Ciboria americana (A)	Leotiomycetes, Helotiales	Sclerotiniaceae	0 iso/0 pl	2 iso/1 pl	0 iso/0 pl
Cladosporium cf subtilissimum (A)	Dothideomycetes, Capnodiales	Davidiellaceae	6 iso/5 pl	3 iso/3 pl	1 iso/1 pl
Cladosporium xylophilum (A)	Dothideomycetes, Capnodiales	Davidiellaceae	41 iso/21 pl	24 iso/11 pl	3 iso/3 pl
Clonostachys rosea f. catenulata (A)	Sordariomycetes, Hypocreales	Bionectriaceae	12 iso/7 pl	7 iso/3 pl	65 iso/34 pl
Cochliobolus homomophorus (A)	Dothideomycetes, Pleosporales	Pleosporaceae	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Colletotrichum phormii (A)	Sordariomycetes,	Glomerellaceae	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Coniolariella sp. (A)	Sordariomycetes, Xylariales	Xylariaceae	0 iso/0 pl	0 iso/0 pl	12 iso/6 pl
Cosmospora vilior (A)	Sordariomycetes, Hypocreales	Nectriaceae	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Cucurbitariaceae sp. (A)	Dothideomycetes, Pleosporales	Cucurbitariaceae	0 iso/0 pl	1 iso/1 pl	0 iso/0 pl
Cylindrocarpon destructans (A)	Sordariomycetes, Hypocreales	Nectriaceae	0 iso/0 pl	0 iso/0 pl	27 iso/18 pl
Cylindrocarpon liriodendri (A)	Sordariomycetes, Hypocreales	Nectriaceae	0 iso/0 pl	0 iso/0 pl	9 iso/5 pl
Cylindrocarpon macrodidymum (A)	Sordariomycetes, Hypocreales	Nectriaceae	0 iso/0 pl	0 iso/0 pl	38 iso/29 pl
Cylindrocarpon pauciseptatum (A)	Sordariomycetes, Hypocreales	Nectriaceae	0 iso/0 pl	0 iso/0 pl	3 iso/3 pl
Cylindrocarpon sp. 1 (A)	Sordariomycetes, Hypocreales	Nectriaceae	0 iso/0 pl	0 iso/0 pl	4 iso/3 pl
Cylindrocarpon sp. 2 (A)	Sordariomycetes, Hypocreales	Nectriaceae	0 iso/0 pl	0 iso/0 pl	9 iso/6 pl
Diaporthe viticola (A)	Sordariomycetes, Diaporthales	Valsaceae	0 iso/0 pl	0 iso/0 pl	20 iso/13 pl
Diplodia seriata (A)	Dothideomycetes, Botryosphaeriales	Botryosphaeriaceae	57 iso/21 pl	41 iso/18 pl	11 iso/7 pl
Epicoccum nigrum (A)	Dothideomycetes, Pleosporales	Didymellaceae	25 iso/12 pl	7 iso/5 pl	37 iso/24 pl
Eucasphaeria sp. (A)	Sordariomycetes, Hypocreales	?	0 iso/0 pl	0 iso/0 pl	18 iso/14 pl
Eutypa lata (A)	Sordariomycetes, Xylariales	Diatrypaceae	54 iso/19 pl	23 iso/10 pl	2 iso/1 pl
Exophiala sp. (A)	Eurotiomycetes, Chaetothyriales	Herpotrichiellaceae	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Fomitiporia mediterranea (B)	Agaricomycetes, Hymenochaetales	Hymenochaetaceae	1 iso/1 pl	4 iso/2 pl	0 iso/0 pl

Table 1 (continued)

Taxon anamorph[a]	Class, Order	Family	Asymptomatic	Esca-symptomatic	Nursery
Fusarium acuminatum (A)	Sordariomycetes, Hypocreales	Nectriaceae	0 iso/0 pl	0 iso/0 pl	7 iso/2 pl
Fusarium avenaceum (A)	Sordariomycetes, Hypocreales	Nectriaceae	6 iso/4 pl	2 iso/2 pl	58 iso/29 pl
Fusarium cf graminearum (A)	Sordariomycetes, Hypocreales	Nectriaceae	0 iso/0 pl	1 iso/1 pl	1 iso/1 pl
Fusarium equiseti (A)	Sordariomycetes, Hypocreales	?	3 iso/3 pl	0 iso/0 pl	11 iso/9 pl
Fusarium oxysporum (A)	Sordariomycetes, Hypocreales	?	5 iso/4 pl	0 iso/0 pl	9 iso/7 pl
Fusarium proliferatum (A)	Sordariomycetes, Hypocreales	Nectriaceae	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Fusarium solani (A)	Sordariomycetes, Hypocreales	Nectriaceae	0 iso/0 pl	0 iso/0 pl	7 iso/4 pl
Fusarium sporotrichioides (A)	Sordariomycetes, Hypocreales	?	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Fusicoccum aesculi (A)	Dothideomycetes, Botryosphaeriales	Botryosphaeriaceae	5 iso/4 pl	2 iso/1 pl	4 iso/3 pl
Geomyces pannorum (A)	Leotiomycetes,	Myxotrichaceae	0 iso/0 pl	0 iso/0 pl	4 iso/3 pl
Geotrichum sp. (A)	Saccharomycetes, Saccharomycetales	Dipodascaceae	0 iso/0 pl	1 iso/1 pl	0 iso/0 pl
Glaera sp. (A)	Leotiomycetes, Helotiales	?	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Gongronella sp. (C)	Mucorales	Mucoraceae	2 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Gymnopus erythropus (B)	Agaricomycetes, Agaricales	Tricholomataceae	0 iso/0 pl	1 iso/1 pl	0 iso/0 pl
Halosphaeriaceae sp. (A)	Sordariomycetes, Microascales	Halosphaeriaceae	5 iso/1 pl	9 iso/2 pl	0 iso/0 pl
Helotiales sp. (A)	Leotiomycetes, Helotiales	?	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Hyphodermella rosae (B)	Agaricomycetes, Polyporales	Phanerochaetaceae	4 iso/1 pl	2 iso/1 pl	0 iso/0 pl
Hypocreales sp. 1 (A)	Sordariomycetes, Hypocreales	?	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Hypocreales sp. 2 (A)	Sordariomycetes, Hypocreales	?	0 iso/0 pl	1 iso/1 pl	0 iso/0 pl
Lecanicillium aphanocladii (A)	Sordariomycetes, Hypocreales	Cordycipitaceae	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Leptosphaerulina australis (A)	Dothideomycetes, Pleosporales	Didymellaceae	0 iso/0 pl	3 iso/1 pl	0 iso/0 pl
Lophiostoma corticola (A)	Dothideomycetes, Pleosporales	Lophiostomataceae	12 iso/5 pl	4 iso/2 pl	2 iso/1 pl
Lophiostoma sp. 1 (A)	Dothideomycetes, Pleosporales	Lophiostomataceae	2 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Lophiostoma sp. 2 (A)	Dothideomycetes, Pleosporales	Lophiostomataceae	2 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Lophiostoma sp. 3 (A)	Dothideomycetes, Pleosporales	Lophiostomataceae	19 iso/7 pl	5 iso/3 pl	0 iso/0 pl
Lophiostoma sp. 4 (A)	Dothideomycetes, Pleosporales	Lophiostomataceae	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Lophiotrema rubi (A)	Dothideomycetes, Pleosporales	Lophiostomataceae	4 iso/3 pl	1 iso/1 pl	0 iso/0 pl
Microdochium bolleyi (A)	Sordariomycetes, Xylariales	?	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Mortierella alpina (C)	Mortierellales	Mortierellaceae	1 iso/1 pl	0 iso/0 pl	4 iso/4 pl
Mortierella elongata (C)	Mortierellales	Mortierellaceae	0 iso/0 pl	0 iso/0 pl	2 iso/1 pl
Mucor circinelloides (C)	Mucorales	Mucoraceae	8 iso/5 pl	2 iso/2 pl	11 iso/10 pl
Mucor hiemalis (C)	Mucorales	Mucoraceae	0 iso/0 pl	0 iso/0 pl	5 iso/4 pl
Mucor sp. (C)	Mucorales	Mucoraceae	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Mycena sp. (B)	Agaricomycetes, Agaricales	Tricholomataceae	0 iso/0 pl	1 iso/1 pl	0 iso/0 pl
Mycoarthris corallinus (A)	Leotiomycetes, Helotiales	?	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Myrothecium verrucaria (A)	Sordariomycetes, Hypocreales	?	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Nectriaceae sp. (A)	Sordariomycetes, Hypocreales	Nectriaceae	2 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Neofabraea malicorticis (A)	Leotiomycetes, Helotiales	Dermateaceae	4 iso/2 pl	0 iso/0 pl	0 iso/0 pl
Neofusicoccum parvum (A)	Dothideomycetes, Botryosphaeriales	Botryosphaeriaceae	0 iso/0 pl	0 iso/0 pl	27 iso/11 pl
Neofusicoccum sp. (A)	Dothideomycetes, Botryosphaeriales	Botryosphaeriaceae	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Neosetophoma sp. 1 (A)	Dothideomycetes, Pleosporales	Phaeosphaeriaceae	0 iso/0 pl	0 iso/0 pl	2 iso/1 pl
Neosetophoma sp. 2 (A)	Dothideomycetes, Pleosporales	Phaeosphaeriaceae	0 iso/0 pl	0 iso/0 pl	5 iso/4 pl
Ophiostoma piceae (A)	Sordariomycetes, Ophiostomatales	Ophiostomataceae	0 iso/0 pl	0 iso/0 pl	42 iso/21 pl
Ophiostoma quercus (A)	Sordariomycetes, Ophiostomatales	Ophiostomataceae	0 iso/0 pl	0 iso/0 pl	18 iso/9 pl
Paecilomyces farinosus (A)	Sordariomycetes, Hypocreales	Cordycipitaceae	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Paraconiothyrium sporulosum (A)	Dothideomycetes, Pleosporales	Montagnulaceae	1 iso/1 pl	5 iso/1 pl	0 iso/0 pl
Paraphaeosphaeria sp. (A)	Dothideomycetes, Pleosporales	Montagnulaceae	2 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Paraphoma chrysanthemicola (A)	Dothideomycetes, Pleosporales	Phaeosphaeriaceae	1 iso/1 pl	2 iso/1 pl	4 iso/3 pl

Table 1 (continued)

Taxon anamorph[a]	Class, Order	Family	Asymptomatic	Esca-symptomatic	Nursery
Paraphoma sp. (A)	Dothideomycetes, Pleosporales	Phaeosphaeriaceae	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Penicillium brevicompactum (A)	Eurotiomycetes, Eurotiales	Trichocomaceae	3 iso/2 pl	2 iso/1 pl	9 iso/9 pl
Penicillium cf decaturense (A)	Eurotiomycetes, Eurotiales	Trichocomaceae	0 iso/0 pl	1 iso/1 pl	0 iso/0 pl
Penicillium crustosum (A)	Eurotiomycetes, Eurotiales	Trichocomaceae	0 iso/0 pl	0 iso/0 pl	6 iso/6 pl
Penicillium expansum (A)	Eurotiomycetes, Eurotiales	Trichocomaceae	1 iso/1 pl	0 iso/0 pl	13 iso/12 pl
Penicillium glabrum (A)	Eurotiomycetes, Eurotiales	Trichocomaceae	8 iso/4 pl	2 iso/1 pl	2 iso/2 pl
Penicillium miczynskii (A)	Eurotiomycetes, Eurotiales	Trichocomaceae	1 iso/1 pl	1 iso/1 pl	0 iso/0 pl
Penicillium olsonii (A)	Eurotiomycetes, Eurotiales	Trichocomaceae	2 iso/2 pl	0 iso/0 pl	2 iso/2 pl
Penicillium sizovae (A)	Eurotiomycetes, Eurotiales	Trichocomaceae	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Penicillium thomii (A)	Eurotiomycetes, Eurotiales	Trichocomaceae	0 iso/0 pl	0 iso/0 pl	25 iso/19 pl
Penicillium waksmanii (A)	Eurotiomycetes, Eurotiales	Trichocomaceae	2 iso/2 pl	0 iso/0 pl	0 iso/0 pl
Pestalotiopsis uvicola (A)	Sordariomycetes, Xylariales	Amphisphaeriaceae	5 iso/4 pl	4 iso/3 pl	2 iso/2 pl
Phaeoacremonium aleophilum (A)	Sordariomycetes, Calosphaeriales	Calosphaeriaceae	8 iso/6 pl	4 iso/3 pl	0 iso/0 pl
Phaeoacremonium mortoniae (A)	Sordariomycetes, Calosphaeriales	Calosphaeriaceae	18 iso/12 pl	6 iso/3 pl	0 iso/0 pl
Phaeoacremonium sp. (A)	Sordariomycetes, Calosphaeriales	Calosphaeriaceae	3 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Phaeoacremonium viticola (A)	Sordariomycetes, Calosphaeriales	Calosphaeriaceae	2 iso/2 pl	14 iso/6 pl	0 iso/0 pl
Phaeomoniella chlamydospora (A)	Eurotiomycetes, Chaetothyriales	Herpotrichiellaceae	102 iso/30 pl	64 iso/16 pl	0 iso/0 pl
Phaeosphaeria sp. (A)	Dothideomycetes, Pleosporales	Phaeosphaeriaceae	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Phialemonium sp. (A)	?	?	3 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Phialophora sp. 1 (A)	Leotiomycetes, Helotiales	?	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Phialophora sp. 2 (A)	Sordariomycetes, Magnaporthales	Magnaporthaceae	0 iso/0 pl	3 iso/1 pl	0 iso/0 pl
Phlebia tremellosa (B)	Agaricomycetes, Corticiales	Corticiaceae	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Phoma bellidis (A)	Dothideomycetes, Pleosporales	Didymellaceae	1 iso/1 pl	3 iso/2 pl	0 iso/0 pl
Phoma eupyrena (A)	Dothideomycetes, Pleosporales	Didymellaceae	0 iso/0 pl	0 iso/0 pl	4 iso/3 pl
Phoma glomerata (A)	Dothideomycetes, Pleosporales	Didymellaceae	0 iso/0 pl	0 iso/0 pl	2 iso/2 pl
Phoma negriana (A)	Dothideomycetes, Pleosporales	Didymellaceae	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Phoma pomorum (A)	Dothideomycetes, Pleosporales	Didymellaceae	3 iso/3 pl	0 iso/0 pl	6 iso/3 pl
Phoma radicina (A)	Dothideomycetes, Pleosporales	Phaeosphaeriaceae	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Phomopsis oblonga (A)	Sordariomycetes, Diaporthales	Valsaceae	0 iso/0 pl	0 iso/0 pl	6 iso/2 pl
Phomopsis sp. 1 (A)	Sordariomycetes, Diaporthales	Valsaceae	0 iso/0 pl	0 iso/0 pl	2 iso/1 pl
Phomopsis viticola (A)	Sordariomycetes, Diaporthales	Valsaceae	30 iso/12pl	23 iso/10 pl	28 iso/18 pl
Pilidiella eucalyptorum (A)	Sordariomycetes, Diaporthales	Melanconidaceae	0 iso/0 pl	1 iso/1 pl	0 iso/0 pl
Pithomyces sp. (A)	Dothideomycetes, Pleosporales	Didymellaceae	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Pleospora sp. (A)	Dothideomycetes, Pleosporales	Pleosporaceae	8 iso/6 pl	3 iso/2 pl	0 iso/0 pl
Pleosporales sp. 1 (A)	Dothideomycetes, Pleosporales	?	0 iso/0 pl	3 iso/1 pl	0 iso/0 pl
Pleosporales sp. 2 (A)	Dothideomycetes, Pleosporales	?	2 iso/1 pl	4 iso/1 pl	0 iso/0 pl
Pleosporales sp. 3 (A)	Dothideomycetes, Pleosporales	?	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Pleosporales sp. 4 (A)	Dothideomycetes, Pleosporales	?	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Pleosporales sp. 5 (A)	Dothideomycetes, Pleosporales	?	8 iso/2 pl	5 iso/1 pl	0 iso/0 pl
Pleosporales sp. 6 (A)	Dothideomycetes, Pleosporales	?	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Pleosporales sp. 7 (A)	Dothideomycetes, Pleosporales	?	0 iso/0 pl	4 iso/1 pl	0 iso/0 pl
Purpureocillium lilacinum (A)	Sordariomycetes, Hypocreales	Ophiocordycipitaceae	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Pyrenochaeta cava (A)	Dothideomycetes, Pleosporales	Pleosporaceae	0 iso/0 pl	3 iso/1 pl	0 iso/0 pl
Pyrenochaeta sp. (A)	Dothideomycetes, Pleosporales	Pleosporaceae	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Rhizoctonia sp. (B)	Agaricomycetes, Cantharellales	Ceratobasidiaceae	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Rhodotorula glutinis (B)	Microbotryomycetes, Sporidiobolales	?	17 iso/10 pl	6 iso/4 pl	17 iso/13 pl
Sistotrema brinkmannii (B)	Agaricomycetes, Corticiales	Corticiaceae	2 iso/2 pl	1 iso/1 pl	0 iso/0 pl
Stagonosporopsis dorenboschii (A)	Dothideomycetes, Pleosporales	Didymellaceae	0 iso/0 pl	0 iso/0 pl	26 iso/17 pl

Table 1 (continued)

Taxon anamorph[a]	Class, Order	Family	Asymptomatic	Esca-symptomatic	Nursery
Stereum rugosum (B)	Agaricomycetes, Russulales	Stereaceae	2 iso/2 pl	1 iso/1 pl	0 iso/0 pl
Thysanophora penicillioides (A)	Eurotiomycetes, Eurotiales	Trichocomaceae	1 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Torula sp. (A)	Dothideomycetes, Pleosporales	?	0 iso/0 pl	1 iso/1 pl	0 iso/0 pl
Trichoderma brevicompactum (A)	Sordariomycetes, Hypocreales	Hypocreaceae	0 iso/0 pl	0 iso/0 pl	5 iso/5 pl
Trichoderma cf viridescens (A)	Sordariomycetes, Hypocreales	Hypocreaceae	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Trichoderma hamatum (A)	Sordariomycetes, Hypocreales	Hypocreaceae	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Trichoderma harzianum (A)	Sordariomycetes, Hypocreales	Hypocreaceae	1 iso/1 pl	3 iso/1 pl	7 iso/7pl
Truncatella angustata (A)	Sordariomycetes, Xylariales	Amphisphaeriaceae	5 iso/4 pl	0 iso/0 pl	14 iso/12 pl
Undetermined fungus 1	?	?	4 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Undetermined fungus 2	?	?	3 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Undetermined fungus 3	?	?	2 iso/1 pl	0 iso/0 pl	0 iso/0 pl
Undetermined fungus 4	?	?	0 iso/0 pl	1 iso/1 pl	0 iso/0 pl
Undetermined fungus 5	?	?	0 iso/0 pl	0 iso/0 pl	2 iso/1 pl
Undetermined fungus 6	?	?	0 iso/0 pl	0 iso/0 pl	2 iso/1 pl
Undetermined fungus 7	?	?	0 iso/0 pl	0 iso/0 pl	3 iso/1 pl
Undetermined fungus 8	?	?	0 iso/0 pl	0 iso/0 pl	1 iso/1 pl
Verticillium nigrescens (A)	Sordariomycetes, Hypocreales	?	2 iso/1 pl	0 iso/0 pl	0 iso/0 pl

[a] As the taxonomy of the OTUs has been inferred from ITS sequences BLAST top scores in GenBank (Online Resource 2) we reported the GenBank classification adopted by the authors for the BLAST top score(s) sequence(s). We are aware that some names may be wrong and consequently their classification

[b] Abbreviations used: (A): Ascomycota; (B): Basidiomycota; (C): Basal fungal lineage

[c] Abundance is the number of fungal strains of a given OTU (iso) isolated from each plant category and incidence is the number of plants (pl) from which an OTU has been isolated in each plant category

GenBank, accepting that this was not aligned with extype. For *Alternaria*, we recovered ITS rDNA genotypes for 216 isolates that differed only in the length of a T-repeat at the end of the ITS2. Sequences with 6, 7 or 8 T-repeats were respectively 100 % similar with GenBank sequences of *Alternaria alternata, A. arborescens*, and *A. mali* (Online resource 2). We considered these 3 ITS rDNA genotypes as a single OTU, the "*Alternaria alternata* species complex" (Table 1). When ITS rDNA sequences exhibited less than 99 % of similarity with any GenBank sequence, we limited the identification to the rank of genus (95–98 % sequence similarity) and only so when the BLAST scores following the top score were part of the same genus. For BLAST scores <95 % we accepted either the family, order, or class rank for identity depending on the consistency of the systematic placement indicated by the BLAST scores following the top score. From 180 grapevine plants, we retrieved 197 different fungal ITS genotypes (Online Resource 2). Using the aforementioned strategy for OTUs delimitation, these genotypes were assigned to 150 operational taxonomic units (OTUs), plus eight undetermined fungal morphotypes for which amplification was unsuccessful (Online Resource 2). As such, a total of 158 OTUs were delimited. The 150 OTUs that could be molecularly delimitated represent 8 fungal classes, 26 orders, and 41 families belonging to various lineages of ascomycetes, basidiomycetes and basal fungal lineages (Table 1). Based on BLAST results, these 150 ITS sequences (Table 1) were distributed in 3 phyla and 6 subphyla: Ascomycota [Pezizomycotina and Saccharomycotina], Basidiomycota [Agaricomycotina, Pucciniomytina and Ustilaginomycotina], and one basal lineage [Mucoromycotina]). The large majority of these OTUs were Ascomycota (5 classes, 16 orders, 31 families, and 130 OTUs) followed by Basidiomycota (3 classes, 8 orders, 8 families, and 14 OTUs), and Mucoromycotina (2 orders, 2 families, and 6 OTUs).

One single vineyard plot harbored a high species richness of wood-inhabiting fungi

The number of OTUs isolated from a single plant, independently of the plant type, ranged from two to 13 (Fig. 1a). Considering each plant type separately, the mean number of OTUs isolated per grapevine plant (Fig. 1b) was very similar for asymptomatic and esca-symptomatic plants (6 OTUs), but higher for nursery plants (8 OTUs). The Simpson index, estimated for each plant type, indicated nevertheless that the fungal species diversity was high (≥0.8) in all plant types (Fig. 1c).

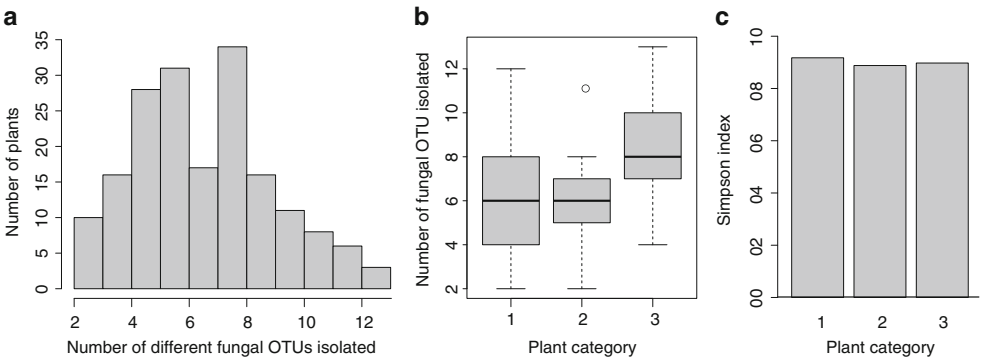

Fig. 1 Fungal diversity indices: **a**. Number of distinct OTUs isolated per plant; **b**. Number of distinct OTUs isolated per plant for each plant type (1. asymptomatic, 2. esca-symptomatic, 3. nursery); **c**. Simpson index estimated for each plant type based on the relative frequencies of the OTUs in the plants (1. asymptomatic, 2. esca-symptomatic, 3. nursery)

Species accumulation curves (Fig. 2) used incidence data (presence or absence of an OTU in a plant) instead of abundance data (number of isolates of an OTU in a plant) to take in account the sampling bias between nursery and adult plants (see Materials and methods section). We were aware that such procedure gave more importance to rarely isolated OTUs than it did for the frequently isolated ones. None of the estimated species accumulation curves for asymptomatic, esca-symptomatic and nursery plants showed any sign of leveling off (Fig. 2), indicating that more sampling effort is required to fully characterize the mycota associated to each plant type.

None of the presumed esca-associated fungi were significantly more invasive in symptomatic plants compared to asymptomatic plants

Among the 150 identified OTUs, 23 OTUs are generally regarded as being associated with the esca and/or young vine decline grapevine trunk diseases: *Eutypa lata, Fomitiporia mediterranea, Phaeomoniella chlamydospora, Stereum rugosum*, anamorphs of the genus *Botryosphaeria* (*Diplodia seriata, Fusicoccum aesculi, Neofusicoccum parvum*), *Cadophora* spp., *Cylindrocarpon* spp., *Phaeoacremonium* spp., and *Phomopsis* spp. (Online Resource 2, Table 1). Only 11 of the 180 plants analyzed (6.1 %) were found to be free from esca and young vine decline associated fungi (asymptomatic: 4, esca-symptomatic: 3, and nursery: 4).

When comparing symptomatic and asymptomatic plants in the Chasselas vineyard, with the exception of basidiomycetes both plant types hosted esca-associated species with medium to high incidence (Fig. 3). Four trunk disease associated fungal species or genera had similar medium to high incidence in adult plants: *P. chlamydospora* (asymptomatic: 43.5 %, esca-symptomatic: 42.1 %), *Phaeoacremonium* spp. (30.4 %, 28.9 %), *E. lata* (27.5 %, 28.9 %) and *Cadophora* (17.4 %, 13.2 %). *Botryosphaeria* anamorphs were more frequently isolated from esca symptomatic plants (50 %) than from asymptomatic ones (36.2 %). The same pattern was observed for *Phomopsis* spp. (esca-symptomatic: 26.3 %, asymptomatic: 17.4 %). The genus *Cylindrocarpon* was absent from adult plants.

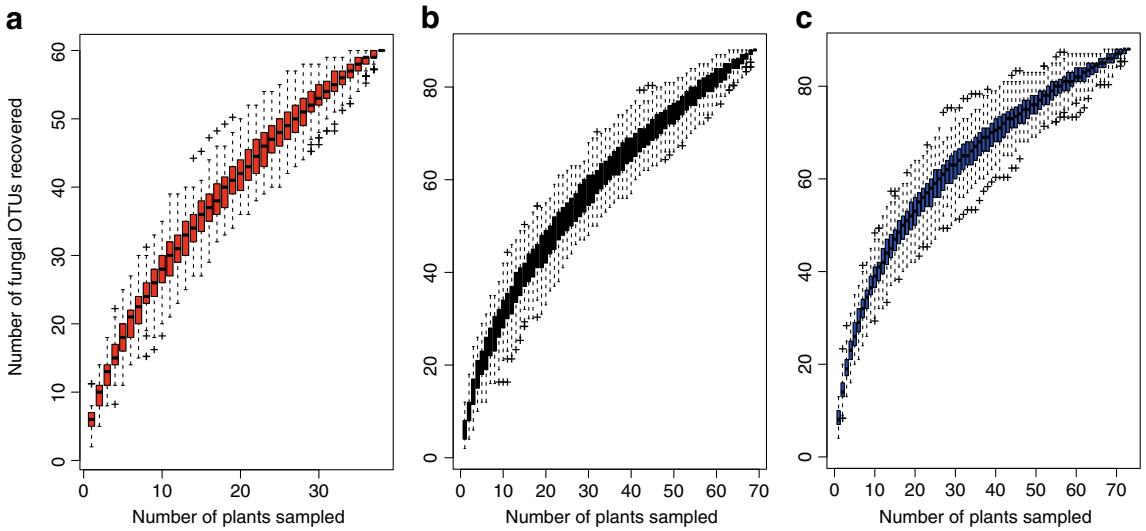

Fig. 2 Species accumulation curves for each plant type. **a**. Asymptomatic plants; **b**. Esca-symptomatic plants; **c**. Nursery plants. Standard deviations for each sampling effort were calculated based on 10,000 resamplings

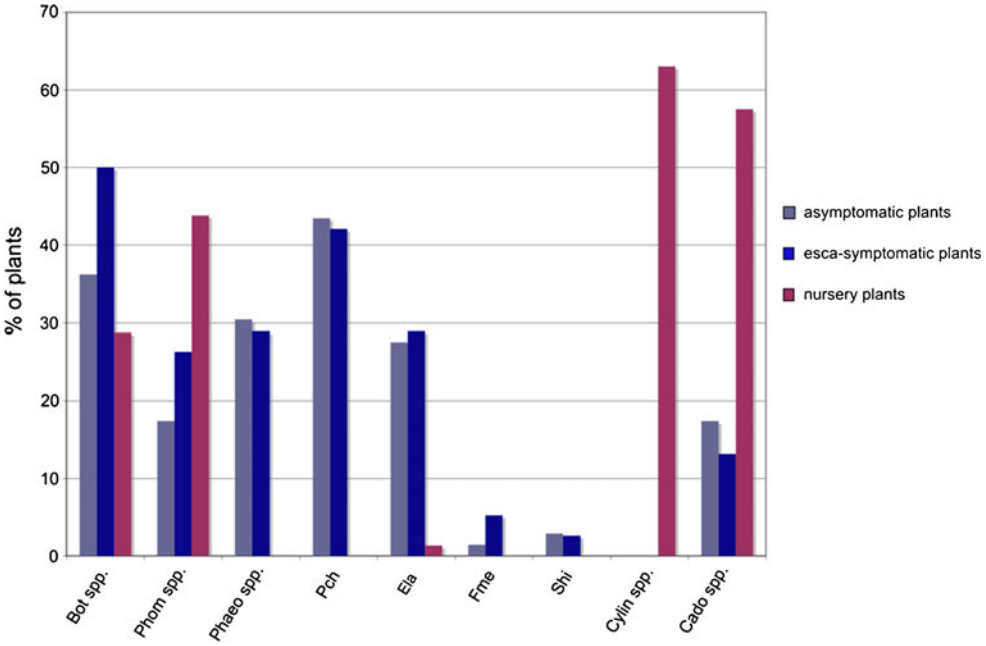

Fig. 3 Incidence of wood disease associated fungi in each plant type. Incidence is defined as the relative frequency of occurrence (presence/absence) of a fungal genus or species in a plant type expressed as a percentage of the total number of plants of each type. Abbreviations used: Bots spp. (*Botryosphaeria* species), Phom spp. (*Phomopsis* species), Phaeo spp. (*Phaeoacremonium* species), Pch (*Phaeomoniella chlamydospora*), Ela (*Eutypa lata*), Fme (*Fomitiporia mediterranea*), Shi (*Stereum hirsutum*), Cylin spp. (*Cylindrocarpon* species) and Cado spp. (*Cadophora* species)

Both esca-symptomatic and asymptomatic plants exhibited a similar abundance of wood disease associated fungi (Fig. 4, esca-symptomatic: 35.8 %, asymptomatic: 31.9 %). The most frequent species, *Phaeoacremonium chlamydospora,* was isolated exclusively from adult plants, (asymptomatic: 10.9 %, and esca-symptomatic: 12.1 %). The second highest abundance in esca-symptomatic plants (7.7 %) was for *Diplodia seriata*, the anamorph of *Botryosphaeria obtusa*, but the number of isolates of that species retrieved from asymptomatic plants was comparable (6.1 %). When considering the other *Botryosphaeria* anamorphs, the relative abundance of *Fusicoccum aesculi* was low (<0.6 %) in both plant types. Cumulative relative abundance of *Botryosphaeria* spp. was slightly higher in esca-symptomatic plants than in asymptomatic ones (respectively 8.1 % and 6.6 %). The next most frequent species in the fungal community associated with adult plants was *Eutypa lata* (asymptomatic: 5.8 % and esca-symptomatic: 4.3 %). The genus *Phomopsis*, was represented in adult plants only by *P. viticola*. Although having a relatively high incidence, this species represented less than 5 % of the fungal community that was associated with asymptomatic (3.2 %) or esca-symptomatic plants (4.3 %). For *Phaeoacremonium* spp., the highest

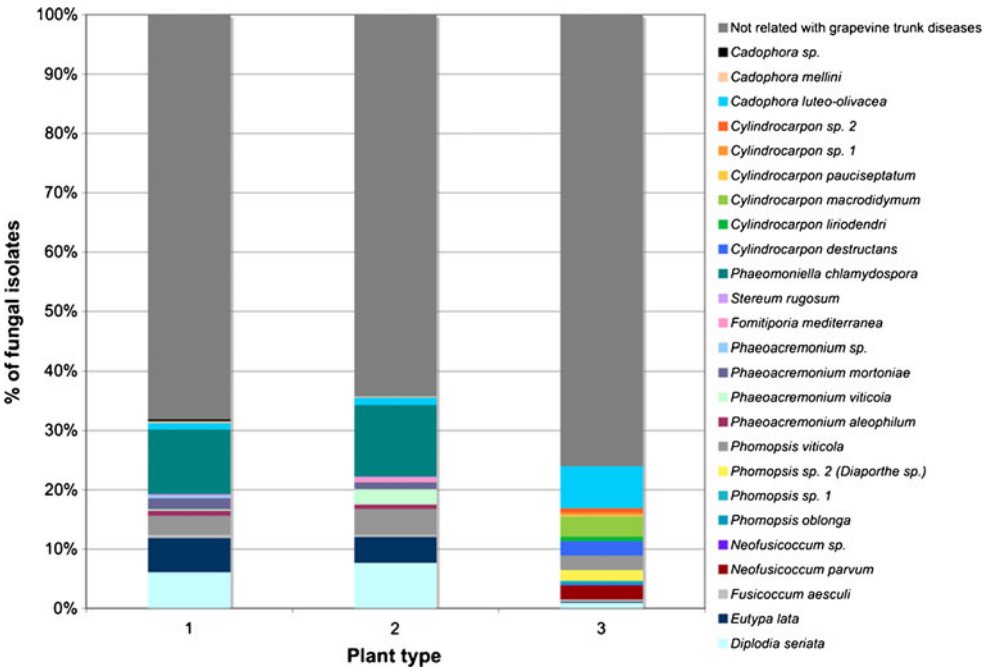

Fig. 4 Abundance of wood disease associated fungi in each plant type. Abundance is defined as the number of fungal isolates of a given OTU as a percentage of the total number of fungal isolates obtained from each plant category. Plant types: 1. asymptomatic, 2. esca-symptomatic, 3. nursery

abundance was noted for *P. viticola* in esca-symptomatic plants (2.6 %), but for *P. mortoniae* in asymptomatic plants (1.9 %). Relative abundance of other species of the same genus was lower than 1 % in adult plants. Overall abundance differences of trunk disease associated fungal species (Fig. 4) were all ≤2 % when comparing esca-symptomatic and asymptomatic plants except for *Phaeoacremonium viticola* (2.4 %). As a result, none of these presumed pathogens was significantly more invasive in esca-symptomatic plants.

Pioneer esca-associated fungi were not transmitted from adult to nursery plants through grafting

Our results (Fig. 3) showed that except for *Phomopsis* and *Botryosphaeria* anamorphs that were hosted respectively by 43.8 and 28.8 % of the nursery plants, esca-associated fungal species were either absent or of very low incidence in plants ready for planting. Nursery plants neither hosted typical esca pioneer species (i.e. *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp.), nor did they host basidiomycetes whereas only very few nursery plants had been contaminated with *Eutypa lata* (1.4 %). While most adult plants contracted esca-associated fungal species, the majority of nursery plants hosted fungi that were more typically associated with young vine decline (Figs. 3, 4), i.e. various species of *Cylindrocarpon* (incidence: 57.5 %, cumulated relative abundance: 8 %), a genus that was completely absent from adult plants. The genus *Cadophora* had a much higher incidence (57.5 %) in nursery plants than in adult plants (asymptomatic: 1.7 %, esca-symptomatic: 1.5 %). Consequently nursery plants hosted presumed fungal pathogens with a high incidence, but there was a clear shift in the involved fungal genera and species during plant maturation (Figs. 3, 4).

The fungal community associated with the wood of adult V. vinifera plants was highly similar in both symptomatic and asymptomatic plants, but very different from nursery plants

Apart from the generally assumed pathogens, other species of the fungal community could be involved in the expression of esca-disease. When comparing the systematic structure of the fungal communities associated with the different plant types (Fig. 5, inferred from Table 1), the most frequently isolated OTUs belonged to the Dothideomycetes and the Sordariomycetes, with a dominance of Dothideomycetes in adults plants (54.9-56.9 % of the fungal isolates). Both classes were equally represented in nursery plants (40.4 % of the isolates are Sordariomycetes and 38.31 % are Dothideomycetes) [Fig. 5a]. Taken together, both classes represented more than 73 % of the isolates in all plant categories. The two other dominant classes in all plant categories were Eurotiomycetes (asymptomatic: 13.8 %, esca-symptomatic: 13.6 %, nursery: 5 %) and Leotiomycetes (asymptomatic: 6.6 %, esca-symptomatic: 5.1 %, nursery: 10.3 %) but with a dominance of the former in adults plants and of the latter in nursery plants. Fungal isolates of the five remaining classes represented less than 6 % of the fungal community of each of the plant types. The comparison of the systematic placement of our fungal isolates revealed a clear shift from nursery plants to adult grapevine plants: Dothideomycetes and Eurotiomycetes increased in frequency at the expense of Leotiomycetes and Sordariomycetes. These frequency shifts were observed for both esca-symptomatic and asymptomatic plants.

The fungal communities hosted by the adult plants, symptomatic or not, were also very similar based on the distribution of the isolates in the different fungal orders (Fig. 5b). If Pleosporales were the most diverse in all plant types (asymptomatic: 27.5 %, esca-symptomatic: 28.6 %, nursery: 32.5 %), the second best represented order was Dothideales for adult plants (asymptomatic: 15.7 %, esca-symptomatic: 15.1 %, nursery: 1.7 %), but Hypocreales for nursery plants (nursery: 26.8 %, asymptomatic: 4.6 %, esca-symptomatic: 3.8 %). Several orders were exclusively found in adult plants (Chaetothyriales, Calosphaeriales, Magnaporthales, Microascales, Agaricales, Corticiales, Hymenochaetales, Polyporales, and Russulales), whereas Ophiostomatales and Atheliales were exclusively present in nursery plants. Most of these orders were represented by singletons or doubletons totaling less than 5 % of the isolated fungi in each plant category. Exceptions were Chaetothyriales in adult plants (asymptomatic: 11.3 %, esca-symptomatic: 12.1 %) and Ophiostomatales in nursery plants (5.3 %). At the ordinal level, the shift in fungal groups from nursery to adult plants showed a considerable decrease of Hypocreales and a complete disappearance of Ophiostomatales. In contrast, Xylariales and particularly Dothideales and Capnodiales increased significantly with plant age.

The principal component analysis (PCA) of OTUs incidence data showed that the indicator species of the fungal community of adult plants were highly similar while nursery plants hosted a very different mycota composition (Fig. 6).

Discussion

To investigate the shift toward pathogenicity of the fungi generally assumed to generate the esca disease symptoms, we compared the fungal communities respectively associated with wood of asymptomatic and esca-symptomatic plants in a single vineyard. As endophyte assemblages of plants are known to vary between sites (Arnold et al. 2003), we limited our experiment to a single adult vineyard. To determine if the esca-associated fungi were transmitted through the grafting process we also analyzed the fungal community associated

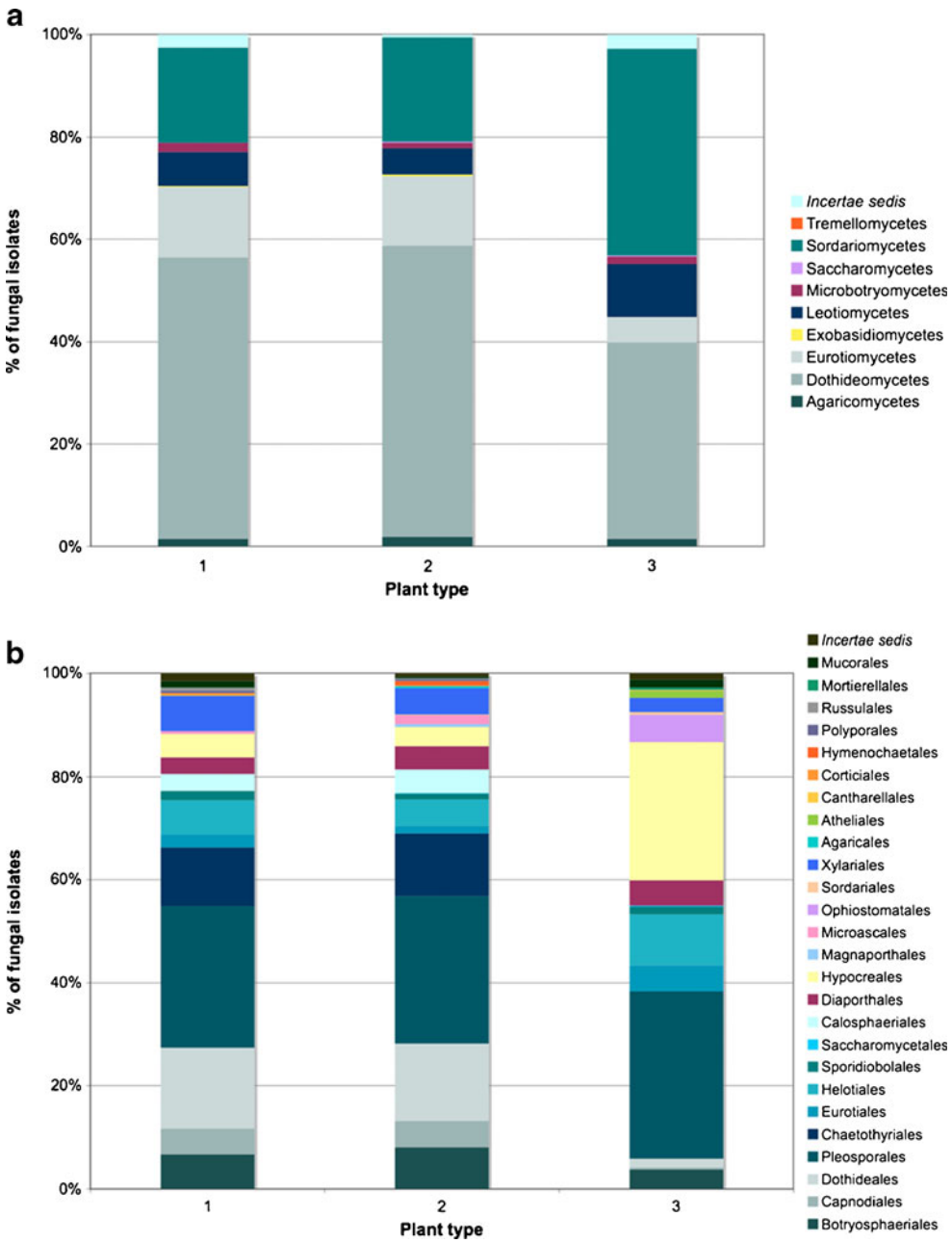

Fig. 5 Systematic structure of the fungal communities respectively associated with the different plant types. **a**. Distribution of the fungal isolates in the different classes; **b**. Distribution of the fungal isolates in the different orders. Plant types: 1. asymptomatic, 2. esca-symptomatic, 3. nursery

with nursery plants that were not hot water treated, and grafted with material sampled in the same vineyard and on the identical rootstock as the adult plants.

The fungal biodiversity (158 OTUs—Online Resource 2) was estimated using direct identification and comparison of ITS sequences with those in GenBank. Using GenBank to identify some genera to species level must be treated with caution unless the sequence is derived from an extype strain (Cai et al. 2011a,b; Ko Ko et al. 2011; Maharachchikumbura et al. 2011, Manamgoda et al. 2011; Tempesta et al. 2011; Udayanga et al. 2011; Wikee et al. 2011; Yang et al. 2011). We adopted a 99 % sequence BLAST similarity threshold to determine species names (Gazis et al. 2011) because recent studies used more variable gene regions than the ITS to delimitate species that were frequent in our sampling (Aveskamp et al. 2009; Aveskamp et al. 2010; Chaverri et al. 2011; Schubert et al. 2007).

Recovering more OTUs in the wood of nursery plants than in the wood of adult plants (Fig. 1b) was not expected because the diversity of endophytes has been shown to increase with plant age (McCutcheon et al. 1993; Zabalgogeazcoa 2008). However, this fact can be explained by the sampling bias mentioned in the Materials and methods section: compared to nursery plants, the isolation of fungi from the wood of adult plants was likely to be biased toward the repeated recovery of the same species, since a single sample of wood was more

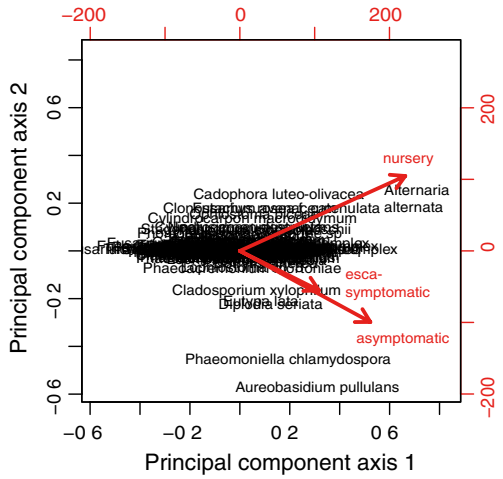

Fig. 6 Biplot of the principal component analyses showing the relative contribution of the plant samples to the main axes (nursery, esca-symptomatic and asymptomatic). The relative contributions of the fungal species are shown in black. The community composition was assessed based on species occurrence (presence-absence scoring) in each plant type

likely to be completely occupied by the same fungal species. The diversity of fungi isolated from the wood of 180 grapevine plants was nevertheless unexpectedly high for each of the plant types analyzed (Simpson index ≥0.8, Fig. 1c), more than two times higher than the one found to be associated not only with wood, but also with shoots and leaves of several cultivars of *V. vinifera* at different ages in the whole of the area surrounding Madrid, Spain (Gonzáles and Tello 2010). These divergent results may partially be explained by the different locations of the experiments, but are more likely related to the methodology used to isolate the fungi from the plants and to the sampling effort (Hyde and Soytong 2008). Species accumulation curves of each plant type (Fig. 2) also suggest that the cultivable part of the fungal community associated with the wood of grapevine in a single vineyard plot or with nursery plants is still far from completely sampled. Consequently, the diversity of fungal endophytes that can associate with *V. vinifera* remains probably largely unknown.

When comparing asymptomatic and esca-symptomatic plants, the incidence and abundance of esca-related fungi were high independently of the plant type, and adult plants, diseased or not, carried the same fungal parasitic load (Figs. 3, 4). We observed no significant difference in the systematic structure of the mycota associated with asymptomatic and esca-symptomatic plants, this at different systematic ranks (Fig. 5). Finding the same taxa in both diseased and healthy plants also suggests that they are part of the normal mycota associated with adult *V. vinifera* plants (Frias-Lopez et al. 2002; Toledo-Hernández et al. 2008). If the group of generally accepted, esca-associated fungi were indeed latent pathogens, the emergence of symptoms of the disease would be the consequence of a shift in species abundance in favor of pathogenic species, leading to the typical discoloration of the leaves associated with esca (Surico et al. 2006). Our results suggest that the esca-associated fungi are probably not pathogens, but more likely either true endophytes *sensu* Mostert *et al*. (2000) or latent saprobes *sensu* Promputtha *et al.* (2007), or else some non-specific saprobes that are part of a fungal decomposer community specialized in the decay of senescent or dead wood material. Our study provides the first empirical evidence for this hypothesis.

There have been three major arguments in favor of the pathogenicity-hypothesis for fungi associated with esca and young vine decline, the first of which concerns the world-wide increase of the incidence of esca and young vine decline since the ban of sodium arsenite. It is true that before the ban of sodium arsenite, esca and young vine decline were considered to be negligible diseases (Bertsch et al. 2009; Mugnai et al. 1999; Graniti et al. 2000). However, even if sodium arsenite can reduce the severity of esca symptoms, it does not contribute significantly towards esca incidence and plant mortality (Fussler et al. 2008). This fungicide has never been registered and therefore has never been used in Switzerland, nor in Germany. Yet, the emergence of the esca disease followed a very similar pattern in these two countries compared to the other European countries (Fischer and Kassemeyer 2003; Viret et al. 2004). Also, when a restricted use of sodium arsenite was still allowed in France, Portugal and Spain, esca was nevertheless already widespread in these countries (Mugnai et al. 1999). The causal link between the ban of sodium arsenite and esca emergence appears therefore entirely circumstantial.

The two other arguments in favor of a presumed pathogenicity of the esca-associated fungi are the repeated isolation of the same fungal groups from grapevine wood necroses and, finally, the ability of some of these fungi to decompose grapevine wood in vitro and to generate necroses in vivo. Many past and present studies on esca have presented lists of fungi that were repeatedly isolated from necrotic wood. Consequently, these fungi were thought to be involved in the esca disease (Armengol et al. 2001; Bertsch et al. 2009; Gramaje and Armengol 2011; Larignon and Dubos 1997; Surico et al. 2006), even though one could also argue that all these studies have essentially shown that esca-related fungi are frequently associated with dead wood in *V. vinifera*. Pathogenicity tests inoculating sterilized wood pieces of grapevine plants with one or several of the esca- or young vine decline-associated fungi showed that some of these were able to colonize dead wood (Chiarappa 1997; Larignon and Dubos 1997; Mugnai et al. 1996; Úrbez-Torres et al. 2009), without demonstrating that these fungi were able to generate wood necroses *in vivo*. However, field inoculation experiments showed that wood-streaking and vessel discoloration were induced months after the

inoculation with *P. chlamydospora* and *P. aleophilum* and that these species could then be isolated back from the margin of the extending necroses (Eskalen et al. 2007). Vacuum inoculations of young vine decline associated fungi in hot water treated rootstock cuttings resulted in a decrease of number of plants emerging from dormancy, in an increase of wood necroses months after inoculation but not for all fungal species and all cultivars (Gramaje et al. 2010). Yet, inoculation experiments generally failed to reproduce the typical foliar symptoms of esca (Mugnai et al. 1999, Gramaje et al. 2010).

In inoculation tests with pathogenic fungi, tylose development around the inoculation region has been interpreted as a defense reaction of the plant preventing free movement of the pathogens in the plant's xylem, fungi being not able to degrade suberine (Clerivet et al. 2000). More recently Sun *et al.* (2006) showed that the mere wounding of *V. vinifera* wood tissues, without pathogen inoculation, causes very abundant tylose development in stems of grapevines resulting in the occlusion of approximately 40 % of the vessels. These authors suggested that tylose formation associated with infection might result from the inoculation wound itself and not from a defense reaction against a pathogen. The same authors also observed that the literature tacitly assumes that tyloses form in functional vessels, but that this assumption has never been proven. In a more recent study, the same authors showed that, while grapevine summer pruning leads to the production of tylose, winter pruning essentially leads to the secretion of gels that have pectin as a major component (Sun et al. 2008). Pectin is a perfect substrate for decomposition by fungi (Green et al. 1996; Green and Clausen 1999). Several esca-associated wood-rot fungi, e.g. *Eutypa lata, Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*, have been shown to invade grapevine wood essentially during winter, the infection being more serious with early winter pruning (Larignon and Dubos 2000; Munkvold and Marois 1995). Frost injuries should also induce the production of pectin gels in the damaged wood of grapevines and create favorable niches for fungal development. The above findings, coupled with the traditional winter pruning practiced worldwide, therefore suggest that even healthy grapevine is likely to contain high amounts of senescent or dead wood, although precise data on the amounts of dead wood in healthy *V. vinifera* plants are not available.

If tylose and pectin gels do not form in functional vessels of grapevine, our hypothesis of a specialized fungal wood decomposer community that develops in grapevine, which is pruned on a yearly basis, provides an explanation for the fact that none of the presumed esca-related species becomes more invasive in symptomatic plants. The assumption of a wood decomposer community that is specific to damaged plant tissues may also explain why we did not find any of the early esca-associated fungi in nursery plants that were grafted with identical rootstock as the adult plants and with healthy scions sampled from the same adult plants studied here. Indeed, the major risk for potential transmission of fungi associated with esca and young vine decline is generally assumed to be during the grafting process of the grapevine cultivar on a new rootstock (Gramaje and Armengol 2011; Giménez-Jaime et al. 2006; Aroca et al. 2010). However, if pioneer esca species were indeed fungal saprobes specialized in wood decay, grapevine healthy shoots of the rootstock mother plant and of the selected cultivar used for grafting are unlikely to host any of these fungi. Once the grafting process terminated, nursery plants do contain damaged tissues that can be invaded by these fungal saprobes. In fact, several earlier studies reported *Phaeomoniella chlamydospora* and *Phaeoacremonium* species from nursery plants (Chicau et al. 2000; Edwards and Pascoe 2004; Giménez-Jaime et al. 2006; Halleen et al. 2003). However, Halleen *et al.* (2003) observed that these esca-associated fungal species were mostly associated with either the rootstock or the graft union. We concur with Halleen et al. (2003) in that the best explanation for this result was the availability of sufficient weakened plant tissue due to the grafting process or through aerial contamination by fungal spores during the grafting process. Weeds sampled in grapevine rootstock mother fields have been shown to host *Phaeomoniella chlamydospora*, *Cylindrocarpon macrodidymum* and *Cadophora luteo-olivacea* (Agustí-Brisach et al. 2011). The high occurrence of *Cylindrocarpon* in newly planted grapevines has been attributed to mechanical injuries of the young root callus during the planting process, exposing grapevine cuttings to infection by these soil-borne fungi (Halleen et al. 2003). A presumed saprotrophy for the esca fungi is also in line with observations that esca development is generally patchy in a vineyard and does not spread from a particular point of infection (Mugnai et al. 1999; Surico et al. 2006). Disease incidence and identity of presumed trunk disease-associated fungi have been shown to vary in function of studied grapevine cultivars, geography, soil type and climate (Armengol et al. 2001; Bertsch et al. 2009; Casieri et al. 2009; Edwards et al. 2001; Larignon 2012; Larignon and Dubos 1997; Marchi 2001; Mugnai et al. 1999; Surico et al. 2006). At the same time, the host specificity of esca-associated fungal species is very broad and nearly all identified fungi that were recovered in this study have also been reported from other hosts (Online Resource 2). Therefore, fungal infection should be primarily dependent on the environmentally available species pool, including the presumed trunk disease associated species, and this for both young and adult grapevine plants.

In more general terms, our study questions the presumed pathogenic status of fungi involved in other newly emerging diseases of plants and animals in cases where no significant

differences were observed between the fungal communities that inhabit healthy and diseased individuals. For example, a study comparing the occurrence of fungi between necrotic and non-symptomatic shoots of the tree *Fraxinus excelsior* found that the same fungal taxa were dominant in all shoots, diseased or not, and that moderate to high similarity of fungal communities was observed in all shoots independent from symptoms (Bakys et al. 2009). An example from zoology is the study by Zuluaga-Montero et al. (2010), focusing on sea fans (*Gorgonia ventalina*), in which the results indicated that the fungal community composition did not differ significantly between healthy and diseased tissues in each reef and that the differences in fungal communities were more attributable to differences between reefs than to the health of the studied colonies.

Defining a fungus as a pathogen implies a difference in its incidence and certainly in its abundance between healthy and diseased individuals. The appearance of the disease symptoms should be the consequence of the increasing proliferation of the causal pathogen and this should have an impact on the fungal community structure. In the case of esca, such a shift in fungal community structure is not observed. In our study, however, a single fungal OTU (based on ITS similarity) possibly embraces very closely related species, subspecies or strains that have a different virulence and could be differentially associated with healthy or diseased plants, as for instance in the case of *Alternaria* (Table 1, Pryor and Michailides 2002), *Phaeomoniella chlamydospora* (Mostert et al. 2006) or *Phaeoacremonium angustius* (Santos et al. 2005). Also, cumulated small differences in abundance of several OTUs might eventually differentiate between healthy and diseased plants, but such slight differences in abundance are, each taken separately, too small to contribute to a significant distinction between healthy and diseased plants in a PCA analysis (Fig. 6). Nevertheless, our experiment was conducted in a single, small vineyard plot, making it unlikely to observe differences in virulence between strains or subspecies associated with adjacent plants. If some strains were indeed more virulent within a single OTU, this would have resulted in an increase of the abundance of such an OTU in diseased grapevine plants, as a more virulent strain is expected to be more invasive than less virulent ones. Neither is it likely that unculturable fungi are responsible for the emergence of esca in the sense that a shift toward pathogenicity - and consequently invasiveness - of these fungi should also have an impact on the culturable part of the fungal community associated with grapevine, which is not the case in our study. Nevertheless, there remains an urgent need to characterize the genotypes of the fungi associated with esca disease in more detail before we can firmly exclude fungi as the principal cause of esca. Other organisms, such as bacteria, may be involved in esca but eventual differences between the bacterial communities associated with diseased or healthy grapevines have never been studied. As suggested by Bertsch *et al.* (2009), environmental parameters may also play an important role in the emergence of grapevine trunk diseases, as may changes in vineyard management and cultural practices (Graniti et al. 2000) or differences between grapevine genotypes (Santos et al. 2005).

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References

Agustí-Brisach C, Gramaje D, León M, García-Jiménez J, Armengol J (2011) Evaluation of vineyard weeds as potential hosts of black-foot and Petri disease pathogens. Plant Dis 95(7):803–810

Armengol J, Vicent A, Torné L, García-Figueres F, García-Jiménez J (2001) Fungi associated with esca and grapevine decline in Spain: a three-year survey. Phytopathol Mediterr 40:325–329

Arnold AE, Mejí LC, Kyllo D, Rojas EI, Maynard Z, Robbins M, Herre EA (2003) Fungal endophytes limit pathogen damage in a tropical tree. Proc Natl Acad Sci U S A 100(26):15649–15654

Aroca A, Gramaje D, Armengol J, Garcia-Jiménez J, Rasposo R (2010) Evaluation of the grapevine nursery propagation process as a source of *Phaeoacrmonium* spp. and *Phaeomoniella chlamydospora* and occurence of trunk disease pathogens in rootstock mother vines in Spain. Eur J Plant Pathol 126:165–174

Aveskamp MM, Verkley GJM, de Gruyter J, Murace MA, Perelló A, Woudenberg HC, Groenewald JZ, Crous PW (2009) DNA phylogeny reveals polyphyly of *Phoma* section *Peyronellaea* and multiple taxonomic novelties. Mycologia 101:363–382

Aveskamp MM, de Gruyter J, Woudenberg JHC, Verkley GJM, Crous PW (2010) Highlights of the Didymellaceae: a polyphasic approach to characterise *Phoma* and related pleosporalean genera. Stud Mycol 65:1–60

Bakys R, Vasaitis R, Barklund P, Thomsen IM, Stenlid J (2009) Occurrence and pathogenicity of fungi in necrotic and non-symptomatic shoots of declining common ash (*Fraxinus excelsior*) in Sweden. Eur J Forest Res 128:51–60

Bertsch C, Larignon P, Farine S, Clément C, Fontaine F (2009) The spread of grapevine trunk disease. Science 324:721

Brugali L (2009) Materials and methods for vineyard plant. Teatro Naturale International year 1 (1). http://www.teatronaturale.com/article/39.html. Accessed 8 March 2012.

Cai L, Giraud T, Zang N, Begerow D, Guohong C, Shivas RG (2011a) The evolution of species concepts and species recognition criteria

in plant pathogenic fungi. Fungal Divers. doi:10.1007/s13225-011-0127-8
Cai L, Udayanga D, Manamgoda DS, Maharachchikumbura SSN, Liu XZ, Hyde KD (2011b) The need to carry out re-inventory of plant pathogenic fungi. Trop Plant Pathol 36:205–213
Casieri L, Hofstetter V, Viret O, Gindro K (2009) Fungal communities associated with the wood of different cultivars of young *Vitis vinifera* plants. Phytopathol Mediterr 48(1):73–83
Chaverri P, Salgado C, Hirooka Y, Rossman AYG, Samuels J (2011) Delimitation of *Neonectria* and *Cylindrocarpon* (Nectriaceae, Hypocreales, Ascomycota) and related genera with *Cylindrocarpon*-like anamorphs. Stud Mycol 68:57–78
Chiarappa L (1997) *Phellinus igniarius*: the cause of spongy wood decay of black measles ("esca") disease of grapevines. Phytopathol Mediterr 36:109–111
Chicau G, Aboim-Inlez M, Cabral S, Cabral JPS (2000) *Phaeoacremonium chlamydosporum* and *Phaeoacremonium angustius* associated with esca and grapevine decline in Vinho Verde grapevines in northwest Portugal. Phytopathol Mediterr 39:80–86
Clerivet CA, Deaon V, Alami I, Lopez F, Geiger JP, Nicole M (2000) Tyloses and gels associated with cellulose accumulation in vessels are responses of plane tree seedlings (*Platanus acerifolia*) to the vascular fungus *Ceratocystis fimbriata* f. sp. *platani*. Trees 15:25–31
Crous PW, Swart L, Coertze S (2001) The effect of hot-water treatment on fungi occurring in apparently healthy grapevines cuttings. Phytopathol Mediterr 40:S464–S466
Edwards J, Pascoe IG (2004) Occurrence of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* associated with Petri disease and esca in Australian grapevines. Aust Plant Pathol 33:273–279
Edwards J, Marchi G, Pascoe IG (2001) Young esca in Australia. Phytopathol Mediterr 40:S303–S310
Eskalen A, Feliciano AJ, Gubler WD (2007) Susceptibility of grapevine pruning wounds and symptom development in response to infection by *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora*. Plant Dis 91:1100–1104
Ferreira JHS, Van Wyk PS, Calitz FJ (1999) Slow dieback of grapevine in South Africa: stress-related predisposition of young vines for infection by *Phaeoacremonium chlamydosporum*. SAJEV 20:43–46
Fischer M, Kassemeyer H-H (2003) Fungi associated with esca disease of grapevine in Germany. Vitis 42(3):109–116
Frias-Lopez J, Zerkle AL, Bonheyo GT, Heikoop JM, Fouke BW (2002) Partitioning of bacterial communities between seawater and healthy, black band diseased, and dead coral surfaces. Appl Environ Microbiol 68:2214–2228
Fussler L, Kobes N, Bertrand F, Maumy M, Grosman J, Savary S (2008) A characterization of grapevine trunk diseases in France from the national grapevine wood diseases survey. Phytopathology 98:571–579
Gazis R, Rehner S, Chaverri P (2011) Species delimitation in fungal endophyte diversity studies and its implications in ecological and biogeographic inferences. Mol Ecol 20(14):3001–3013
Giménez-Jaime A, Aroca A, Raposo R, Garcia-Jiménez J, Armengol J (2006) Occurrence of fungal pathogens associated with grapevine nurseries and the decline of young vines in Spain. J Phytopathol 154:598–602
Gonzáles V, Tello ML (2010) The endophytic mycota associated with *Vitis vinifera* in central Spain. Fungal Divers 47(1):29–42
Gramaje D, Armengol J (2011) Fungal trunk pathogens in the grapevine propagation process: potential inoculum sources, detection, identification, and management strategies. Plant Dis 95(9):1040–1055
Gramaje D, Garcia-Jiménez J, Armengol J (2010) Field evaluation of grapevine rootstocks inoculated with fungi associated with Petri disease and esca. Am J Enol Vitic 61(4):512–520
Graniti A, Surico G, Mugnai L (2000) Esca of grapevine: a disease complex or a complex of diseases? Phytopathol Mediterr 39:16–20
Green F III, Clausen CA (1999) Production of polygalacturonase and increase of longitudinal gas permeability in southern pine by brown-rot and white-rot fungi. Holzforschung 53(6):563–568
Green F III, Kuster TA, Highley TL (1996) Pectin degradation during colonization of wood by brown-rot fungi. Rec Res Devel Plant Pathol 1:83–93
Guo LD, Hyde KD, Liew ECY (2001) Detection and taxonomic placement of endophytic fungi within front tissues of *Livistona chinensis* based on rDNA sequences. Mol Phyl Evol 19:1–13
Halleen F, Crous PW, Petrini O (2003) Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. Aust Plant Pathol 32:47–52
Halleen F, Fourie PH, Crous P (2006) A review of black foot disease of grapevine. Phytopathol Mediterr 45:S55–S67
Higgins KL, Coley PD, Kursar TA, Arnold AE (2011) Culturing and direct PCR suggest prevalent host generalism among fungal endophytes of tropical grasses. Mycologia 103(2):247–260
Hyde KD, Soytong K (2008) The fungal endophyte dilemma. Fungal Divers 33:163–173
International Organisation of Vine and Wine (2011). State of the vitiviniculture world market. OIV annual report, March. Available: http://www.indianwineacademy.com/2011_note_conj_mars_EN.pdf. Accessed 8 March 2012.
Ko Ko TW, McKenzie EHC, Bahkali AH, To-anun C, Chukeatirote E, Promputtha I, Abd-Elsalam KA, Soytong K, Wulandari NF, Sanoamuang N, Jonglaekha N, Kodsueb R, Cheewangkoon R, Wikee S, Chamyuang S, Hyde KD (2011) The need for re-inventory of Thai phytopathogens. Chiang Mai J Sci 38(4):1–13
Kuntzmann P, Villaume S, Larignon P, Bertsch C (2010) Esca, BDA and eutypiosis: foliar symptoms, trunk lesions and fungi observed in diseased vinestocks in two vineyards in Alsace. Vitis 49(2):71–76
Larignon P (2012) Maladies cryptogamiques du bois de la vigne: symptomatologie et agents pathogènes. http://www.vignevin.com/menu-haut/actualites/article.html?tx_ttnews%5Btt_news%5D=368&tx_ttnews%5BbackPid%5D=918&cHash=2c0eccd030. Accessed 29 March 2012.
Larignon P, Dubos B (1997) Fungi associated with esca disease in grapevine. Eur J Plant Pathol 103:147–157
Larignon P, Dubos B (2000) Preliminary studies on the biology of *Phaeoacremonium*. Phytopathol Mediterr 39:184–189
Maharachchikumbura SSN, Guo LD, Chukeatirote E, Bahkali AH, Hyde KD (2011) *Pestalotiopsis*—morphology, phylogeny, biochemistry and diversity. Fungal Divers 50:167–187
Manamgoda DS, Cai L, Bhkali AH, Chukeatirote E, Hyde KD (2011) *Cochliobolus*: an overview and current status of species. Fungal Divers 51(S1):3–42
Marchi G (2001) Susceptibility to esca of various grapevine (*Vitis vinifera*) cultivars grafted on different rootstocks in a vineyard in the province of Siena (Italy). Phytopathol Mediterr 40:27–36
Martin MT, Cobos R (2007) Identification of fungi associated with grapevine decline in Castilla y Leon (Spain). Phytopathol Mediterr 46:18–25
McCutcheon TL, Carrol GC, Schwab S (1993) Genotypic diversity in populations of a fungal endophyte from douglas fir. Mycologia 85 (2):180–186
Mostert L, Crous PW, Petrini O (2000) Endophytic fungi associated with shoots and leaves of *Vitis vinifera*, with specific reference to the *Phomopsis viticola* complex. Sydowia 52:46–58
Mostert L, Ablen ECA, Halleen F, Crous PW (2006) Genetic diversity among isolates of *Phaeomoniella chlamydospora* on grapevines. Aust Plant Pathol 35(4):453–460
Mugnai L, Contesini AM, Surico G, Graniti A, Imbriani R, Bianco N (1996) Recenti progressi nella conoscenza del "mal dell'esca"

della vite in Italia, in Convegno nazionale 'Arsenico, Sí-No', Codroipo, Udine, 14 dicembre 1995, Forum Fitoiatrici, ERSA, Udine, pp 115–122.

Mugnai L, Graniti A, Surico G (1999) Esca (black measles) and brown wood-streaking: two old and elusive diseases of grapevines. Plant Dis 83(5):404–418

Munkvold GP, Marois JJ (1995) Factors associated with variation in susceptibility of grapevine pruning wounds to infection by *Eutypa lata*. Phytopathology 85:249–256

Neubert K, Mendgen K, Brinkmann H, Wirsel SGR (2006) Only a few fungal species dominate highly diverse mycofloras associated with the common red. Appl Environ Microbiol 72:1118–1128

O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R (2005) Fungal community analysis by large-scale sequencing of environmental samples. Appl Environ Microbiol 71:5544–5550

Phillips AJL (2000) Excoriose, cane blight and related diseases of grapevines: a taxonomic review of the pathogens. Phytopathol Mediterr 39(3):341–356

Promputtha I, Lumyong S, Dhanasekaran V, McKenzie EHC, Hyde KD, Jeewon R (2007) A phylogenetic evaluation of whether endophytes become saprotrophs at host senescence. Microb Ecol 53:579–590

Pryor BM, Michailides TJ (2002) Morphological, pathogenic, and molecular characterization of *Alternaria* isolates associated with *Alternaria* late blight of pistachio. Phytopathology 92(4):406–416

R Development Core Team (2006) R: a language and environment for statistical computing. Available: http://www.r-statistics.org.

Reisenzein H, Berger N, Nieder G (2000) Esca in Austria. Phytopathol Mediterr 39:26–34

Rolshausen P, Kiyomoto R (2011) The status of grapevine trunk diseases in the Northeastern United States. Available: http://www.newenglandvfc.org/pdf_proceedings/status_grapevinetrunkdisease.pdf. Accessed 8 March 2012.

Sánchez S, Bills GF, Zabalgogeazcoa I (2007) The endophytic mycobiota of the grass *Dactylis glomerata*. Fungal Divers 27:171–195

Sánchez S, Bills GF, Zabalgogeazcoa I (2008) Diversity and structure of the fungal endophytic assemblages from two sympatric coastal grasses. Fungal Divers 33:87–100

Santos C, Fragoerio S, Phillips A (2005) Physiological response of grapevine cultivars and a rootstock to infection with *Phaeoacremonium* and *Phaeomoniella* isolates: an in vitro approach using plants and calluses. Sci Hortic 103(2):187–198

Scheck H, Vasquez S, Fogle D, Gubler WD (1998) Grape growers report losses to black foot and grapevine decline. Calif Agric 52(4):19–23

Schubert K, Groenewald JZ, Braun U, Dijksterhuis J, Starink M, Hill CF, Zalar P, de Hoog GS, Crous PW (2007) Biodiversity in the *Cladosporium herbarum* complex (Davidiellaceae, Capnodiales), with standardisation of methods for *Cladosporium* taxonomy and diagnostics. Stud Mycol 58(1):105–156

Sosnowski M, Wicks T, Edwards J, Scott E, Lardner R (2005) What's happening in the world of grapevine trunk diseases? The Australian and New Zeeland Grapegrower and Winemaker 498:18–21

Sosnowski MR, Shtienberg D, Creaser ML, Wicks TJ, Lardner R et al (2007) The influence of climate on foliar symptoms of eutypa dieback in grapevines. Phytopathology 97:1284–1289

Sun Q, Rost LR, Matthews MA (2006) Pruning-induced tylose development in stems of current-year shoots of *Vitis vinifer*a (Vitaceae). Am J Bot 93(11):1567–1576

Sun Q, Rost LR, Matthews MA (2008) Wound-induced vascular occlusions in *Vitis vinifera* (Vitaceae): tyloses in summer and gels in winter. Am J Bot 95(12):1498–1505

Surico G, Marchi G, Braccini P, Mugnai L (2000) Epidemiology of esca in some vineyards in Tuscany (Italy). Phytopathol Mediterr 39(1):190–205

Surico G, Mugnai L, Marchi G (2006) Older and more recent observations on esca: a critical overview. Phytopathol Mediterr 45: S68–S86

Tempesta S, Rubini A, Pupulin F, Rambelli A (2011) *Pestalotiopsis* endophytes from leaves of two orchid species collected in Costa Rica. Cryptog Mycol 32(3):315–321

Toledo-Hernández C, Zuluaga-Montero A, Bones-González A, Sabat AM, Bayman P (2008) Fungi in healthy and diseased sea fans (*Gorgonia ventalina)*: Is *Aspergillus sydowii* always the pathogen? Coral Reefs 27:707–714

U'Ren JM, Lutzoni F, Miadlikowska J, Arnold AE (2010) Community analysis reveals close affinities between endophytic and endolichenic fungi in mosses and lichens. Microb Ecol 60:340–353

Udayanga D, Liu X, McKenzie EHC, Chukeatirote E, Bahkali AHA, Hyde KD (2011) The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. Fungal Divers 50:189–225

Urbez-Torres JR, Leavitt GM, Voegel TM, Gubler WD (2006) Identification and distribution of *Botryosphaeria* spp. associated with grapevine cankers in California. Plant Dis 90(12)):1490–1503

Úrbez-Torres JR, Adams P, Kamas J, Gubler WD (2009) Identification, incidence, and pathogenicity of fungal species associated with grapevine dieback in Texas. Am J Enol Vitic 60(4):497–507

Van Wyk M, Adawi AOA, Kahn IA, Deadman ML, Jahwari AAA, Wingfield BD, Ploetz R, Wingfield JM (2007) *Ceratocystis manginecans* sp. nov., causal agent of a destructive mango wilt disease in Oman and Pakistan. Fungal Divers 27:213–230

Verhoeff K (1974) Latent infections by fungi. Annu Rev Phytopath 12:99–110

Viret O, Bloesch B, Fabre AL, Taillens J, Siegfried W (2004) L'esca en Suisse: situation en 2001 et évolution en 2004. Available: http://www.vignevin-sudouest.com/publications/itv-colloque/documents/COLLOQUE_Maladies-bois-integral.pdf. Accessed 8 March 2012.

Wikee S, Cai L, Pairin N, McKenzie EHC, Su YY, Chukeatirote E, Thi HN, Bahkali AH, Moslem MA, Abdelsalam K, Hyde KD (2011) *Colletotrichum* species from Jasmine (*Jasminum sambac*). Fungal Divers 46:171–182

Yang Y, Cai L, Yu Z, Liu Z, Hyde KD (2011) *Colletotrichum* species on Orchidaceae in southwest China. Cryptog Mycol 32(3):229–253

Zabalgogeazcoa I (2008) Fungal endophytes and their interaction with plant pathogens. Span J Agric Res 6:138–146, Special issue

Zuluaga-Montero A, Toledo-Hernández C, Rodrígues JA, Sabat AM, Bayman P (2010) Spatial variation in fungal communities isolated from healthy and diseased sea fans Gorgonia ventalina and seawater. Aquat Biol 8:151–160