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# Grapevine trunk diseases in German viticulture. III. Biodiversity and spatial distribution of fungal pathogens in rootstock mother plants and possible relation to leaf symptoms

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## Summary

**Three rootstock mother blocks, planted with cultivars SO4 (planted 2004), 125AA (2005) and 5BB (2005), and located in southwestern Germany were examined for the existence of grapevine trunk disease (GTD) pathogens and related internal and external symptoms between 2011 and 2017. Frequency of leaf symptoms ranged from 0.2 % in six-year old blocks to appr. 1.5 % in 12-year-old blocks. While the typical "tiger stripe pattern" was less common, the majority of affected leaves was characterized by irregularly arranged necrotic spots spread over the leaf surface. Irrespective of leaf symptoms, in cross sections of 9-12 year old vines all sampled trunks (n ≥ 20 for each block) showed the typical GTD symptoms in the wood, with symptoms prevalent in the trunk head compared to the middle section and the basis of trunks. Pathogens were isolated from all trunks, with *Fomitiporia mediterranea* (*Fmed*), *Cadophora luteo-olivacea* (*Clo*), *Phaeomoniella chlamydospora* (*Pch*), *Eutypa lata* (*Elata*), and *Phaeoacremonium aleophilum* (*Pal*) being the most common. Other GTD species included *Cadophora cf. novi-eboraci* (new for German viticulture), *Diaporthe eres, D. nobilis* (new for German viticulture), *D. rudis* (new for German viticulture), *Eutypa laevata* (new for German viticiculture), *Ilyonectria europaea*, *I. liriodendri*, and *Pestalotiopsis* sp. The significance of the once found *Sarocladium strictum* remains unclear. GTD species were revealed from all sampled trunk parts, with maximum diversity and overall frequency in the trunk head. Further species, not related to GTDs, existed in all parts of the trunk. GTD pathogens were also demonstrated for all shoots (two shoots each of ten vines SO4, 125AA and 5BB, with five vines each externally symptomatic and non-symptomatic), but mostly could be detected by molecular means only. *Clo, Pch*, and *Pal* were the predominant species in shoots; further GTD species were *Ilyonectria europaea*, *I. liriodendri*, and *Phaeoacremonium angustius*.**

K e y w o r d s : biodiversity; grapevine trunk diseases; rootstock mother plants; fungal pathogens.

## Introduction

Grapevine trunk diseases (GTDs) affecting young vines and nursery processes include the Esca disease complex (for definition of esca complex diseases see, among others, Surico 2001, 2009), black-foot disease (Agustí-Brisach and Armengol 2013, Agustí-Brisach *et al*. 2013) and Botryosphaeria dieback (reviewed by Bertsch *et al*. 2012). Eutypa dieback, due to several species of diatrypaceous fungi, mostly *Eutypa lata* (*Elata*), is apparent in older vineyards only (Lecomte *et al*. 2000, Rolshausen *et al*. 2014).

Between 25 and 50 millions of graftlings are annually produced by German nurseries (Verband Deutscher Rebenpflanzguterzeuger, pers. comm.). During the bench grafting process the scions, *i.e.* fruit cultivars, by means of matching cuts such as "omega" or "whip" are combined with suitable rootstocks. In viticulture worldwide, the grafting process is often discussed as one of the main reasons for Esca, particularly Petri disease providing entry ports for the associated fungal pathogens by means of open wounds in the wood. For all these diseases, water and air are considered as the main vectors for propagules, i.e. spores/conidia, of the pathogens, which in this way may spread readily within the nursery and between pre-infected and non-infected wood. Rootstock mother plants, grafting tools, water baths, callusing media or soil all have been demonstrated as being potentially contaminated by infectious spores in the past (Rego *et al*. 2001, Whiteman *et al*. 2007, Aroca *et al*. 2006, 2010).

Out of reasons essentially unknown, during the propagation process rootstock wood has been found to be more visually affected than scions, and so emphasis of research always was biased towards the rootstock part. Several potential causal agents have been named in the past, the ones mostly cited the anamorphic fungus *Phaeomoniella chlamydospora* (*Pch*) as well as *Phaeoacremonium aleophilum* (*Pal*). For the latter, *Togninia minima* has been confirmed as the teleomorphic form (Mostert *et al*. 2003; Rooney-Latham *et al*. 2005), and the designation *P. minimum* has been suggested for *Pal* (Gramaje *et al*. 2015). Additional pathogens are discussed in the meantime, with special emphasis on *Cadophora* spp. (Gramaje *et al*. 2009, Travadon *et al*. 2015) or other, partly novel, species of

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*Phaeoacremonium* (Mostert *et al*. 2006, Gramaje *et al*. 2015, Spies *et al*. 2018). For German viticulture, first reports on Petri disease as an emerging disease in young vineyards date back to Fischer and Kassemeyer (2004); over the years, further studies have been conducted by the first author (for instance, see Fischer 2009), but until recently (Fischer *et al*. 2016) results were presented in small scale journals on a regional basis only, mainly directed to the practice side of viticulture. In this study, between 2011 and 2017 three rootstock mother gardens, SO4, 125AA and 5BB, all located in the southwestern region of Germany were monitored for the occurrence of leaf symptoms and the existence of GTD-related fungi in the wood. Samples were taken from i) different sections of trunks of mother plants, and ii) shoots still attached or freshly cut from the mother vines. Examination and assessment of samples was by visual, culturing and molecular means. As a result, new and more comprehensive data are presented with respect to the diversity, the spatial distribution and the frequency of GTD organisms in rootstook mother vines. Besides, several non-GTD fungi were recovered and are reported here for the first time on grapevine.

## Material and Methods

Plantations: Mother gardens comprised the rootstock varieties most common in German viticulture and included cultivars SO4 (planted 2004), 125AA (planted 2005) and 5BB (planted 2005). Mother plants all are raised, *i.e.* with a distinct trunk, and trellised. All blocks are located in the southwestern part of Germany.

Monitoring for leaf symptoms, wood samples from trunks and shoots, and isolation technique (see also Fischer *et al*. 2016): During the period from 2011 to 2017, all blocks were monitored at least twice for leaf symptoms; monitoring was conducted between August and September.

Starting in 2013 mother gardens were repeatedly sampled over the years; with this background, age of plantations during sampling ranged from 9 to 12 years. For sampling of the trunks ($n \geq 20$ for each block, then between nine and twelve years old) wood pieces were taken from visually affected regions in three different cross sections made in: i) the trunk head, ii) the middle section and iii) the basis. Sampled trunks were uprooted in September/October; they were cut into pieces and samples were taken from the above regions. Shoots, i.e. rootstock canes eventually used as propagation material, were sampled at wounds originating from pruning measures or mechanical injuries (both mostly combined with lateral shoots) or hail. Two shoots each of ten plants of SO4, 125AA and 5BB, with five plants each externally symptomatic and non-symptomatic, were examined. Sampling period for shoots was from September through December.

After visual assessment of wood symptoms isolations were made by plating surface sterilised symptomatic grapevine material onto Potato Dextrose Agar (PDA) or malt extract (ME) medium containing 25 $\mu g \cdot mL^{-1}$ chloramphenicol (AppliChem, Darmstadt, Germany; for details see Fischer and Kassemeyer 2003, Cloete *et al*. 2016). Plates were incubated under daylight conditions at appr. 23 °C and were checked once a day for 4 weeks. Hyphal tips of growing colonies were transferred to fresh PDA to obtain pure cultures. Fungal isolates are maintained in the culture collection of the Institute for Plant Protection in Viticulture at the Julius Kühn-Institut, Geilweilerhof, and are stored in tubes at +4 °C conditions.

Molecular studies on fungal pure cultures (see also Fischer *et al*. 2016): Total DNA was extracted from pure cultures as described by Tillett and Neilan (2000). Extracted fungal DNA was subjected to PCR amplification of the ITS region using primers ITS5 and ITS4 (for primer sequences, see White *et al*. 1990). PCR conditions were an initial denaturation for 5 min at 95 °C, followed by 25 cycles at 98 °C for 20 s, 57 °C for 15 s, 72 °C for 20 s and a final extension step for 1 min at 72 °C. Amplification was carried out in a 20 µL volume with the KAPA HiFi™ HotStart PCR Kit (KAPA Biosystems, Wilmington, USA). Reactions contained 1x PCR buffer, 2,0 mM $MgCl_2$, 300 µM of each dNTP, 0,3 µM of each primer, 0,5 U of KAPA HiFi HotStart DNA Polymerase and 1 µL template (20 ng $\mu L^{-1}$). After electrophoretic examination PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Purified PCR products were subsequently sequenced in both directions with primers ITS5 and ITS4 in an Applied Biosystems 3130XL DNA Analyzer using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) at Seqlab (Sequence Laboratories Göttingen, Göttingen, Germany). Assembling and examination of consensus sequences were performed using CLC Main Workbench 7.8.1 (Qiagen Bioinformatics, Qiagen, Aarhus, Denmark). For identification of fungal isolates consensus sequences were analysed with the Basic Local Alignment Tool (BLAST) available from NCBI (National Center for Biotechnology Information, Bethesda, USA), and GenBank numbers were generated for representative isolates of all GTD taxa. Supportive data for identification were derived from microscopy and morphology of pure cultures. For this, fragments of pure cultures were mounted in water and Melzer's reagent and studied at 500x and 1000x under phase contrast optics.

Molecular studies on wood samples (from shoots only): Wood samples of appr. 0.3 x 0.3 x 0.3 cm were taken from the shoots, debarked and surface sterilised by short flaming (Gierl and Fischer 2017). Pieces were ground to a fine powder in a bead mill TissueLyser II (Qiagen, Hilden, Germany) for 20 s at 30 Hz. Total DNA was extracted from 80-100 mg powder using the innuPREP Plant DNA Kit (Analytik Jena AG, Jena, Germany) following the manufacturer's instructions. Identification of species followed the procedure as described above.

## Results

Leaf symptoms in mother blocks (Figs. 1 and 2): Symptomatology was variable

Fig. 1: Eight year old mother vine of rootstock cultivar 5BB. Note the tiger stripe pattern on leaf.

Fig. 2: Eight year old mother vine of rootstock cultivar 5BB. Note the necrotic spots on leaves.

in all blocks, with only the minority of affected leaves showing the typical "tiger stripe pattern" (Fig. 1). Mostly, irregular arranged necrotic spots spread over the leaf surface were apparent (Fig. 2), sometimes accompanied by a distinct wilting of the leaves´ margins. Appearance of symptoms was often inconsistent between shoots of a single plant (Fig. 2; number of shoots per plant usually between two and six to seven). First emergence of symptoms in the blocks was in six year old (5 BB) respectively eight year old plants (125AA, SO4). Appearance of symptoms was inconsistent over the years; that is, symptoms could be apparent in one year and missing in the following. No underlying mechanism, such as climate or number of shoots per plant, was assignable to this phenomenon.

In all blocks, the infestation rate increased i) within the year, with first symptom appearance in July and last emergence of new symptoms in September, and ii) over the years. In 5BB it ranged between 0.2 % (in 2011, then 6 years old) and appr. 1.5 % (in 2017, then 12 years old); in 125AA, it increased from 0.3 % (in 2011, then 6 years old) to 1.6 % (in 2016, then 11 years old), and in SO4 from appr. 0.1 % (in 2012, then 8 years old) it raised to 1.3 % (in 2017, then 13 years old).

Wood symptoms and fungal diversity, including GTD-species new for German viticulture, in mother plants (Figs. 3-6; Tab. 1): Wood symptoms in the trunks were in accordance with symptoms as described from adult vineyard

Fig. 3: Eleven year old mother vine of rootstock cultivar SO4. Discolored vessels, with and without gummosis, are evident in cross section of medial zone of trunk.

Fig. 4: Ten year old mother vine of rootstock cultivar SO4. White rot and gummosis are apparent in cross section of trunk head zone.


Fig. 5: Nine year old mother vine of rootstock cultivar SO4. Mycelial outgrowth assignable to *Fomitiporia mediterranea* is evident in cross section of trunk head.

vines. Discolored vessels, with or without showing signs of gummosis (Fig. 3), and white rot (Fig. 4) were abundant. Upon storage under more humid conditions, mycelial outgrowth assignable to *Fomitiporia mediterranea* (*Fmed*) was regularly observed, even when samples were lacking visible evidence of white rot (Fig. 5).

By means of culturing, a total of 452 isolates representing 41 species were obtained from the samples of trunks and shoots. Out of these, 15 species are considered relevant for GTDs (Tab. 1; mycelial isolates depicted in Fig. 6). With one exception, all species were found in the trunks of mother plants. Only *Cadophora luteo-olivacea* (*Clo*), *Pal*, and *Pch* however could be revealed from all three sampled trunk zones. Others were restricted to the stem head (*Fmed*) or to the basis of trunks (*Ilyonectria*

Table 1

Occurrence of GTD related fungi in trunks and shoots of German rootstock mother plants. Sampled cultivars are 5BB, SO4 and 125AA (n ≥ 20 plants per cultivar)

| Species | trunk zone | | | shoot related to wounds | | Occurrence in rootstock mother plants in other countries |
|---|---|---|---|---|---|---|
| | head | middle | basis | yes | no | |
| *Cadophora luteo-olivacea* | + | + | + | + | + | Halleen *et al*. 2007; South Africa<br>Gramaje *et al*. 2011; Spain. |
| *Cadophora cf. novi-eboraci* [1] | + | + | | | | Not known |
| *Diaporthe eres* | + | | + | | | Cinelli *et al*. 2016; Italy |
| *Diaporthe nobilis* | + | | | | | Not known |
| *Diaporthe rudis* | + | | | | | Not known |
| *Eutypa laevata* | + | + | | | | Not known |
| *Eutypa lata* | + | | + | | | Liminana *et al.* 2009; France |
| *Fomitiporia mediterranea* | + | | | | | Not known |
| *Ilyonectria europaea* | | | + | + | + | Carlucci *et al*. 2017; Italy<br>Agustí-Brisach *et al*. 2013; Spain |
| *Ilyonectria liriodendri* | | | + | + | + | Carlucci *et al*. 2017; Italy<br>Agustí-Brisach *et al*. 2013; Spain |
| *Pestalotiopsis sp.* [1] | + | | | | | Maharachchikumbura *et al*. 2016; France |
| *Phaeoacremonium aleophilum* | + | + | + | + | + | Rego *et al.* 2000; Portugal<br>Edwards *et al*. 2003; Australia.<br>Fourie and Halleen 2004; South Africa<br>Zanzotto *et al*. 2007; Italy |
| *Phaeoacremonium angustius* | + | | | | + | Not known |
| *Phaeomoniella chlamydospora* | + | + | + | + | + | Fourie and Halleen 2002; South Africa<br>Ridgway *et al.* 2002; New Zealand<br>Edwards *et al.* 2003; Australia.<br>Retief *et al*. 2005; South Africa<br>Whiteman *et al*. 2007; New Zealand<br>Zanzotto *et al*. 2007; Italy |
| *Sarocladium strictum* | | | | | + | Not known |

[1:] no unequivocal specific assignment possible based on microscopy, culture characters and ITS sequences as deposited at GenBank.

*spp*.). Seven species also occurred in shoots, both in externally unaffected regions and/or next to injuries due to mechanical measures, such as removing of lateral shoots, or hail. As an exception, *Sarocladium strictum* was demonstrated from shoots only (Tab. 1).

While *Fmed*, *Pch, Pal, Clo*, *Elata* and *Eucasphaeria capensis* have been found in German nursery material, including rootstock mother vines, before (Fischer 2009, Fischer *et al*. 2016, Fischer unpubl. results), all other taxa as mentioned in Tab. 1 are new findings for German rootstock mother vines. These include *Cadophora cf. novi-eboraci*, *Diaporthe eres*, *D. nobilis*, *D. rudis*, *Eutypa laevata*, *Ilyonectria europaea*, *I. liriodendri*, *Pestalotiopsis sp*., *Phaeoacremonium angustius*, and *Sarocladium strictum*. Identification of all these species is based on mycelial isolates followed by molecular measures.

GTD-species spectrum in/on the wood of shoots (Tab. 1): Fungal species on shoots were demonstrated both by isolation of mycelia and/or molecular measures.

Shoots with leaf symptoms: GTD fungi were demonstrated for all plants (n = 15; two shoots each taken from five plants SO4, 125AA and 5BB). A total of seven species was identified, namely *Pch* (from four plants of SO4 and three plants each of 125AA and 5BB), *Pal* (two plants each of SO4 and 5BB, one plant of 125AA), *Clo* (five plants each of SO4, 125AA and 5BB), and *Eucasphaeria capensis* (one shoot of SO4), *Phaeoacremonium angustius* (one shoot of SO4), *Ilyonectria europaea* (one shoot of 5BB), *I. liriodendri* (one shoot of 5BB) and *Sarocladium strictum* (one shoot of 5BB).

Shoots without leaf symptoms: GTD fungi were demonstrated for all plants (n = 15; two shoots each taken from five plants SO4, 125AA and 5BB). Four species were identified, with *Pch* (from three plants each of SO4 and 5BB, one plant of 125AA), *Pal* (three plants of 5BB, one plant of SO4), *Clo* (five plants each for SO4, 125AA and 5BB), and *Sarocladium strictum* (one shoot of 5BB).

Spatial distribution and frequency of GTD fungi isolated from the wood of trunks and possible relation to leaf symptoms in the SO4 mother block – a case study (Tab. 2): In the then 12 year old SO4 mother block 10 plants each with and without leaf symptoms were analyzed by taking wood samples from the head, middle section, and basis of the trunks. GTD-related species were apparent in all trunk zones, with the majori-

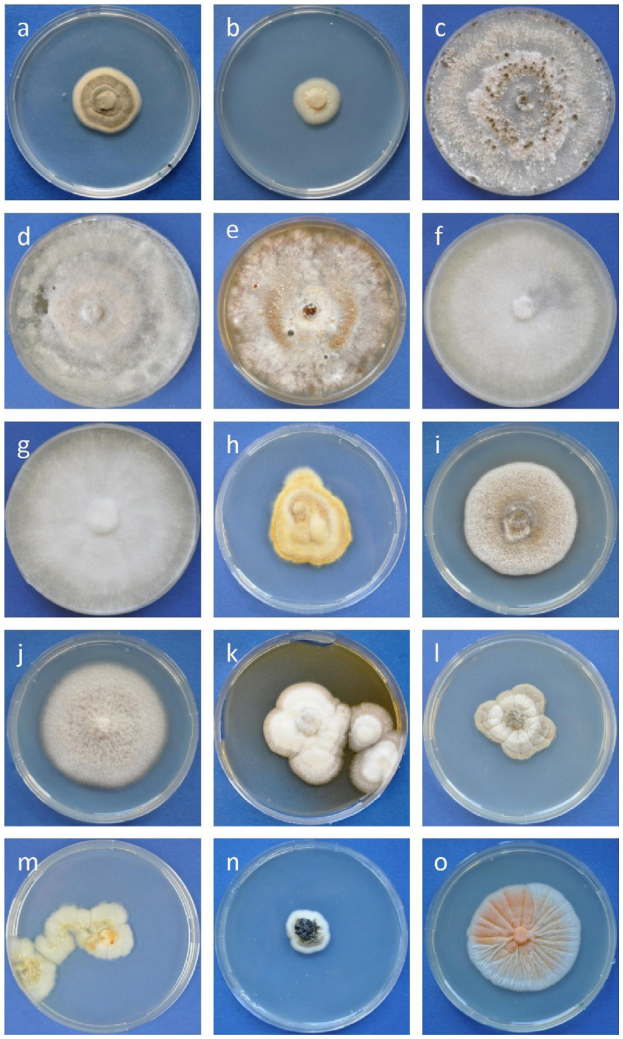


Fig. 6: GTD related fungi and *Sarocladium strictum* derived from rootstock mother plants, cultivars SO4, 125AA and 5BB. Cultures are on PDA after 14 d of incubation at appr. 23 °C under daylight conditions. **a**: *Cadophora luteo-olivacea*; **b**: *Cadophora cf. novi-eboraci*; **c**: *Diaporthe eres*; **d**: *Diaporthe nobilis*; **e**: *Diaporthe rudis*; **f**: *Eutypa laevata*; **g**: *Eutypa lata*; **h**: *Fomitiporia mediterranea*; **i**: *Ilyonectria europaea*; **j**: *Ilyonectria liriodendri*; **k**: *Pestalotiopsis sp.*, **l**: *Phaeoacramonium aleophilum* (after 28 d); **m**: *Phaeoacremonium angustius* (after 28 d); **n**: *Phaeomoniella chlamydospora*; **o**: *Sarocladium strictum*.

ty of diversity and overall frequency in the head (Tab. 2). Both overall number as well as spatial distribution of GTD species inside the trunk were found to have some impact on the development of leaf symptoms: i) trunks with leaf symptoms hosted a higher number of species (ten vs. seven), and ii) more species were isolated from the stem head (nine vs. four).

Mother plants without leaf symptoms (Tab. 2, trunks 1-10): GTD fungi were isolated from all plants and from all trunk zones, with some overlapping of species between zones (see also Tab. 1). In total, seven different species were recovered, with four species each in the trunk head, in the middle section and in the basis. All trunks were infested with *Fmed* (stem head only). *Pch* in seven vines was revealed from the stem head, in three vines from the middle section, and in one vine from the basis. *Pal* appeared in one single vine only, where it was isolated from the stem head and the middle section. Other relevant taxa were *Elata* (in four plants; from stem head and basis), *Clo* (in three plants; from stem head, middle section, and basis), *Cadophora malorum* (in one plant; from middle section), and *Diaporthe eres* (in one plant; from basis).

Non-GTD taxa appeared in different trunk sections and included *Alternaria alternata, Coniochaeta velutina, Cosmospora flavoviridis, Fusarium cf. avenaceum, Heterobasidion annosum, Neofabraea kienholzii*, and *Periconia macrospinosa*.

Mother plants with leaf symptoms (Tab. 2, trunks 11-20): GTD fungi were isolated from all plants, and from all trunk zones, with some overlapping of species between zones (see also Tab. 1).

In total, ten different species were isolated, with nine species in the trunk head, two species in the middle section, and three species in the basis. *Fmed* was isolated from all vines (stem head only). *Pch* in three vines was isolated from the stem head, in three vines from the middle section, and in two vines from the basis. *Pal* in two vines was isolated from the stem head. Other relevant taxa were *Elata* (in one plant; from steam head), *Eutypa laevata* (one plant; from middle section), *Clo* (two plants; from stem head and basis), *C. cf. novi-eboraci* (one plant; from stem head), *D. nobilis* (one plant; from stem head), *D. rudis* (one plant; from stem head), and *Pestalotiopsis* sp. (one plant; from stem head).

Non-GTD taxa were isolated from all trunk sections and included *Eucasphaeria capensis*, *Fusarium cf. avenaceum, Neofabraea kienholzii, Peniophora incarnata*, *Resinicium bicolor*, and *Trichoderma harzianum*.

Notable non-GTD species newly reported for German rootstock mother plants (Tab. 2): These include *Alternaria alternata* (a weak pathogen on different parts of grapevine), *Cosmospora* (*Nectria*) *flavoviridis* (often fungicol, *i.e.* living on other fungi), *Heterobasidion annosum* (important root pathogen on forest and ornamental trees), *Periconia macrospinosa* (a so-called "dark septate endophyte"), *Lophiostoma* (*Massarina*) *corticola* (a widespread saprophyte), *Neofabraea kienholzii* (common in our study; causal agent of "bull´s eye rot" on apple), *Coniochaeta velutina* (putative endophyte and possibly antifugal), *Fusarium cf. avenaceum* (opportunistic weak parasite, also on roots), *Peniophora incarnata* (commonly found on dead wood of grapevine; see Fischer and Kassemeyer 2003), *Resinicium bicolor* (wood decaying on processed wood), and *Trichoderma harzianum* (antifungal and in some products used as a biological antagonist).

## Discussion

Leaf symptoms - rootstock mother plants *vs.* vineyard plants: Apparently, the number of externally symptomatic vines is lower in rootstock mother blocks then it is in vineyards of the same age. This might explain the surprisingly little information that is available about the incidence and frequency of leaf symptoms in such plantations. Information is essentially limited

Table 2

Occurrence of GTD related species (bold letters) and other notable species in different zones of 12 year old trunks of SO4 rootstock mother plants, and possible relation to leaf symptoms in 2016. Ten plants each with and without leaf symptoms were sampled

| Trunk number | Leaf symptoms | Trunk zone | | |
|---|---|---|---|---|
| | | Head | Middle | Basis |
| 1 | - | ***Eutypa lata***<br>***Fomitiporia mediterranea***<br>***Phaeomoniella chlamydospora***<br>*Coniochaeta velutina*<br>*Eucasphaeria capensis* | ***Cadophora luteo-olivacea***<br>***Cadophora cf. novi-eboraci*** [1]<br>***Phaeoacremonium aleophilum*** | |
| 2 | - | ***Fomitiporia mediterranea***<br>*Fusarium cf. avenaceum* [1]<br>*Neofabraea kienholzii* | *Fusarium cf. avenaceum* [1] | |
| 3 | - | ***Fomitiporia mediterranea*** | | ***Diaporthe eres***<br>***Cadophora luteo-olivacea*** |
| 4 | - | ***Fomitiporia mediterranea***<br>***Phaeomoniella chlamydospora*** | *Lophiostoma (Massaria) corticola* | |
| 5 | - | ***Fomitiporia mediterranea***<br>***Phaeomoniella chlamydospora*** | | |
| 6 | - | ***Eutypa lata***<br>***Fomitiporia mediterranea***<br>***Phaeomoniella chlamydospora*** | *Alternaria alternata*<br>*Fusarium cf. avenaceum* [1] | |
| 7 | - | ***Fomitiporia mediterranea***<br>***Phaeomoniella chlamydospora*** | | |
| 8 | - | ***Cadophora luteo-olivacea***<br>***Eutypa lata***<br>***Fomitiporia mediterranea***<br>***Phaeomoniella chlamydospora***<br>*Cosmospora (Nectria) flavoviridis* | ***Phaeomoniella chlamydospora*** | *Alternaria alternata*<br>*Periconia macrospinosa* |
| 9 | - | **Fomitiporia mediterranea**<br>*Cosmospora (Nectria) flavoviridis* | ***Phaeomoniella chlamydospora*** | ***Phaeomoniella chlamydospora*** |
| 10 | - | ***Fomitiporia mediterranea***<br>***Phaeomoniella chlamydospora*** | ***Phaeomoniella chlamydospora*** | ***Eutypa lata***,<br>*Heterobasidion annosum* |
| Summary | | **- four GTD taxa:**<br>***Clo* (in 1 trunk), *Elata* (3);**<br>***Fmed* (10), *Pch* (7).**<br>- all trunks (n=10) GTD-infested<br>- six non-GTD taxa | **- four GTD taxa:**<br>***Clo* (1); *Cad. malorum* (1),**<br>***Pal* (1), *Pch* (3).**<br>- four trunks GTD-infested<br>- three non-GTD taxa | **- four GTD taxa:**<br>***Clo* (1); *Diaporthe eres* (1),**<br>***Elata* (1), *Pch* (1).**<br>- three trunks GTD-infested<br>- three non-GTD taxa |
| 11 | + | ***Fomitiporia mediterranea*** | | |
| 12 | + | ***Diaporthe nobilis***<br>***Fomitiporia mediterranea***<br>***Phaeomoniella chlamydospora***<br>*Neofabraea kienholzii* | | ***Phaeomoniella chlamydospora*** |
| 13 | + | ***Fomitiporia mediterranea*** | | *Resinicium bicolor* |
| 14 | + | ***Cadophora novi-eboraci***<br>***Eutypa lata***<br>***Fomitiporia mediterranea***<br>***Phaeoacremonium aleophilum*** | | |
| 15 | + | ***Fomitiporia mediterranea***<br>***Pestalotiopsis sp.*** [1] | *Neofabraea kienholzii* | ***Phaeomoniella chlamydospora*** |
| 16 | + | ***Fomitiporia mediterranea***<br>*Fusarium avenaceum* [1] | | |
| 17 | + | ***Cadophora luteo-olivacea***<br>***Fomitiporia mediterranea***<br>***Phaeoacremonium aleophilum*** | | ***Cadophora luteo-olivacea***<br>***Phaeoacremonium aleophilum*** |
| 18 | + | ***Fomitiporia mediterranea***<br>***Phaeomoniella chlamydospora*** | ***Phaeomoniella chlamydospora*** | *Neofabraea kienholzii* |
| 19 | + | ***Fomitiporia mediterranea***<br>***Phaeomoniella chlamydospora*** | ***Eutypa laevata***<br>***Phaeomoniella chlamydospora*** | |
| 20 | + | ***Diaporthe rudis***<br>***Fomitiporia mediterranea***<br>*Peniophora incarnata*<br>*Trichoderma harzianum* | ***Phaeomoniella chlamydospora*** | |
| Summary | | **- nine GTD taxa:**<br>***Clo* (1), *Cad. malorum* (1),**<br>***Diaporthe nobilis* (1), *D. rudis***<br>**(1), *Elata* (1), *Fmed* (10), *Pal* (2),**<br>***Pch* (3), *Pestaloptiopsis* sp. (1).**<br>- all trunks (n=10) GTD-infested<br>- three non-GTD taxa | **- two GTD taxa:**<br>***E. laevata* (1), Pch (3).**<br>- three trunks GTD-infested<br>- one non-GTD taxon | **- three GTD taxa:**<br>***Clo* (1), *Pal* (1), *Pch* (2)**<br>- three trunks GTD-infested<br>- two non-GTD taxa |

[1]: no unequivocal specific assignment possible based on microscopy, culture characters and ITS sequences as deposited at GenBank.

to the existence of pathogens in the wood, both demonstrated by isolation and molecular techniques. In this way, Whiteman *et al*. (2007) found an infection rate of > 80 % (n = 100) in mother vines of cultivar 3309 in New Zealand, but don´t give any data on the plantations age and symptomatic status of leaves. Fourie and Halleen (2002) took samples from "symptomless, 1-year-old shoots" from, among others, two mother blocks of 101-14 Mgt (14 years old by time of sampling) respectively Richter 99 (18 years old), but it remains unclear if this is synonymous with a lack of visibly affected mother plants. In the present study, first symptoms rarely but regularly appeared in plantations less than 10 years old; symptom frequency increased over the years and was over 1% in 11-13 years old blocks. With this background, there is some economic loss for the producers, as aberrant vines will be marked during the season eventually leading to an exclusion from further reproduction processes.

Wood symptoms and fungal pathogens - rootstock mother plants vs. vineyard plants: As is well known from adult vineyard plants, also in rootstock mother vines the infestation frequency in the trunks was very high in this study, *i.e.* 100 % for all cultivars. In our blocks (9 to 12 years old by the time of sampling) not a single plant had remained unaffected by GTD pathogens. As a striking contrast, Aroca *et al*. (2010) only in 16.4 % (n = 140 plants, sampled in 2006) respectively 30 % of plants (n = 140, sampled in 2007) found trunk disease pathogens, mostly *Pch* and species of *Phaeoacremonium* and within the *Botryosphaeriaceae*. Age of sampled mother fields is not indicated in Aroca *et al*. (2010). Calzarano and Di Marco (2007) in vineyard plants of 32 and 36 years age found infection rates of appr. 70 % (32 years) and approx. 75 % (36 years), with *Fmed, Pch, Pal*, and *Botryosphaeria obtusa* being the most common species. In none of the above studies, species of *Cadophora* (common in the present study; see also Gramaje *et al*. 2011) or rarer taxa such as *Sarocladium strictum* (Arzanlou *et al*. 2013) were reported.

The symptomatology as well as the fungal spectrum on the large does not differ from that in vineyard plants, with *Fmed*, *Pch* and *Pal* being the most abundant pathogens, followed by *Clo* (the prevailing species in the shoots) and *Elata* (Tab. 2). Out of reasons unknown, not a single species assignable to the Botryosphaeriaceae family was isolated from the mother vines, and this applies both to adult plants and shoots. This finding is in contradiction with former studies carried out in South Africa (Fourie and Halleen 2004) and Spain (Aroca *et al*. 2008, 2010; see above). Also in German vineyards and even on non-*Vitis* hosts several members of the Botryosphaeriaceae have been confirmed recently (Fischer *et al*. 2016, Gierl and Fischer 2017).

White rot was ubiquitous in the stem head, indicating that infections by *Fmed* mostly/exclusively are *via* pruning wounds. The significance of these wounds as main entrance for pathogens also is underlined by the large number of fungal species existing in this particular trunk zone, although this was less evident in plants without leaf symptoms. Only *Pch* and *Clo* were isolated from all three sampled trunk zones, and two different reasons may account for this: i) the particular species may be able to spread readily inside the vascular system eventually leading to a fast colonization of the host plant (for instance, see Edwards *et al*. 2003), and/or ii) infections by these species also are by sucker wounds (Makatini 2014) and/or roots. Existence of *Pch* and *Clo* in the soil has repeatedly proven in the past, both in vineyards (*Pch*: Whiteman *et al*. 2002, Damm and Fourie 2005, *Clo*: Halleen *et al*. 2007, Gramaje *et al.* 2011, Travadon *et al*. 2015) and, for *Clo* only, in orchards (Manici *et al*. 2013).

Mother plants and propagation material as an infection source in viticulture: As one of the main reasons behind the emergence and spread of GTDs including Esca, infected rootstock mother plants and propagation material are discussed to be among the primary inoculum sources (for instance, see Mugnai *et al*. 1999, Halleen *et al*. 2003, Retief *et al*. 2006, Bertsch *et al*. 2012, Fontaine *et al*. 2016). With this background, one would expect to find symptom incidence and severity in the grafted vines to be most obvious next to the cutting wounds and, above all, related to the grafting junction. In the wood of adult vineyard plants however both in the rootstock part and in the grafting junction area symptoms usually are less pronounced and less extended spatially when compared to the scion part, especially the trunk head (Fig. 7 a, b).

While pre-infected rootstock material harbors pathogenic inoculum such as spores and/or mycelial fragments, it merely acts as a source of inoculum during the plant propagation process, where it may infect other, and possibly yet unaffected, cuttings. If this state of infection and colonization alone would eventually lead to the visible outbreak of the disease and to which degree remains an open question. In adult plants taken from the field however, and this is evident from the distribution of symptoms in longitudinal and cross sections of the scion part of trunks, multiple new infection events hit the plant year by year, with the annual pruning wounds acting as main portal. In contrast, symptoms are less obvious in the rootstock part of such plants and this might be due to both i) a reduced number of infection processes and/or ii) a comparatively limited spread of the pathogens in this plant part. It is interesting to note that, as was shown in this study for rootstock mother plants, the biodiversity of related fungi is maximum in the pruning wound zone, with several species – most prominent *Fmed* – being preferably isolated from this part of the host (Tab. 2).

Phytosanitary/preventive measures: No curative treatment is presently known for Esca and related diseases (see overview in Gramaje *et al*. 2018) and so control ideas rest on phytosanitary and preventive measures, such as the usage of the biological control agent, *Trichoderma* (for instance, Di Marco *et al*. 2004, Halleen and Fourie 2016, Mutawila *et al*. 2016). Quite surprisingly, members of this ubiquitous genus only once were recovered in the present study, namely *T. harzianum* in the stemhead of a leaf symptomatic 12-year old SO4, where it co-existed with the GTD pathogens *Fmed* and *Diaporthe rudis* and the saprophytic *Peniophora incarnata*.

Fig. 7: Cross sections of 12 years old *Vitis vinifera* 'Pinot noir'. **a** (left): Rootstock part (125AA); note that wood is essentially free of GTD-related symptoms. **b** (right): Stemhead region; note distinct white rot and wood discoloration due to infection by GTD fungi.

Leaf symptoms in the mother blocks under study here were first noted in 6 year old plantations, with the original infection processes supposedly occurring several years before. All this underlines the significance of a "prior to infection" treatment of pruning wounds with any wound protection product; once the pathogens have invaded the host plant any remedial measures become increasingly difficult. Other preventive measures discussed more recently include electrospun polymers (Buchholz *et al*. 2016) or paints/pastes supplemented or not with fungicides (Rolshausen and Gubler 2005, Sosnowski *et al.* 2008; for an overview, see Gramaje *et al*. 2018). For all this, long term trials in the field, conducted in different viticultural areas and preferably under practice conditions, are still largely lacking.

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## References

Agustí-Brisach, C.; Armengol, J.; 2013: Black-foot disease of grapevine: an update on taxonomy, epidemiology and management strategies. Phytopathol. Mediterr. **52**, 245-261.

Agustí-Brisach, C.; Gramaje, D.; García-Jiménez, J.; Armengol, J.; 2013: Detection of black-foot disease in the grapevine nursery process in Spain. Eur. J. Plant Pathol. **137**, 103-112.

Aroca, Á.; Garcia-Figueres, F.; Bracamonte, L.; Luque, J.; Raposo, R.; 2006: A survey of trunk disease pathogens within rootstocks of grapevines in Spain. Eur. J. Plant Pathol. **115**, 195-202.

Aroca, Á.; Gramaje, D.; Armengol, J.; García-Jiménez, J.; Raposo, R.; 2010: Evaluation of the grapevine nursery propagation process as a source of *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* and occurrence of trunk disease pathogens in rootstock mother vines in Spain. Eur. J. Plant Pathol. **126**, 165-174.

Aroca, Á.; Raposo, R.; Gramaje, D.; Armengol, J.; Martos, S.; Luque, J.; 2008: First report of *Lasiodiplodia theobromae* on rootstock mother grapevines in Spain. Plant Dis. **92**, 832.

Arzanlou, M.; Moshari, S.; Salari, M.; Badali, H.; 2013: Molecular characterisation and pathogenicity of *Phaeoacremonium* spp. associated with esca disease of grapevine in Northern Iran. Arch. Phytopathol. Plant Protect. **46,** 375-388.

Bertsch, C.; Ramírez-Suero, M.; Magnin-Robert, M.; Larignon, P.; Chong, J.; Abou-Mansour, E.; Spagnolo, A.; Clément, C.; Fontaine, F.; 2012: Grapevine trunk diseases: complex and still poorly understood. Plant Pathol. **62**, 243-265.

Buchholz, V.; Molnar, M.; Wang, H.; Reich, S.; Agarwal, S.; Fischer, M.; Greiner, A.; 2016: Protection of vine plants against Esca disease by breathable electrospun antifungal nonwovens. Macromol. Biosci. **16**, 1391-1397.

Calzarano, F.; Di Marco, S.; 2007: Wood discoloration and decay in grapevines with esca proper and their relationship with foliar symptoms. Phytopathol. Mediterr. **46**, 96-101.

Carlucci, A.; Lops, F.; Mostert, L.; Halleen, F.; Raimondo, M. L.; 2017: Occurrence of fungi causing black foot on young grapevines and nursery rootstock plants in Italy. Phytopathol. Mediterr. **56**, 10-39.

Cinelli, T.; Mondello, V; Marchi, G.; Burruano, S.; Alves, A.; Mugnai, L.; 2016: First report of *Diaporthe eres* associated with cane blight of grapevine (*Vitis vinifera*) in Italy. Plant Dis. **100**, 532.

Cloete, M.; Fischer, M.; Mostert, L.; Halleen, F.; 2016: A new species of *Phellinus sensu stricto* associated with esca on grapevine in South Africa. Mycolog. Progr. **15**, 1-9.

Damm, U.; Fourie, P. H.; 2005: Development of a cost-effective protocol for molecular detection of fungal pathogens in soil. S. Afr. J. Sci. **101**, 135-139.

Di Marco, S.; Osti, F.; Cesar, A.; 2004: Experiments on the control of esca by *Trichoderma*. Phytopathol. Mediterr. **43**, 108-115.

Edwards, J.; Pascoe, I. G.; Salib, S.; Laukart, N.; 2003: *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* can spread into grapevine canes from trunks of infected mother vines. Abstracts 3rd Int. Workshop Grapevine Trunk Dis., 29. Lincoln University, Canterbury, New Zealand.

Fischer, M.; 2009: Untersuchungen zu den Übertragungswegen der Esca-Erkrankung im Weinbau und Erarbeitung von Verfahren zur Erzeugung gesunden Rebenpflanzguts. Abschlußbericht zum Forschungsvorhaben BLE-06HS022.

Fischer, M.; Kassemeyer, H. H.; 2003: Fungi associated with Esca disease of grapevine in Germany. Vitis **42**, 109-116.

Fischer, M.; Kassemeyer, H. H.; 2004: Esca - Vorkommen und Symptomatik. Der Deutsche Weinbau, 26-29.

Fischer, M.; Schneider, P.; Kraus, C.; Molnar, M.; Dubois, C.; d´Aguiar, D.; Haag, N.; 2016: Grapevine trunk disease in German viticulture: occurrence of lesser known fungi and first report of *Phaeoacremonium viticola* and *P. fraxinopennsylvanicum*. Vitis **55**, 145-156.

Fontaine, F.; Gramaje, D.; Armengol, J.; Smart, R.; Nagy, Z.A.; Borgo, M.; Rego, C.; Corio-Costet, M. F.; 2016: Grapevine trunk diseases. A review. OIV Publ., Paris.

Fourie, P. H.; Halleen, F.; 2002: Investigation on the occurrence of *Phaeomoniella chlamydospora* in canes of rootstock mother vines. Australas. Plant Pathol. **31**, 425-426.

Fourie, P. H.; Halleen, F.; 2004: Occurrence of grapevine trunk disease pathogens in rootstock mother plants in South Africa. Australas. Plant Pathol. **33**, 313-315.

Gierl, L.; Fischer, M.; 2017. Grapevine trunk disease in German viticulture II. Associated fungi occurring on non-*Vitis* hosts, and first report of *Phaeoacremonium angustius*. Vitis **56**, 103-110.

Gramaje, D.; Muñoz, R. M.; Lerma, M. L.; García-Jiménez, J.; Armengol, J.; 2009: Fungal grapevine trunk pathogens associated with Syrah decline in Spain. Phytopathol. Mediterr. **48**, 396-402.

Gramaje, D.; Mostert, L.; Armengol, J.; 2011: Characterization of *Cadophora luteo-olivacea* and *C. melinii* isolates obtained from grapevines and environmental samples from grapevine nurseries in Spain. Phytopathol. Mediterr. **50**, 112-126.

Gramaje, D.; Mostert, L.; Groenewald, J.Z.; Crous P.W.; 2015: *Phaeoacremonium*: From esca disease to phaeohyphomycosis. Fungal Biol. **119**, 759-783.

Gramaje, D.; Úrbez-Torres, J. R.; Sosnowski, M. R.; 2018: Managing grapevine trunk diseases with respect to etiology and epidemiology: current strategies and future prospects. Plant Dis. **102**, 12-39.

Halleen, F.; Crous, P.W.; Petrini, O.; 2003: Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. Australas. Plant Pathol. **32**, 47-52.

Halleen, F.; Fourie, P. H.; 2016: An integrated strategy for the pro-active management of grapevine trunk disease pathogen infections in grapevine nurseries. S. Afr. J. Enol. Vitic. **37**, 104-114.

Halleen, F.; Mostert, L.; Crous, P.; 2007: Pathogenicity testing of lesser-known vascular fungi of grapevine. Australas. Plant Pathol. **36**, 277-285.

Lecomte, P.; Peros, J. P.; Blancard, D.; Bastien, N.; Délye, C.; 2000: PCR assays that identify the grapevine dieback fungus *Eutypa lata*. Appl. Environ. Microbiol. **66**, 4475-4480.

Liminana, J. M.; Pacreau, G.; Boureau, F.; Menard, E.; David, S.; Himonnet, C.; Fermaud, M.; Goutouly, J. P.; Lecomte, P.; Dumot, V.; 2009: Inner necrosis in grapevine rootstock mother plants in the Cognac area (Charentes, France). Phytopathol. Mediterr. **48**, 92-100.

Maharachchikumbura, S. S. N.; Larignon, P.; Hyde, K. D.; Al-Sadi, A. M.; Liu, Z.; 2016: Characterization of *Neopestalotiopsis*, *Pestalotiopsis* and *Truncatella* species associated with grapevine trunk disease in France. Phytopathol. Mediterr. **55**, 380-390.

Makatini, G. J.; 2014: The Role of Sucker Wounds as Portals for Grapevine Trunk Pathogen Infections. MSc Thesis, University of Stellenbosch, South Africa.

Manici, L. M.; Kelderer, M.; Franke-Whittle, I. H.; Rühmer, T.; Baab, E.; Nicoletti, F.; Caputo, F.; Topp, A.; Insam, H.; Naef, A.; 2013: Relationship between root-endophytic microbial communities and replant disease in spezialised apple growing areas in Europe. Appl. Soil Ecol. **72**, 207-214.

Mostert, L.; Crous, P. W.; Groenewald, J.; Gams, W.; Summerbell, R. C.; 2003: *Togninia* (Calosphaeriales) is confirmed as the teleomorph of *Phaeoacremonium* by means of morphology, sexual compatibility and DNA phylogeny. Mycologia **95**, 646-659.

Mostert, L.; Halleen, F.; Fourie, P.; Crous, P.W.; 2006: A review of *Phaeoacremonium* species involved in Petri disease and esca of grapevines. Phytopathol. Mediterr. **45S**, 12-29.

Mugnai, L.; Graniti, A.; Surico, G.; 1999: Esca (Black Measles) and Brown Wood-Streaking: Two old and elusive diseases of grapevines. Plant Dis. **83**, 404-418.

Mutawila, C.; Vinale, F.; Halleen, F.; Lorito, M.; Mostert, L.; 2016: Isolation, production and *in vitro* effects of the major secondary metabolite produced by *Trichoderma* species used for the control of grapevine trunk diseases. Plant Pathol. **65**, 104-113.

Rego, C.; Carvalho, A.; Nascimento, T.; Oliveira, H.; 2001: First approach on the understanding of inoculum sources of *Cylindrocarpon destructans* and *Phaeomoniella chlamydospora* concerning grapevine rootstocks in Portugal. IOBC/WPRS Bull. **24**, 67-72.

Rego, C.; Oliveira, H.; Carvalho, A.; Phillips, A.J.L.; 2000: Involvement of *Phaeoacremonium* spp. and *Cylindrocarpon destructans* with grapevine decline in Portugal. Phytopathol. Mediterr. **39**, 76-79.

Retief, E.; Damm, U.; van Niekerk, J.; Mcleod, A.; Fourie, P.; 2005: A protocol for molecular detection of *Phaeomoniella chlamydospora* in grapevine wood. S. Afr. J. Sci. **101**, 139-142.

Retief, E.; McLeod, A.; Fourie, P. H.; 2006: Potential inoculum sources of *Phaeomoniella chlamydospora* in South African grapevine nurseries. Eur. J. Plant Pathol. **115**, 331-339.

Ridgway, H. J.; Sleight, B. E.; Stewart, A.; 2002: Molecular evidence for the presence of *Phaeomoniella chlamydospora* in New Zealand nurseries, and its detection in rootstock mother vines using species-specific PCR. Australas. Plant Pathol. **31**, 267-271.

Rolshausen, P. E.; Baumgartner, K.; Travadon, R.; Fujiyoshi, P.; Pouzoulet, J.; Wilcox, W. F.; 2014: Identification of *Eutypa* spp. causing Eutypa dieback of grapevine in Eastern North America. Plant Dis. **98**, 483-491.

Rolshausen, P.; Gubler, D.; 2005: Use of boron for the control of Eutypa dieback of grapevines. Plant Dis. **89**, 734-738.

Rooney-Latham, S.; Eskalen, A.; Gubler, W.D.; 2005: Teleomorph formation of *Phaeoacremonium aleophilum*, cause of esca and grapevine decline in California. Plant Dis. **89**, 177-184.

Sosnowski, M. R.; Creaser, M. L.; Wicks, T. J.; Lardner, R.; Scott, E.; 2008: Protection of grapevine pruning wounds from infection by *Eutypa lata*. Aust. J. Grape. Wine Res. **14**, 134-142.

Spies, C. F. J.; Moyo, P.; Halleen, F.; Mostert, L.; 2018: *Phaeoacremonium* species diversity on woody hosts in the Western Cape Province of South Africa. Persoonia **40**, 26-62.

Surico, G.; 2001: Towards commonly agreed answers to some basic questions on esca. Phytopathol. Mediterr. **40**, S487-490.

Surico, G.; 2009: Towards a redefinition of the diseases within the esca complex of grapevine. Phytopathol. Mediterr. **48**, 5-10.

Tillett, D.; Neilan, B. A.; 2000: Xanthogenate nucleic acid isolation from cultured and environmental cyanobacteria. J. Phycol. **36**, 251-258.

Travadon, R.; Lawrence, D. P.; Rooney-Latham, S.; Gubler, W. D.; Wilcox, W. F.; Rolshausen, P. E.; Baumgartner, K.; 2015: *Cadophora* species associated with wood-decay of grapevine in North America. Fungal Biol. **119**, 53-66.

White, T. J.; Bruns, T. D.; Lee, S.; Taylor, J. W.; 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White (Eds): PCR protocols, 315-322. Academic Press, San Diego, U.S.A.

Whiteman, S. A.; Jaspers, M. V.; Stewart, A.; Ridgway, H. J.; 2002: Detection of *Phaeomoniella chlamydospora* in soil using species-specific PCR. N. Z. Plant Protect. **55**, 139-145.

Whiteman, S. A.; Steward, A.; Ridgway, H. J.; Jaspers, M. V.; 2007: Infection of rootstock mother-vines by *Phaeomoniella chlamydospora* results in infected young grapevines. Australas. Plant Pathol. **36**, 198-203.

Zanzotto, A.; Autiero, F.; Bellotto, D.; Dal Cortivo, G.; Lucchetta, G.; Borgo, M.; 2007: Occurrence of *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* in grape propagation materials and young grapevines. Eur. J. Plant Pathol. **119**, 183-192.

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