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Fungal communities living in the wood of different cultivars of young *Vitis vinifera* plants

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Summary. The fungal communities associated with five *Vitis vinifera* cultivars grown in Switzerland ('Humagne', 'Chasselas', 'Arvine', 'Gamaret' and 'Gamay') were examined. Of the 703 fungal isolates obtained in pure culture, 66 operational taxonomic units (OTUs) were defined. The results show that: the great majority of the fungi isolated in this study were ascomycetes, with a high proportion of Sordariomycetes (mainly Hypocreales, Sordariales and Diaporthales); different fungal OTUs were associated with different cultivars; graft and rootstock contributed equally to the fungal community composition; Esca- or Petri-related species occurred sporadically in the different cultivars, with some of them occupying specific tissues or parts of the plant (e.g.: Botryosphaeriaceous species, *Phaeomoniella chlamydospora* and *Phomopsis viticola*); almost 25% of OTUs occurred in different plant parts in most cultivars, which suggests an easy spread outwards from the infected material (graft or rootstock), which might be explained by the fungal propagules being transported through the xylem vessels.

Key words: endophytes, grapevine, esca disease, vessel lumen.

Introduction

Fungal diseases of grapevine wood, such as young vine decline (Petri disease) and esca, cause severe losses in newly planted as well as in established vineyards in most vine-producing countries (Bertelli *et al.*, 1998; Scheck *et al.*, 1998; Ferreira *et al.*, 1999; Mugnai *et al.*, 1999; Pascoe and Cottral, 2000; Whiting *et al.*, 2001; Giménez-Jaime *et al.*, 2006). Studies on the fungi associated with *Vitis vinifera* have so far focused essentially on the detection and identification of the fungal species present in necrotic wood. *Phaeomoniella chlamydospora* and species of the genera *Phaeoacremonium* and *Fomitiporia* are considered to cause esca disease (Crous *et al.*, 1996, 2001; Larignon and Dubos, 1997; Armengol *et al.*, 2001; Rumbos and Rumbou, 2001). More recently, Gimenez-Jaime *et al.* (2006) reported that young vine decline was associated with the same fungal species as esca, with different fungal species predominating depending on the geographical area. As result, more attention was paid to material selection and to plant production in nurseries. Nowadays the presence of these fungi in nursery material is thought to be responsible for poor vine vigor and lower yields in newly established vineyards (Morton, 1999). However, our knowledge of the interactions between these fungal pathogens in the wood remains very limited. They may act simultaneously or in succession (Graniti *et al.*, 2002). Also *V. vinifera,* as virtually all other land plants, hosts many more fungal endophytes than had been thought (Schweigkofler and Prillinger, 1999). These endophytes may play an important role in balancing the fungal community living in *V. vinifera*, as latent pathogens or as protective endophytes some of which could also be used as biocontrol agents (Król and Machowicz-Stefaniak, 2008). The identification and location of the fungi associated with *V. vinifera* in its

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early stages of growth is a preliminary but essential step to a better understanding of the emergence of wood diseases in grapes.

In this study, we isolated and identified the fungi occurring in grafted plants ready for planting of five *V. vinifera* cultivars commonly grown in grapevine producing areas in Switzerland. After isolation and identification of the fungi in pure culture, fungal communities were compared between cultivars to determine: 1) which major groups of fungi were found in young vine wood, 2) which fungi, if any, preferred certain cultivars, or particular tissues (wood or pith) and/or plant parts (basal end, pruning wounds and graft with grafting point). Finally we investigated whether wood physiology, and more specifically the size of the xylem vessels, could explain the different fungal communities associated with the different cultivars.

Materials and methods

The fungi associated with *V. vinifera* were isolated from five cultivars: '*Gamay*' and '*Chasselas*' (among the most frequent cultivars in Switzerland); '*Arvine*' and '*Humagne*' (two ancient endemic varieties) and 'Gamaret' (a recently registered cultivar resistant to *Botrytis cinerea*, which was selectively bred by Agroscope Changins-Wädenswil, Nyon, Switzerland). The plant material, obtained from different nurseries in Switzerland, consisted of one-year-old rooted plants grafted on the commercial rootstock 3309.

Eight to twelve plants of each cultivar were de-barked and surface-sterilized (3.5% NaOCl for 20 min) after removal of the roots, the soil and the residual waxes. Four wood pieces (4 to 6 cm length) were aseptically taken from three zones of every plant (basal end, pruning wounds and graft with grafting point). Pieces of pith and wood were placed separately on 15-cm Petri dishes containing potato dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA) amended with aureomycin (12.5 mg l^{-1}). After inspecting the plates for the emergence of fungi during 10 days, fungal isolates were grown on PDA at room temperature. The fungi were identified based on their macro- and micro-morphology. To confirm the identification and define the isolates not clearly identified molecular techniques were used, based on the closest match of their internal transcribed spacer (ITS) sequence with the ITS data deposited in GenBank (a sequence similarity ≥98% was interpreted as the threshold for species delimitation) (White *et al.*, 1990). Fungal genomic DNA was isolated from fresh mycelium stored in SDS 3% buffer. The ITS1-5.8S-ITS2 were amplified and sequenced using the ITS1-F and ITS4 primers (the sequences of these primers are available at: http://www.biology.duke.edu/fungi/mycolab/primers.htm). Representative isolates of the different operational taxonomic units (OTUs) were deposited in the mycological collection of the Mycology group in the Federal research station Agroscope Changins-Wadenswil, Nyon, Switzerland.

Sequencing used the reagents and conditions of the BibDye®Terminator v3.1 Cycle sequencing and an automated capillary sequencer ABI 3700 DNA analyzer (Perkin Elmer, Applied Biosystems, Foster City, CA, USA).

Xylem vessels were examined by scanning electron microscopy (SEM). Wood discs (diam.=1 cm) were cut from some canes of each *V. vinifera* cultivar and fixed under osmium tetroxide vapor (aqueous solution of 2% OsO_4 (w:v) and 3% CrO_3 (w:v) for 10 h at room temperature in a humid chamber), following the method described by Gindro *et al.* (2003). The discs were then gradually dehydrated in an acetone series of 10-30-50-70-90-100% (v:v), keeping them on ice for 20 min at each step. After critical point drying (CPD030; Bal-Tech, Balzers, Liechtenstein), the samples were coated with platinum in a S150B sputter coater (Edwards, Wilmington, MA, USA) and inspected with a Jeol JSM-6300 F scanning electron microscope at 5 KV (Jeol GmbH, D-85386 Eching, Germany). The pictures were digitized using SemAfore 5.0 digitizer software (Jeol).

Vessel lumina were measured using ImageJ image analysis software (http://rsb.info.nih.gov/ij/index.html) on a minimum of 4 digital pictures of wood discs for each cultivar and rootstock. The number of vessels measured ranged from 565 ('Gamay') to 837 ('Arvine'). The proportional vessel lumen (*Vl*) was calculated for each cultivar (*Vl*=sum of vessel lumen/total surface of xylem zone). Vessel lumen data were tested for normal distribution using the Shapiro-Wilk test (XLSTAT, Addinsoft, 1995–2004).

Results

A total of 703 fungal isolates was obtained (Table 1), the number of isolates per cultivar ranged from 121 ('Arvine') to 159 ('Chasselas'). Based on morphology, microscopy and blast of the

ITS sequence data in Genbank, 682 isolates were identified to the level of species (50 spp.), while 15 isolates could only be identified to the level of genus, and 7 isolates to the level of class (Tables 2, 3 and 4). Sixty-six OTUs were defined.

The fungal isolates are representative of three of the major lineages of Fungi: Ascomycota, Basidiomycota, and Zygomycota (Table 2, 3 and 4). The great majority of the isolates are ascomycetes (87.5%) (Fig.1). Of these, the best-represented class was the Sordariomycetes comprising 55.1% of isolates (33.8% 'Humagne' ; 59.7% 'Chasselas'), followed by the Eurotiomycetes (22.6% of isolates; 14.9% 'Arvine' ; 26.7% 'Gamay'), the Dothideomycetes (12% of isolates; 5.3% 'Gamay' ; 21.9% 'Humagne'), and the Leotiomycetes (9.9% of isolates; 3.8% 'Chasselas' and 'Gamay' ; 20.7% 'Arvine'). The Sordariomycetes isolates were mainly Hypocreales (12 OTUs), Sordariales (4 OTUs), and Diaporthales (5 OTUs). Only a few species belonged to the basidiomycetes (1.7% of isolates) and zygomycetes (10.8% of isolates).

As regards the OTU composition of the fungal communities associated with the different cultivars, 24 of the 66 OTUs defined by ITS blast in Genbank and/or by micro- and micromorphology (36.4%) were found in only one cultivar (Table 3). Seven of the OTUs were isolated from all cultivars (*Apiospora montagnei*, *Alternaria* sp., *Cadophora luteo-olivacea*, *Fusarium* sp., *Ophiostoma piceae, Penicillium* sp. and *Trichoderma* sp.), 5 from four cultivars (*Chaetomium globosum*, *Clonostachys rosea*, *Mortierella hyalina*, *Mucor hiemalis and Truncatella angustata*), 8 from three cultivars, and 22 from two cultivars. The esca and Petri related isolates (*"Botryosphaeria"* spp., *Pa. chlamydospora*, *Cylindrocarpon liriodendrii*) and some other species causing grapevine diseases (*Cadophora* spp. and *Phomopsis viticola* [cane and leaf spot disease of grapes]) were included in these infrequently occurring fungal species in the five cultivars examined. Some other fungal pathogens, however never previously reported on symptomatic vines, were also isolated, such as *Ca. luteo-olivacea*, *Co. truncatum*, *Lecythophora hoffmannii*, *Rhizopus stolonifer*, *Alternaria* spp., *Diaporthe* spp., and *Phoma* spp. (Table 3). The highest number of fungal pathogens was found in 'Humagne' (23 OTUs), while the other cultivars generally hosted fewer

Table 1. Number of isolates from the different types of tissue isolated from each cultivar.

Grape cultivar	No. of fungal isolates		
	Wood	Pith	Total
Chasselas	92	67	159
Humagne	87	64	151
Gamaret	76	67	141
Gamay	72	59	131
Arvine	70	51	123

Table 2. Fungal classes isolated from each cultivar.

Fungal class	No. of orders in each class and cultivar				
	Chasselas	Humagne	Gamaret	Gamay	Arvine
Agaricomycetes	1	2	-	1	-
Dothideomycetes	3	3	2	2	2
Eurotiomycetes	2	3	1	1	2
Leotiomycetes	1	1	1	1	1
Pezizomycetes	-	-	-	-	1
Sordariomycetes	6	5	5	4	5
Zygomycetes	2	2	1	2	2

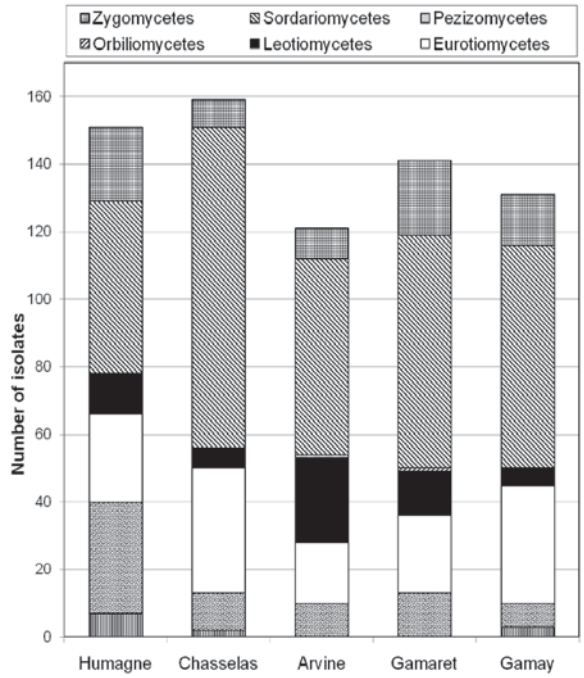

Fig. 1. Number of isolates belonging to different fungal classes in each *Vitis vinifera* cultivar.

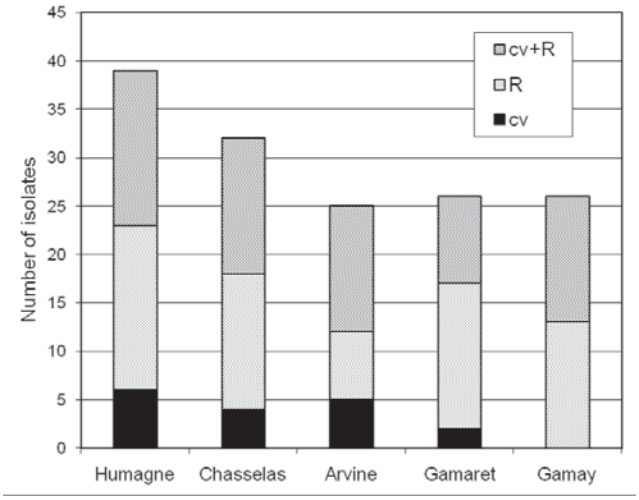

Fig. 2. Number of isolates from graft (cv.), rootstock (R) and from both (cv.+R) of each *Vitis vinifera* cultivar.

pathogens: between 12 OTUs ('Arvine') and 16 OTUs ('Chasselas').

More isolates were retrieved from the wood than from the pith (Table 1). However in all cultivars the number of OTUs isolated from the wood (W) and the number of OTUs isolated from the pith (P) were very similar ('Humagne': W=28, P=27; 'Chasselas': W=25, P=22; 'Arvine': W=17, P=17; 'Gamaret': W=20, P=19) except 'Gamay' (W=24, P=15) (Table 3). Only three fungi were wood or pith-specific colonizers: *A. montagnei* occurred only in the wood of 4 out of 5 cultivars, and *Ch. globosum* and *M. hyalina* only in the wood of 3 out of 5 cultivars. Some species occurred in both wood and pith of nearly all cultivars (e.g.: several *Ophiostoma* and *Penicillium* species). Other species colonized only the wood or the pith of some cultivars (e.g. *Alternaria* spp. colonized only the wood of 'Arvine' and 'Gamaret'), while in all other cultivars occurred in both wood and pith. Different species of the same genus sometimes colonized different tissues of the same cultivar. For instance, in 'Humagne', "*Botryosphaeria*" *obtusa* colonized both the wood and the pith, whereas *B. parva* colonized only the pith. However, *B. parva* occurred in both the wood and the pith of 'Arvine'. In 'Chasselas', *Diaporthe medusaea* preferentially colonized the wood, *Diaporthe eres* the pith.

The number of OTUs isolated was much the same across the different parts of the plant (a, graft and grafting point; b, pruning wound; c, rootstock. Table 3, Fig. 2) in all cultivars ('Humagne': a=22, b=25; c=22; 'Chasselas': a=17, b=20; c=22; 'Arvine': a=18, b=16; c=12; 'Gamay': a=13, b=19; c=16) except 'Gamaret', in which the fungi mainly colonized the pruning wound and the rootstock (a=9, b=20; c=16). The number of OTUs found in all plant parts of a cultivar (Table 4) represented approximately a quarter of the total number of OTUs isolated for that cultivar ('Humagne': 26%; 'Chasselas': 25%; 'Arvine': 29%; Gamey: 31%) except again for 'Gamaret' (15%). Fungi specific to a particular part of a vine comprised 37% of OTUs in 'Arvine' to 48% in 'Chasselas' (calculated as: [number of OTUs isolated in the cultivar from a single part of a vine/total number of OTUs isolated from that vine] × 100). Very few OTUs occurred only in the grafting zone plus the rootstock (a+c, Table 4).

When wood physiology was compared between cultivars, the vessels occupied from 21.8% ('Chasselas') to 47.2% ('Humagne') of the xylem lumen in cross section (Table 4 and Fig. 3), with mean values from 6.32E+03 μm^2 ('Chasselas') to 8.4E+03 μm^2

Table 3. Presence of each operational taxonomic unit (OUT) in different plant parts and tissues of each cultivar.

OTUs names	Class/Order[a]	Grape cultivar										Occ.[d]
		Humagne		Chasselas		Arvine		Gamaret		Gamay		
		a/b/c[b]	W/P[c]	a/b/c	W/P	a/b/c	W/P	a/b/c	W/P	a/b/c	W/P	
Alternaria arborescens	Doth/Pleosporales	+/+/-	+/+	- /- /-		- /- /-		- /- /-		- /- /-		1
Alternaria sp. * x[e]	Doth/Pleosporales	**+/+/-**	**+/+**	**+/- /+**	**+/+**	**+/- /-**	**+/-**	**- /+/+**	**+/-**	**+/+/+**	**+/+**	**5**
Apiospora montagnei #	Sord/Sordariomycetidae	**+/-/+**	**+/-**	**+/+/+**	**+/-**	**+/+/-**	**+/-**	**- /+/-**	**+/-**	**+/+/-**	**+/+**	**5**
Arthrobotrys sp. □	Orb/Orbiliales	-/-/-		- /- /-		- /- /-		- /- /+	- /+	- /- /-		1
Aspergillus niger group □	Eur/Eurotiales	-/-/+	+/-	- /- /+	+/-	- /- /-		- /- /-		- /- /-		2
Aspergillus sp. □	Eur/Eurotiales	-/-/+	- /+	- /- /-		+/- /-	- /+	- /- /-		+/+/-	+/+	3
Bionectria ochroleuca □	Sord/Hypocreales	-/-/-		- /- /-		- /- /-		- /- /-		- /- /+	+/-	1
"Botryosphaeria" obtusa * #	Doth/Botryosphaeriales	+/+/+	+/+	+/- /-	+/-	- /- /-		- /- /-		- /- /-		2
"Botryosphaeria" parva * #	Doth/Botryosphaeriales	+/-/-	- /+	- /- /-		+/+/-	+/+	- /- /-		- /- /-		2
Botrytis cinerea *	Leo/Helotiales	-/-/-		- /- /-		- /- /+	- /+	+/- /-	+/-	- /+/+	+/+	3
Cadophora luteo-olivacea * # x	Leo/Helotiales	**+/+/+**	**- /+**	**- /+/+**	**- /+**	**+/+/+**	**+/+**	**- /+/+**	**+/+**	**+/+/-**	**+/-**	**5**
Cadophora fastigiata x □	Leo/Helotiales	-/-/-		- /+/+	- /+	- /+/+	+/+	- /- /-		- /- /-		2
Ceratobasidium sp. #	Aga/Cantharellales	-/-/-		- /- /-		- /- /-		- /- /-		+/- /+	+/+	1
Chaetomium globosum x	Sord/Sordariales	**-/+/-**	**+/-**	**+/+/+**	**+/+**	**- /- /-**		**- /+/-**	**+/-**	**- /+/-**	**+/-**	**4**
Chaetomium nigricolor x	Sord/Sordariales	-/-/-		- /- /-		- /- /-		- /+/-	+/-	- /- /-		1
Chaetomium sp. x	Sord/Sordariales	-/-/-		+/+/-	+/+	- /+/+	+/+	- /+/-	+/-	- /- /-		3
Cladosporium cladosporioides x □	Doth/Capnodiales	-/+/-	- /+	- /- /-		- /- /-		- /- /-		- /- /-		1
Cladosporium sphaerospermum x □	Doth/Capnodiales	-/-/-		+/+/-	+/+	- /- /-		- /- /-		- /+/-	- /+	2
Clonostachys rosea x	Sord/Hypocreales	**-/-/-**		**+/+/+**	**+/+**	**+/+/+**	**+/+**	**- /- /+**	**+/-**	**+/+/+**	**+/+**	**4**
Colletotrichum truncatum #	Sord/Glomerellaceae	-/-/-		- /- /-		- /- /-		- /- /-		+/+/+	+/+	1
Coprinellus radians x	Aga/Agaricales	+/-/-	- /+	+/+/-	+/-	- /- /-		- /- /-		- /- /-		2
Cylindrocarpon liriodendri * #	Sord/Hypocreales	+/-/+	+/+	- /- /-		- /- /-		- /+/+	- /+	- /- /-		2
Cylindrocarpon sp. * #	Sord/Hypocreales	-/-/+	+/+	- /- /-		- /- /-		- /- /-		- /- /+	+/-	2
Diaporthe eres * #	Sord/Diaporthales	-/-/-		- /- /+	- /+	- /- /-		- /- /-		- /- /-		1
Diaporthe medusae * #	Sord/Diaporthales	-/-/+		- /- /+	+/-	- /- /-		- /- /-		- /- /-		1
Diaporthe phaseolorum * #	Sord/Diaporthales	-/+/-	+/+	- /- /-		- /- /-		- /- /-		- /- /-		2
Diaporthe sp. * #	Sord/Diaporthales	-/-/+	+/+	- /- /-		- /- /-		- /+/+	+/+	- /- /-		2
Epicoccum nigrum # x	Doth/Pleosporales	-/+/-	+/-	- /- /-		- /- /-		- /- /-		- /- /+	+/-	2

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(Table 3 continued)

Fusarium sp. * # □	Sord/Hypocreales	**+/+/+**	**+/+**	**+/+/-**	**+/+**	**+/+/+**	**+/+**	**+/+/+**	**+/+**	**+/+/+**	**+/-**	**5**
Geomyces pannorum □	Leo/Myxotrichaceae	+/+/-	+/+	- /- /-		- /- /-		- /+/+	+/+	- /- /-		2
Haematonectria haematococca #	Sord/Hypocreales	-/-/-		- /- /-		+/+/+	+/-	- /- /-		- /- /-		1
Hypocrea parapilulifera x □	Sord/Hypocreales	-/-/-		- /- /-		+/+/-	+/+	- /- /-		- /- /-		1
Leptosphaeria sp. #	Doth/Pleosporales	-/-/-		- /+/+	- /+	- /- /-		- /- /+	- /+	- /- /-		2
Lecythophora hoffmannii x	Sord/Coniochaetales	-/+/+	- /+	- /- /-		- /- /-		- /- /-		- /- /-		1
Massarina corticola □	Doth/Pleosporales	+/+/+	- /+	- /- /+	- /+	- /- /-		- /+/+	+/+	- /- /-		3
Microdochium bolleyi #	Doth/Pleosporales	-/+/-	+/-	- /- /-		- /- /-		- /- /-		- /- /-		1
Mortierella hyalina □	Zyg/Mortierellales	**+/-/-**	**+/-**	**- /- /+**	**+/-**	**+/+/+**	**+/+**	**- /- /-**		**- /+/-**	**+/-**	**4**
Mucor circinelloides □	Zyg/Mucorales	-/-/-		+/- /-	+/+	- /- /-		- /- /-		- /+/-	+/-	2
Mucor hiemalis □	Zyg/Mucorales	+/+/+	+/+	+/- /-	+/+	- /- /-		+/+/+	+/+	+/+/+	+/+	4
Mucor plumbeus □	Zyg/Mucorales	+/-/+	+/+	- /- /-		- /- /-		- /- /-		- /+/-	- /+	2
Mucor racemosus □	Zyg/Mucorales	-/-/-		+/+/+	+/+	- /- /+	- /+	+/- /-	- /+	- /- /-		3
Mycovellosiella fulva #	Doth/Capnodiales	+/+/-	+/+	- /- /-		- /- /-		- /- /+	- /+	- /- /-		2
Nectria fuckeliana * #	Sord/Hypocreales	- /-/-		- /- /+	+/+	- /- /-		- /- /-		- /- /-		2
Nectria ramulariae * #	Sord/Hypocreales	-/-/-		- /- /-		+/- /-	+/-	- /+/+	+/+	- /- /-		2
Neonectria macrodidyma * #	Sord/Hypocreales	-/+/-	- /+	+/+/+	+/+	- /- /-		- /- /-		- /- /-		2
Neoplaconema sp. * #	Doth/?	-/+/-	+/-	- /- /-		- /- /-		- /- /-		- /- /-		1
Ophiostoma piceae #	Sord/Ophiostomatales	**+/-/-**	**+/-**	**+/+/+**	**+/+**	**+/- /-**	**+/+**	**+/+/+**	**+/+**	**+/+/+**	**+/+**	**5**
Ophiostoma quercus #	ord/Ophiostomatales	+/+/+	+/+	- /- /-		- /- /-		+/+/-	+/+	+/+/-	+/+	3
Ophiostoma subalpinum #	ord/Ophiostomatales	-/-/-		- /- /-		- /- /-		- /+/-	+/+	- /- /-		1
Ophiostoma sp. #	ord/Ophiostomatales	-/-/-		- /+/-	+/+	- /- /-		- /- /-		- /- /-		1
Penicillium sp. □	Eur/Eurotiales	**+/+/+**	**+/+**	**+/+/+**	**+/+**	**+/+/+**	**+/+**	**+/+/+**	**+/+**	**+/+/+**	**+/+**	**5**
Phaeomoniella chlamydospora *	Eur/Chaetothyriales	-/-/-		- /+/+	- /+	- /- /-		- /- /-		- /- /-		1
Phoma glomerata * #	Doth/Pleosporales	+/-/-	+/-	- /- /-		- /- /-		- /- /-		- /- /-		1
Phoma sp. * #	Doth/Pleosporales	+/+/+	+/-	- /+/+	+/+	- /- /-		+/+/-	+/+	- /- /-		3
Phomopsis viticola *	Sord/Diaportales	-/-/-		+/- /-	+/-	+/- /-	+/-	- /- /-		- /- /-		2
Rhizoctonia sp. #	Aga/Cantharellales	-/+/+	+/+	- /- /-		- /- /-		- /- /-		- /- /-		1
Rhizopus stolonifer * # □	Zyg/Mucorales	-/-/-		- /- /-		- /+/+	+/+	- /- /-		- /- /+	+/-	2
Sclerotinia sclerotiorum #	Leo/Helotiales	-/+/-	+/-	- /- /-		- /- /-		- /- /-		- /- /-		1

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(Table 3 continued)

Sordaria fimicola □	Sord/Sordariales	-/-/-		- /+/-	+/-	- /- /-		- /- /-		- /- /-		1
Sordariomycetes of incertae sedis	Sord/?	-/-/-		- /- /-		+/+/-	- /+	- /+/+	+/+	- /- /+	+/+	3
Trichocladium asperum x □	Sord/Sordariales	-/+/+	- /+	- /- /-		+/+/+	+/+	- /- /-		- /- /-		2
Trichoderma sp. □	Sord/Sordariales	**+/+/+**	**+/+**	**- /+/+**	**+/-**	**+/- /-**	**+/-**	**+/+/-**	**+/+**	**+/+/+**	**+/+**	**5**
Truncatella angustata #	Sord/Xylariales	**+/+/+**	**+/+**	**+/+/+**	**+/+**	**+/+/-**	**+/-**	**- /- /-**		**- /+/-**	**+/-**	**4**
Umbelopsis isabellina □	Zyg/Mucorales	-/-/+	- /+	- /- /-		- /- /-		- /- /-		- /- /-		1
Verpa bohemica	Pez/Pezizales	-/-/-		- /- /-		- /+/-	- /+	- /- /-		- /- /-		1
Zygorhynchus moelleri □	Zyg/Mucorales	-/-/-		- /- /-		- /- /-		- /- /-		- /- /+	+/-	1
Zone-specific OTUs		**5/8/6**		**4/2/7**		**6/1/2**		**2/5/4**		**0/6/6**		

[a] Aga, Agaricomycetes; Doth, Dothideomycetes; Eur, Eurotiomycetes; Leo, Leotiomycetes; Orb, Orbiliomycetes; Pez, Pezizomycetes; Sord, Sordariomycetes; Zyg, Zygomycetes. When Order is "incertae sedis" Subclass or Family name are reported underlined.
[b] a, graft and grafting point; b, pruning wounds; c, basal end.
[c] W, wood; P, pith.
[d] Occ., occurrence: No. of cultivars in which the species occurs.
[e] *, *Vitis* pathogen; # pathogen of other plants; x, secondary colonizer with ligninolytic capacity; Δ, saprotrophic.
+, present; -, absent.
[f] In bold the OTUs isolated from at least 4 cultivars.

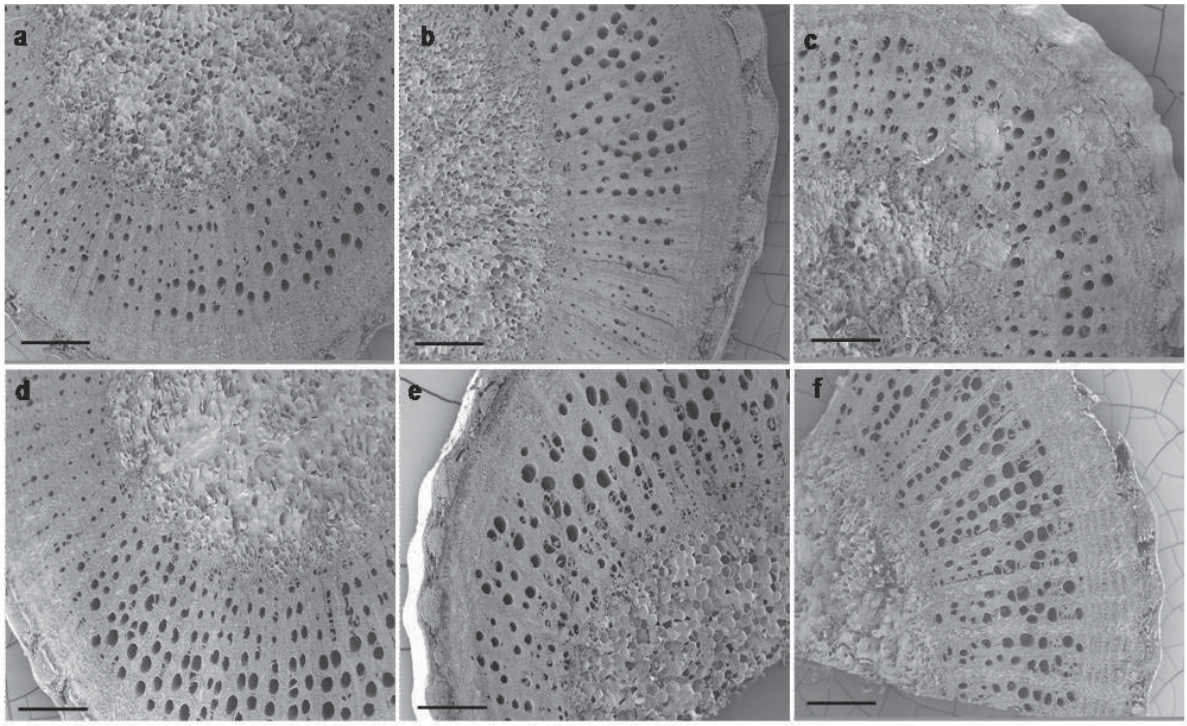

Fig. 3. Example SEM images of vessels surface in semi-thin section dormant canes. a, Chasselas; b, Gamay; c, 3309 (rootstock); d, Gamaret; e, Arvine; f, Humagne. Scale bar=1 mm.

Table 4. Operational taxonomic units (OTUs) isolated from more than one plant part in each cultivar.

Plant part [a]	Fungal species				
	'Humagne'	'Chasselas'	'Arvine'	'Gamaret'	'Gamay'
a+b+c OTUs (+/+/+)	*"Botryosphaeria" obtusa* *Cadophora luteo-olivacea* *Fusarium* sp. *Massarina corticola* *Mucor hiemalis* *Ophiostoma quercus* *Penicillium* sp. *Phoma* sp. *Trichoderma* sp. *Truncatella angustata*	*Apiospora montagnei* *Clonostachys rosea* *Fusarium* sp. *Mucor racemosus* *Neonectria macrodidyma* *Ophiostoma piceae* *Penicillium* sp. *Truncatella angustata*	*Cadophora luteo-olivacea* *Chaetomium globosum* *Clonostachys rosea* *Fusarium* sp. *Haematonectria haematococca* *Mortierella hyalina* *Penicillium* sp. *Trichocladium asperum*	*Fusarium* sp. *Mucor hiemalis* *Ophiostoma piceae* *Penicillium* sp.	*Alternaria* sp. *Clonostachys rosea* *Colletotrichum truncatum* *Fusarium* sp. *Mucor hiemalis* *Ophiostoma piceae* *Penicillium* sp. *Trichoderma* sp.
a+b OTUs (+/+/-)	*Alternaria arborescens* *Alternaria* sp. *Geomyces pannorum* *Mycovellosiella fulva*	*Chaetomium* sp. *Cladosporium sphaerospermum* *Fusarium* sp.	*Apiospora montagnei* *Botryosphaeria parva* *Hypocrea parapilulifera* *Sordariomycetes of incertae sedis* *Truncatella angustata*	*Trichoderma* sp. *Phoma* sp. *Ophiostoma quercus*	*Apiospora montagnei* *Cadophora luteo-olivacea* *Ophiostoma quercus* *Aspergillus* sp.
b+c OTUs (-/+/+)	*Lecythophora hoffmannii* *Rhizoctonia* sp. *Trichocladium asperum*	*Cadophora luteo-olivacea* *Cadophora fastigiata* *Leptosphaeria* sp. *Phaeomoniella chlamydospora* *Phoma* sp. *Trichoderma* sp.	*Rhizopus stolonifer* *Chaetomium* sp. *Cadophora fastigiata*	*Alternaria* sp. *Cadophora luteo-olivacea* *Cylindrocarpon liriodendri* *Diaporthe* sp. *Geomyces pannorum* *Massarina corticola* *Nectria ramulariae* *Sordariomycetes of incertae sedis*	*Botrytis cinerea*
a+c OTUs (+/-/+)	*Mucor plumbeus* *Cylindrocarpon liriodendri* *Apiospora montagnei*	*Alternaria* sp.			*Ceratobasidium* sp.

[a] a, graft and grafting point; b, pruning wounds; c, basal end.

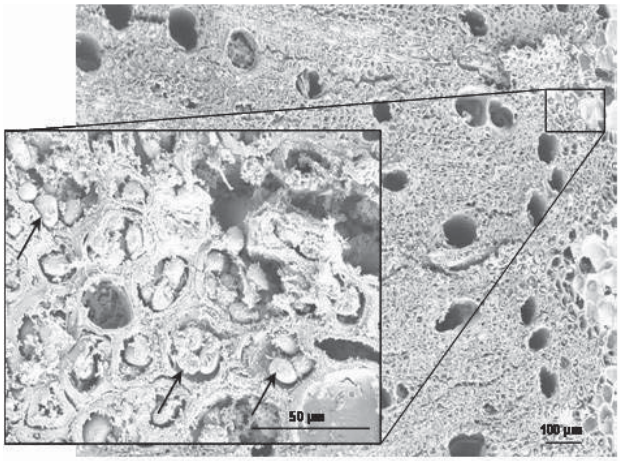

Fig. 4. Representative SEM image of starch granules accumulated into cells of the xylematic zone in Chasselas cultivar. Arrows show starch granules.

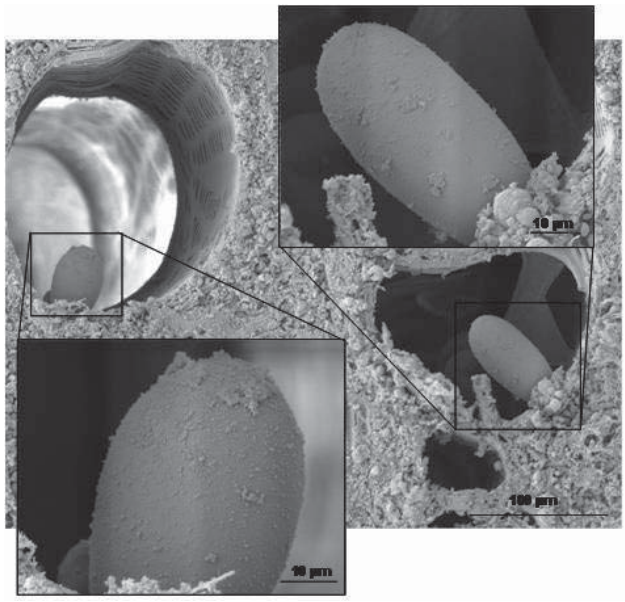

Fig. 5. Representative SEM image of fungal conidia found in xylematic vessels of Gamaret cultivar.

('Arvine'). SEM observations showed many starch granules in most of the cells surrounding the vessels (Fig. 4) and in a few cases there were fungal conidia in the xylem vessels (Fig. 5).

Discussion

The study describes the fungal communities occurring in the wood of five different *V. vinifera* cultivars grown in Switzerland. The fungal community associated with *V. vinifera* generally, and with individual cultivars, is dominated by the ascomycotes, which comprise 87.5% of the 703 fungal isolates obtained in pure culture. Within the ascomycotes, the best-represented class is the Sordariomycetes (55.1% of the ascomycetes isolated), in which numerous isolates belong to the Hypocreales, the Sordariales and the Diaporthales. Although the number of vines sampled was limited (8–12 per cultivar), the results are consistent with the very few studies that have investigated fungal communities in wood (Evans *et al.*, 2003; Giordano *et al.*, 2009). Most other endophyte studies so far have concentrated on the leaves, twigs and branches of plants and reported high proportions of Diaporthales, Dothideales, Helotiales, Pezizales and Xylariales (Arnold *et al.*, 2001, 2007; Hoffman and Arnold, 2008). This pattern is apparently different in the plant stems.

Although the same fungal classes and orders predominate in all cultivars, the composition of OTUs differs between cultivars. Almost 30% of OTUs identified in this study was found in all cultivars. Considering the OTUs isolated in a single cultivar as incidental, approximately half of the species would still not be found in more than two cultivars. These results suggest that different fungal species and genera are associated with the different cultivars. As the plants studied came from different nurseries, and as fungal communities in different soils, and fungal propagules in the air of different areas, are likely to be different, this result is not surprising.

The low occurrence of esca- or Petri disease-associated fungi does not support the findings of Vigues *et al.* (2007), who reported a very high occurrence of *Pa. chlamydospora* in a nursery, while in our study this species was isolated only in a single cultivar ('Chasselas'). These results are however not contradictory since the plants studied in this study and those examined by Vigues *et al.* (2007) came from different nurseries, in which plants could have been contaminated by *Pa. chlamydospora* in varying degrees. Moreover, in our study the plants were de-barked before isolation, a procedure not followed by Vigues and co-authors; *Pa. chlamydospora* may not have been present in the wood but only in the bark. Furthermore *Pa. chlamydospora* is a pretty slow growing fungus, and could have been overgrown by faster growing species.

More isolates were retrieved from the wood than from the pith, while the number of OTUs isolated from these two types of tissue was much the same in all cultivars. This could be explained by the high starch content in the wood cells.

When the number of OTUs isolated from the different parts of the plant (a, graft and grafting point; b, pruning wound; c, basal end) were compared, the number of fungal species and genera isolated from each of the three plant parts in each cultivar were much the same except for 'Gamaret', in which the fungi were mainly found in pruning wounds and the basal ends. This suggests that in most cases, all three plant parts contributed equally to the composition of the fungal communities in the cultivars. In our study, several species were more common in certain tissue or plant parts of a cultivar than in others. For instance, species in the genus "*Botryosphaeria*" are found in different tissues and plant parts of 'Humagne', 'Chasselas' and 'Arvine' vines; *Pa. chlamydospora* is found exclusively in the pith of the rootstock in 'Chasselas', while *Ph. viticola* is isolated only from the wood of the graft in 'Chasselas' and 'Arvine'. These findings are consistent with those reported by other authors. Halleen *et al.* (2001) found several fungal species preferentially associated with a particular plant part in healthy grapevine cuttings. Similarly, Rumbos and Rumbou (2001) reported strong differences in the isolation frequencies of some fungal species between different plant parts of 'Mavrodafni', with "*Botryosphaeria*" spp. isolated from 0.8 to 2.1% in the graft union and the basal end, respectively.

Approximately a quarter of the OTUs identified occurred in more than one plant part, while very few OTUs occurred only in the grafting zone and the rootstock. This suggests that not many OTUs are introduced in the plants through independent inoculations, or that individual species, independently introduced into the rootstock and the graft rapidly colonize (one year in this study) the whole plant. If this last supposition is correct, it would

mean that the transportation of fungal propagules in the xylem vessels is facilitated. To examine this possibility, we examined the proportional xylem lumen and their mean diameter. The mean vessel lumen values range from 6.32E+03 μm^2 ('Chasselas') to 8.4E+03 μm^2 ('Arvine'). Thus the smallest vessel diameter observed in 'Chasselas' would be around 89.7 μm, if the vessels are considered regular round pipes; this would be bigger than the diameter of the fungal spores produced by the species isolated in our study. Our results confirm what reported by Bruno and Sparapano (2007) who found viable fungal conidia and mycelium fragments in the xylem sap of diseased vines.

We confirm the presence of numerous fungal species, several of them reported as *Vitis* pathogens or causing diseases on other plants. Remarkably the number of pathogens is greater in 'Humagne' than in other cultivars. Possible explanations of what found could be: the higher number of isolates in 'Humagne' (and in 'Chasselas'); the greater number of vessels in the xylem zone (*Vl*=47.2%, Table 4) allowing a faster movement of fungi in the plant and an homogenization of the fungal community (greater number of OTUs isolated in all plant parts). Alternatively, since the chemistry of the wood (e.g. tannin content) and the defense responses (e.g. stilbens produced systemically after pathogen attack) differ between cultivars, this may have produced the differences in the growth of different fungi in the vine wood.

Some of the species found in the present study have been reported as being suitable bio-control agents against grapevine pathogens. For instance, *Cl. rosea*, a mycoparasite of several plant-pathogenic fungi including *Botrytis* spp. (Pachenari and Dix, 1980; Yu and Sutton, 1997; Li *et al.*, 2002), was reported as one of a number of promising bio-control agents for *B. cinerea* (Sutton *et al.*, 1997; Köhl *et al.*, 1998; Cotaa *et al.*, 2008; Sutton *et al.*, 2008). *Trichoderma* species have also been reported as good candidates for the bio-control of disease because of their capacity to parasitize pathogens and also to trigger plant defense responses (Dumas and Boyoski, 1992; Yedidia *et al.*, 1999; Carsolio *et al.*, 1999; Zellinger and Omann, 2007). Of the fungi found in *V. vinifera* in this study, therefore, some may play an active role in balancing the fungal community and/or enhancing the host response in order to avoid an excessive growth of pathogenic fungi.

Further research is needed to better understand the role that the most frequently isolated species play in the fungal communities of grapevine. Investigation of endophyte interactions in vine wood is an essential step to counter the wood diseases of grapevine more efficiently, and to select and apply fungal bio-control agents.

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