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Grapevine trunk disease in German viticulture II. Associated fungi occurring on non-*Vitis* hosts, and first report of *Phaeoacremonium angustius*

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Summary

Fifteen species of wood colonizing fungi are presented that have been collected from various non-*Vitis* hosts in the vicinity of vineyards located in southern Palatinate, Germany. Information is provided on their geographic distribution, epidemiology, host range, life strategy, symptoms and diagnosis. Their role as possible pathogens within the complex of grapevine trunk diseases (GTDs) is discussed. The following species are reported for the first time in Germany: *Botryosphaeria sarmentorum, Cadophora malorum, Cadophora novi-eboraci, Collophora africana, Collophora hispanica, Cytospora chrysosperma, Diaporthe foeniculina, Dothiorella iberica,* and *Phaeoacremonium angustius*. *Diplodia seriata, Diplodia mutila, Dothiorella iberica, Cytospora chrysosperma, and Dothiorella iberica* were proven by airborne inoculum, and could be demonstrated throughout the duration of our study, *i.e.* from March through September. The study points to a possible significance of non-*Vitis* hosts as additional inoculum source in GTDs. Also, the existence of airborne spores early in the year might be relevant with regard to the pruning period of vines.

K e y w o r d s : fungal pathogens; grapevine trunk diseases; host range; inoculum source; molecular diagnosis.

Introduction

Xylematic parts of the vascular bundles of flowering plants may be affected by so-called tracheomycosis: fungal pathogens are able to invade the vessels and, subsequently, adjacent cells. By plugging of the vessels and formation of mycotoxins diseases may be developed, resulting in phenomena such as wilt or dieback (Surico *et al*. 2008, Andolfi *et al*. 2011, Pouzoulet *et al*. 2014). Besides Esca and related Grapevine Trunk Diseases (GTDs) prominent examples of tracheomycosis are Dutch elm disease, with *Ceratocystis (novo-) ulmi* as causal agent, *Verticillium* wilt, caused by *Verticillium dahliae* and/or *V. albo-atrum*, or Coffee wilt disease, due to *Giberella (Fusarium) xylarioides*. While well adapted to a highly specific environment *in planta* these pathogens show a vast diversity of adaptation in relation to host range, which ranges from strictly specific (*G. xylarioides*; Rutherford 2006) to more or less non-specific (*V. dahliae, V. albo-atrum*; Pegg and Brady 2002).

After 20 years of intensive studies, Esca related pathogens *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. are known from a variety of host plants, with *Vitis vinifera* considered the main host (Mugnai *et al*. 1999, Bertsch *et al*. 2013, Gramaje *et al*. 2015, overview under http://nt.ars-grin.gov/fungaldatabases/). Number of studies on possible non-*Vitis* hosts for these particular pathogens however is restricted and is often limited to certain geographic areas (Damm *et al*. 2008, Cloete *et al*. 2011, Úrbez-Torres *et al*. 2013, Sami *et al*. 2014, Moyo *et al*. 2016). With respect to other GTD related fungi (for an overview, see Bertsch *et al*. 2013; for German viticulture, see Fischer *et al*. 2016) this lack of information is even more evident. However, the possible occurrence of these pathogens on non-*Vitis* hosts is relevant in different aspects: i) they may provide an additional source of inoculum, putting extra infection pressure on neighboring vineyards; ii) both external and internal symptoms might be developed on the non-*Vitis*-hosts and might lead to better understanding of host-pathogen relationships, and, iii) pathogens might be identified previously not or only rarely detected for German viticulture, in this way inducing further steps to studying their significance and related control measures.

In conformity with the previous study (Fischer *et al*. 2016) obtained fungal isolates both from wood samples and spore traps were identified by culturing on artificial medium. Obtained pure cultures were roughly classified by microscopic examination followed by molecular based analyses. Fifteen taxa were selected on the basis of their putative association with GTDs and are presented in more detail with regard to pathogenic significance, geographic distribution, epidemiology, host range, life strategy and symptoms as well as diagnosis. Cultural phenotypes are illustrated by photographs.

Material and Methods

W o o d s a m p l e s a n d c u l t u r i n g : Sampling was done from March through August 2016 in the southern Palatinate area of Germany based on a selection of de-

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ciduous trees, with one – two specimens each of *Corylus avellana, Cydonia oblonga, Ficus carica, Juglans regia, Malus domestica, Prunus armeniaca, P. cerasus, P. domestica, P. dulcis, P. persica, P. spinosa,* and *Pyrus communis* (see also the Table). Trees were all living and standing, and were located in the proximity of vineyards. Estimated age of trees was between 20 and 50 years. Each host was sampled three times, in March, May, and August. Isolation sites comprised wood lesions and/or sections of necrotic wood on different major branches. Only in *Cydonia*, *Malus* and *Prunus domestica*, each with a minimum age of 40 years, some signs of external decline were notable.

Isolations were made by plating surface sterilised symptomatic material onto Potato Dextrose Agar (PDA) or malt extract (ME) medium (for details see Fischer and Kassemeyer 2003, Cloete *et al*. 2016). Dishes were incubated at 25 °C under permanent dark conditions. Fungal growth was checked once a day; resulting pure colonies were transferred onto separate Petri dishes containing PDA. Fungal isolates are maintained in the culture collection of the Institute for Plant Protection in Viticulture at the Julius Kühn Institute, Geilweilerhof, and are stored in tubes at +4 °C conditions. Growth studies and documentation of mycelia were conducted on Petri dishes (9.5 cm in diam.) containing PDA at appr. 23 °C under daylight conditions. For first-hand classification, pieces of cultured mycelia were mounted in water or Melzer's reagent and studied at 500x and 1000x under phase contrast optics using a Leica DM 750 microscope.

Spore trapping: Spore trapping was conducted from March through September in 2016, with one trap each placed in the above selection of suspected host plants (Table; see also Fischer *et al*. 2016). Traps consisted of glass slides covered with Vaseline (Balea, DM Germany) affixed on branches next to externally affected wood. Slides were exchanged on a weekly basis. Sampling of the spore traps was based on the filter method described by Eskalen and Gubler (2001). Obtained cultures were processed as described above.

DNA extraction, PCR and sequencing: Whole cell DNA was isolated from cultured mycelium as described by Tillett and Neilan (2000). Prior to DNA extraction, cultures were grown on PDA at appr. 23 °C under daylight conditions. Quantity and quality of the DNA were examined using a Spectrophotometer (Nanodrop 2000c, Thermo Scientific, Waltham, USA). Isolated DNA was diluted to a final concentration of maximum 100 $ng \cdot \mu L^{-1}$ in distilled water. The polymerase chain reaction (PCR) was used to amplify a portion of the nuclear encoded ribosomal DNA unit defined by the primer combination prITS5 and prITS4 (for primer sequences, see White *et al*. 1990). The fragment spans the entire ITS1 region, the 5.8S rRNA gene, and the ITS2 region. The PCR reactions were set up in 20 µL volumes. Hot start PCR was applied throughout using a KAPAHiFi™ Hot Start Polymerase (PEQLAB Biotechnologie GmbH, Erlangen, Germany): twenty five cycles were performed on a SimpliAmp™ Thermal Cycler (Applied Biosystems, Darmstadt, Germany) using the following parameters: 95 °C initial denaturation step (5 min), 98 °C denaturation step (20 sec), 53 °C annealing step (15 sec), 72 °C primer extension (20 sec). A final incubation step at 72 °C (1 min) was added after the final cycle. Five µL of each PCR reaction were mixed with 2 µL of 6x loading dye and were electrophoresed on 1.5 % agarose gels. A 100 bp+ DNA ladder (PEQLAB Biotechnologie GmbH, Erlangen, Germany) was used as standard. The amplified products were purified with the QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA was suspended in 30 µL Tris-HCl buffer (10 mM, pH 8.5). PCR primers, i.e. prITS5 and prITS4, were used for sequencing. Cleaned products were sequenced using ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA, USA). Products were then analyzed on an ABI Prism 3130XL DNA sequencer (Perkin-Elmer, Norwalk, CN, USA). Sequences were processed as described before (Cloete *et al.* 2016); one each per taxon was deposited at GenBank (www.ncbi.nlm.nih.gov/genbank/).

Results

General remarks: A total of 73 taxa were obtained during our sampling period, *i.e.* March through September 2016. With exception of the white rot fungi *Antrodiella hoehnelii*, *Bjerkandera adusta*, *Phellinus tuberculosus* and *Trametes hirsuta* (all Basidiomycetes) only members of the Ascomycetes were revealed, many of them classified as endophytes and therefore not included in our list of disease related fungi. In the end we selected fifteen disease relevant fungi whose connection with the sampled plant species specimen is summarized in the Table. The Table also contains the species *Collophora paarla* and *Leucostoma persoonii,* but these have been treated in the former study (Fischer *et al*. 2016). *Paraconiotyrium fuckelii* (Verkley *et al*. 2014) is omitted due to uncertain origin of isolate. GTD candidates were isolated from seven out of twelve plant species, namely *Corylus avellana* (fungal isolate from spore trap), *Cydonia oblonga* (wood), *Ficus carica* (wood), *Malus domestica* (spore trap), *Prunus armeniaca* (wood, spore trap), *Prunus spinosa* (wood, spore trap), and *Prunus dulcis* (wood). Pathogens were also derived from *Prunus cerasus*, *P. domestica* and *P. persica* (see Damm *et al.* 2008, for further information on *Prunus* related pathogens). No disease relevant fungi were obtained from *Pyrus*, probably due to incomplete sampling (one wooden sample only taken from this particular host). To our knowledge some of the presented fungi, such as species of *Collophora*, have not been reported from *Vitis*. However, corresponding sampling sites in the wood showed clear similarity to GTDs such as brown wood streaking and so we decided to include them in our list.

Airborne inoculum: Out of the taxa considered relevant for GTDs, *Diplodia seriata, Diplodia mutila, Dothiorella iberica,* and *Cytospora chrysosperma* were demonstrated by spore traps, and throughout first proof was as early as calendar week 11, *i.e.* before middle of March. All these species were recorded throughout the whole study, from March through August.

Table

Grapevine Trunk Diseases (GTDs) in Germany: selected fungal species related to plant diseases isolated from different host plants during 2016. Fungi discussed in the literature as being associated with GTDs are in bold letters

Host plant	Trivial name	Selected fungi associated with plant diseases (species in **bold letters** discussed as pathogens in GTDs)
Malus domestica	apple	*Cadophora malorum* (W[1]) ***Cadophora novi-eboraci*** (W) ***Diaporthe eres*** (W) ***Diplodia seriata*** (S)
Cydonia oblonga	quint	*Botryosphaeria sarmentorum* (S) ***Eutypa lata*** (W) ***Phaeoacremonium angustius*** (W)
Ficus carica	fig	*Aureobasidium pullulans* (S) ***Diaporthe foeniculina*** (W)
Prunus armeniaca	apricot	*Aureobasidium pullulans* (S) *Collophora africana* (S) *Collophora hispanica* (W) ***Diplodia mutila*** (W, S) ***Diplodia seriata*** (W) ***Dothiorella iberica*** (S) *Leucostoma persoonii* (S)
Prunus spinosa	blackthorn	***Cytospora chrysosperma*** (W, S)
Prunus domestica	mirabelle	*Leucostoma persoonii* (W)
Prunus dulcis	almond	*Collophora africana* (W) ***Stereum hirsutum*** (W)
Prunus persica	peach	*Collophora paarla* (W) *Leucostoma persoonii* (W)
Prunus cerasus	cherry	*Collophora paarla* (W)
Juglans regia[2]	walnut	*Paraconiothyrium fuckelii* (W)
Corylus avellana	hazelnut	***Dothiorella iberica*** (S)
Pyrus communis	pear	*Aureobasidium pullulans* (S, W)

[1] origin of isolate: W = wood sample; S = spore trap
[2] removed branch lying on the ground next to tree of *Juglans regia*

Annotated list of species:

Aureobasidium pullulans (De Bary) G. Arnaud ex Cif., Ribaldi & Corte: The ubiquitous species was often recovered from spore traps, from *Ficus carica*, *Prunus armeniaca*, and *Pyrus communis*, and once from wood, from the latter host. For spore traps, occurrence was throughout the sampling period, from March through September. Recently, we have confirmed *A. pullulans* from wood, spore traps and xylem sap in German vineyards (Fischer *et al*. 2016) of grapevine; existence on *Ficus*, *Prunus* and *Pyrus* is new for Germany, but has been reported from other countries (http://nt.ars-grin.gov/fungaldatabases/). Colony morphology: Figure, a. See also Fischer *et al*. 2016. ITS 5-4 sequence: GenBank accession KX034050.

Botryosphaeria sarmentorum A.J.L. Phillips, J. Luque & A. Alves: This is the first report of this species in Germany, from any host. Our single collection is from a spore trap on quint, *Cydonia oblonga*, in March. In the original literature it has been described from several deciduous trees, including *Malus*, *Prunus* or *Pyrus* (Phillips *et al*. 2005; see also Phillips *et al*. 2013, as *Dothiorella sarmentorum*). Occurrence of the species on grapevine is not confirmed yet, even though it is regarded as plurivorous and cosmopolitan by Phillips *et al*. (2013). In general, species of *Botryosphaeria* are considered as saprophytes, pathogens or endophytes on a variety of hosts, including *Vitis* (Larignon *et al*. 2001, Phillips *et al*. 2013). Colony morphology: Figure, b. ITS 5-4 sequence: GenBank accession KY556683.

Cadophora malorum (Kidd & Beaumont) W. Gams: While *Cadophora* as a pathogen has gained increasing interest in the last few years, relatively little is known about its diversity and exact role in GTDs (see Travadon *et al*. 2015 for an overview). In our recent study (Fischer *et al*. 2016) we recovered *C. luteo-olivacea* and *C. fastigiata*, both being most prominent in nurseries. The lesser known *Cadophora malorum* (Gams 2000) had a "first report" on kiwi plants in Chile recently, where it was found to be associated with cordon dieback (Diaz *et al*. 2016; see also Prodi *et al*. 2008). On apples and pears, the species causes the so-called side rot (Spadaro *et al*. 2011). In our studies we isolated it from a necrotic branch of a 40 years plus apple tree located in close proximity to vineyards. Recently, in an experiment with apple plants

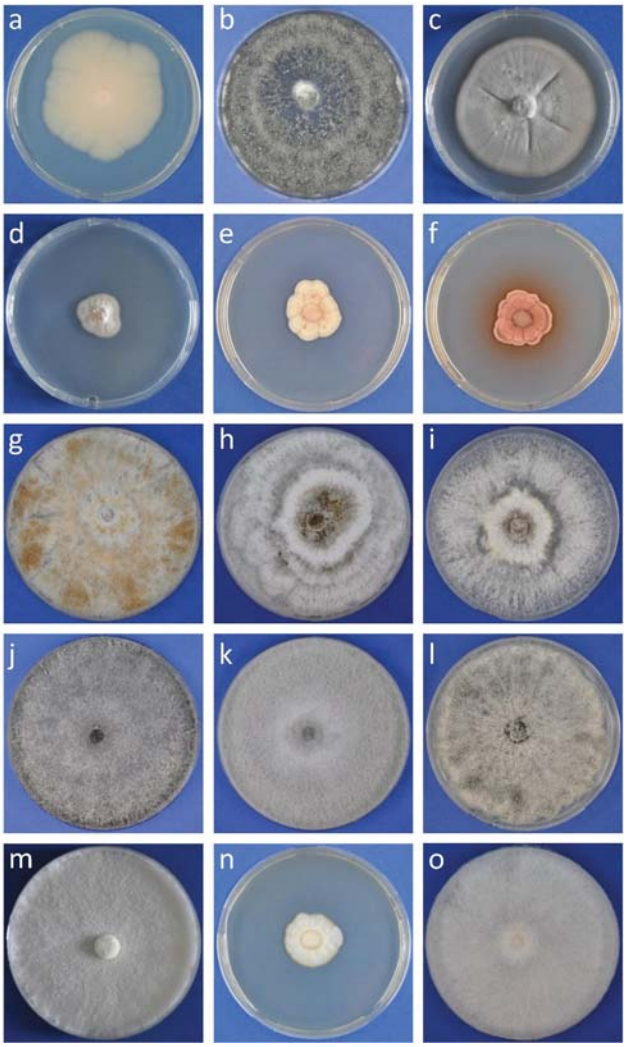

Figure: Fungi derived from non-*Vitis* hosts in Germany. Species in **bold letters** are discussed as related to grapevine trunk diseases. Cultures are on PDA after 14 d of incubation at approx. 23 °C under daylight conditions. **a**: *Aureobasidium pullulans*; **b**: *Botryosphaeria sarmentorum*; **c**: *Cadophora malorum*; **d**: ***Cadophora novi-eboraci***; **e**: *Collophora africana*; **f**: *Collophora hispanica*; **g**: ***Cytospora chrysosperma***; **h**: ***Diaporthe eres***; **i**: *Diaporthe foeniculina*; **j**: ***Diplodia mutila***; **k**: ***Diplodia seriata***; **l**: ***Dothiorella iberica***; **m**: ***Eutypa lata***; **n**: *Phaeoacremonium angustius*; **o**: ***Stereum hirsutum.***

grown in soil of replant diseased orchards from Northern Germany, the species has also been obtained from roots (Popp and Grunewaldt-Stöcker, University of Hannover, pers. comm.). With this background it seems worth wile to investigate root systems for the recovery of *C. malorum* as well. Association of *C. malorum* with grapevine in Germany is probable but needs further documentation (Haag and Fischer, in prep.). Colony morphology: Figure, c. ITS 5-4 sequence: GenBank accession KY549800.

Cadophora novi-eboraci Travadon, Lawrence, Rooney-Latham, Gubler, Wilcox, Rolshausen & K. aumgartner: The species has been described very recently, based on isolates collected from wood canker of *Vitis labruscana* 'Concord' in the U.S.A. (Travadon *et al*. 2015). Another isolate of the species has been derived from discoloured wood of the kiwi fruit tree, *Actinidia chinensis*, in Italy. Our finding, from necrotic wood of *Malus domestica*, is the first finding worldwide from this particular host, and the first finding from any host in Germany. Interestingly, *C. novi-eboraci* in our study was found on the same tree as the above mentioned *C. malorum*. Sampling sites in the wood however were different. As with *C. malorum* association with grapevine is probable (Haag and Fischer, in prep.). Colony morphology: Figure, d. ITS 5-4 sequence: GenBank accession KY549801.

Collophora africana Damm & Crous; *Collophora hispanica* Gramaje, Armengol & Damm: These are two more representatives of *Collophora* detected for the first time in Germany (for first report of *C. paarla*, see Fischer *et al*. 2016). In accordance with the symptoms described in the original literature (Damm *et al*. 2008) we isolated both species from discolored wood lesions on branches of *Prunus* spp. Occurrence on *Vitis* in Germany, and anywhere, has not been confirmed so far. Colony morphology: Figure, e (*C. africana*) and f (*C. hispanica*). ITS 5-4 sequence: GenBank accession KY556684 (*C. africana*); KY556685 (*C. hispanica*).

Cytospora chrysosperma (Pers.) Fr.: The multi-species genus *Cytospora* (appr. 110 species presently acknowledged) comprises endophytes (Gonzalez and Tello 2011), saprophytes and destructive canker pathogens (Adams *et al*. 2005, Lawrence *et al*. 2016) on numerous woody plant species, mostly species of *Populus*, *Prunus* or *Malus*. As one of the related symptoms of *Cytospora* pathogens a loss of hydraulic conductivity within the xylem has been mentioned (Arzanlou and Narmani 2015, Lawrence *et al*. 2016).

Cytospora chrysosperma (with the teleomorph, *Valsa sordita*) is considered as a cosmopolitan species and has been classified both as endophyte (Gonzales and Tello 2011) and pathogen (Arzanlou and Narmani 2015, Lawrence *et al*. 2016). Related species, such as the newly described *C. vinacea* and *C. viticola* (Lawrence *et al*. 2016), were isolated from wood cankers of grapevine, and, based on pathogenicity tests, were classified as possible pathogens and they may act in synergy with other genera of wood-colonizing fungi within the GTD complex.

To our best of knowledge, our collection from necrotic wood of *Prunus spinosa* is the first report from this host plant worldwide. It was also repeatedly isolated from a spore trap on this host, from March through September. Findings from grapevine exist in Spain (Gonzalez and Tello 2011, Garcia Benavides *et al*. 2013; in the latter case associated with the borer, *Xylotrechus arvicola*), Iran (Arzanlou and Narmani 2015) and the U.S.A. (Lawrence *et al*. 2016). It was not detected in our previous survey (Fischer *et al*. 2016). Colony morphology: Figure, g. ITS 5-4 sequence: GenBank accession KY549802.

Diaporthe eres Nitschke: *Diaporthe*, with the anamorphs within the well known *Phomopsis*, is a cosmopolitan genus inhabiting a wide range of host plants, some of them economically important. *Diaporthe eres* has been documented worldwide, including Germany, from a variety of deciduous plants (Wehmeyer 1933). Occurrence on grapevine however has been established only recently (Casieri *et al*. 2009 for Switzerland, Kaliterna *et al*. 2013

for Croatia, Cinelli *et al*. 2016 for Italy); the species was found to be related to "diseased wood" and in pathogenicity trials was assessed as "a moderate pathogen" (Kaliterna *et al*. 2013), while cane blight was assigned to the species in the Italian studies.

Our specimen was isolated from a branch of a *Malus domestica* tree (close to 50 years old) and to our knowledge represents the first finding from this host in Germany. Isolation site was next to an extended lesion, affected wood was dark and clearly separated from the surrounding tissue.

A detailed description of the species is provided in Gomes *et al*. (2013). Colony morphology: Figure, h. ITS 5-4 sequence: GenBank accession KY549803.

Diaporthe foeniculina Niessl: This cosmopolitan species occurs on many deciduous plants, both annual and perennial (Gomes *et al*. 2013). Interestingly, the original description has been made from the well known herb, fennel (*Foeniculum vulgare*). *Vitis vinifera* has been mentioned as host plant of *D. foeniculina* by Udayanga *et al*. in South Africa (2014) and Lawrence *et al*. in California (2015). Statements on the exact pathogenic significance are lacking though.

Our findings from a branch lesion on fig (*Ficus carica*, appr. 20 years old) resulted in two mycelial colony types (the faster growing phenotype, also characterized by strong development of aerial mycelium, is depicted in Figure i), even though ITS sequences turned out to be identical. They represent the first report of *D. foeniculina* from fig worldwide and are the first ones from any host in Germany. Existence on grapevine in Europe remains uncertain at the moment. Colony morphology: Figure, i. ITS 5-4 sequence: GenBank accession KY549796.

Diplodia mutila (Fr.) Mont: The well known *Diplodia mutila* (with the teleomorphic state *Botryosphaeria stevensii*) was shown to cause the "Black Dead Arm" disease of grapevine already in 1974 (Lehoczky 1974). Since then, several other species of *Botryosphaeria* were reported to be related with the described symptoms (among others see Larignon and Dubos 2001). Both geographic distribution and host range of *Diplodia mutila* are large (Phillips *et al*. 2013). Our finding is based both on spore trap and wood of *Prunus armeniaca*, with no other trees around but within 10 m distance to the next vineyard. The species exists in several viticultural areas of Germany, and in some cases was associated with external Esca symptoms (Fischer and Kassemeyer 2003, Fischer, unpubl. results); however, it never acted as a sole agent and was in co-existence with Esca pathogens such as *Phaeomoniella chlamydospora* and/or *Fomitiporia mediterranea*. Pathogenicity tests demonstrated *D. mutila* (as *B. stevensii*) as a weak pathogen on grapevine (Phillips 1998); it also may exist as endophyte and related symptoms may vary between regions. Colony morphology: Figure, j. ITS 5-4 sequence: GenBank accession KY549804.

Diplodia seriata De Not.: This species, with the teleomorphic state *Botryosphaeria obtusa*, has been extensively treated by Phillips (2002), Úrbez-Torres *et al*. (2012) and Phillips *et al*. (2013) and is commonly isolated out of GTD affected vines. However, it is not clear if it acts as pathogen or saprobe; frequently is has been found in pre-damaged plant tissue (Phillips 2002). For the occurrence in German vineyards, see Fischer *et al*. (2016). In the present study we repeatedly isolated *D. seriata* from necrotic wood of apple (*Malus domestica*) and apricot (*Prunus armeniaca*), both positioned close to vineyards. In addition, a culture was derived from a spore trap affixed to the same apple tree, from March through August. *Malus* as a host plant for *D. seriata* (*B. obtusa*) has been cited for different countries in Europe, such as Bulgaria or Portugal (Phillips *et al*. 2012). *Prunus armeniaca* has been reported for South Africa (Damm *et al*. 2007) and Spain (Gramaje *et al*. 2012). Colony morphology: Figure, k. ITS 5-4 sequence: GenBank accession KY549805.

Dothiorella iberica A.J.L. Phillips, J. Luque & A. Alves: *Dothiorella iberica*, with the teleomorph *Botryosphaeria iberica,* has a wide host range, including both coniferous and deciduous trees (Phillips *et al*. 2013), and in this way is conform to other members of the genus. Disease symptoms related to *D. iberica* have been reported from almond (*Prunus dulcis*) trees in California (Doll *et al*. 2015) as well as from grapevine in California (Úrbez-Torres and Gubler 2007, 2009) and Spain (Martin and Cobos 2007). In California, symptoms on the different hosts include dead spurs as well as cordon and trunk dieback due to canker formation in the vascular tissue. In the Spanish survey *D. iberica* was exclusively associated with symptoms of vines younger than five years, where its percentage among *Botryosphaeria*-like taxa was 6 %; the species was not recovered from older vines, *i.e.* older than 5 years.

Our report is based on two collections from spore traps, placed on *Corylus avellana* and *Prunus armeniaca*. Branches of the sampled *P. armeniaca* showed signs of dieback next to the spore traps and wood discoloration was evident in cross sections; still, we were not able to obtain *D. iberica* isolates from this wood portion. Colony morphology: Figure. l. ITS 5-4 sequence: GenBank accession KY549806.

Eutypa lata (Pers.) Tul. & C. Tul.: In our study, *E. lata* was revealed once, in visibly affected wood of a branch of *Cydonia oblonga* (quint), located some 100 m away from the next vineyard. Quite unique, infected wood was separated from surrounding tissue by a distinct black line of demarcation. Occurrence of *E. lata* on a huge variety of host plants is well known and list of hosts also includes *Cydonia oblonga* (Rappaz 1987, Cook and Dubé 1989, Carter 1991). In the meantime, a considerable number of taxonomic novelties has been described as being very closely related to *E. lata* (Rolshausen *et al*. 2006, Trouillas and Gubler 2010, Trouillas *et al*. 2010), and so the actual identity as given in the former reports may be uncertain. Our report is the first one from *Cydonia* in Germany.

Pathogenicity of *E. lata* on non-*Vitis* hosts such as *Diospyros kaki* (persimmon) has been assessed in the comprehensive study of Moyo *et al*. (2016) in South African Western Cape orchards. Lesion lengths caused by *E. lata* were different between orchards and were also variable between mature wood and green shoots; they were consistently larger than in the agar plug treated controls. Colony

morphology: Figure, m. ITS 5-4 sequence: GenBank accession KY549799.

Phaeoacremonium angustius W. Gams, Crous & M.J. Wingfield: The collection of this species was obtained in the last round of our wood-sampling period, *i.e.* August, and represents an all-new report, both with regard to geographic area (Germany) and host plant, *i.e.* wood of *Cydonia oblonga* (worldwide). As part of their molecular studies, Gramaje *et al*. (2015) place *P. angustius* in one clade together with *P. viticola* (for occurrence in Germany, see Fischer *et al*. 2016) and *P. roseum* (known from Canada only; Úrbez-Torres *et al*. 2014). *Phaeoacremonium angustius* has been previously reported from Italy (Crous and Gams 2000), Portugal (Chicau *et al*. 2000), Spain (García-Benavides 2013), and U.S.A. (Groenewald *et al*. 2001). Hosts include *Vitis, Malus, Olea*, and – with the present finding – *Cydonia*. Little is known about the pathogenicity of *P. angustius* against its host plants. Aroca and Raposo (2007, 2009) tested nine species of *Phaeoacremonium* on grapevine seedlings and cuttings and found *P. angustius* to cause foliar symptoms including interveinal chlorosis and stunted leaves.

Our finding on quint does not allow any strict conclusions about the possible occurrence in German vineyards. Together with the well known *P. aleophilum* and the recently (Fischer *et al*. 2016) discovered *P. fraxinopennsylvanicum* and *P. viticola* it brings the number of *Phaeoacremonium* species in Germany up to four. Colony morphology: Figure, n. ITS 5-4 sequence: GenBank accession KY549797.

Stereum hirsutum (Willd.) Pers.: *Stereum hirsutum* has been frequently mentioned in the specialized literature (Larignon and Dubos 1997, Mugnai *et al*. 1999, Armengol *et al*. 2001) as a, less important, pathogen among the Esca disease fungi. In the meantime it is usually considered a saprobe or a weak facultative parasite only, usually living on dead or strongly decayed wood (Fischer and González García 2015). With this background occurrence of the species is nearly ubiquitous (Eriksson *et al*. 1984). Our finding on *Prunus dulcis* is the second only to be found in the literature (Adaskaveg and Ogawa 1990, reported it from California). Colony morphology: Figure, o. ITS 5-4 sequence: GenBank accession KY549798.

Discussion

Hosts and pathogenic fungi: Except for *Pyrus communis*, all sampled tree species were found to be connected with plant pathogenic fungi. Seven tree species were associated with a total of ten GTD related pathogens, with some of the pathogens spread over different hosts. The host-pathogen relations were as follows: *Corylus avellana* (one GTD pathogen), *Cydonia oblonga* (two), *Ficus carica* (one). *Malus domestica* (three), *Prunus armeniaca* (three), *P. dulcis* (one), and *P. spinosa* (one). From the fungal side, the majority of GTD candidates were isolated out of visibly affected wood. However, spore trap based isolates only were obtained for *Diplodia seriata* on apple and *Dothiorella iberica* on almond and hazelnut; therefore, exact host – pathogen relationship remains somewhat uncertain here.

Pathogenic significance of detected GTD fungi is variable according to the literature and in some cases cannot be fully assessed at the moment: while it is subordinate in, for example, *Stereum hirsutum*, it is considered moderate to high in other species such as *Diplodia mutila*, *Dothiorella iberica*, or *Eutypa lata* (Larignon and Dubos 1997, Mugnai *et al.* 1999, Graniti *et al*. 2000, Halleen *et al*. 2007, Surico *et al*. 2008, Bertsch *et al*. 2013, Gramaje *et al*. 2012, Phillips *et al*. 2013, Úrbez-Torres *et al*. 2009, 2013, Fischer *et al*. 2016).

Spore traps *vs*. wood samples: Among the suspected GTD pathogens, *Dothiorella iberica* was the only one demonstrated solely by spore traps. Repeated wood sampling from the related trees, *Corylus avellana* and *P. armeniaca*, was without success and no mycelial isolates were obtained. With this background it only can be stated that *D. iberica*, in some way, exists in the area, with *Corylus* and *Prunus* as likely host candidates besides grapevine. Proof of fungi *via* spore traps does not result in strict statements concerning the host – fungus relationship; on the other hand, it allows more accurate insights into the fungus' sporulation period and in the end may lead to more accurate control measures.

Durability of fungal spores under field conditions: very little is known about the durability and germination rates of GTD related conidia/spores in the field. Under laboratory conditions conidia of different strains of *Phaeomoniella chlamydospora* stored in sterile tap water showed a germination rate between < 0.1% and > 5% after storage at -20 °C for two months; also temperatures of > 40 °C were tolerated for several weeks (Fischer, unpubl. data; see also Gramaje *et al*. 2008). In our study, isolates of *Aureobasidium pullulans* or *Dothiorella iberica* were obtained *via* spore traps only. Traps were changed on a weekly basis, and so the viability of respective spores may be predicted as several days at a minimum. We do not know about other fungi though: some might have lost the germination ability altogether within the above time interval and so won't appear in cultivation based tests. Both reducing of the time interval as well as accompanying molecular diagnosis are indispensable to get a more complete picture of the fungal spectrum on spore traps. With this background it is interesting to note that only two of our fungi, *i.e. Diplodia mutila* and *Cytospora chrysosperma*, could be demonstrated both from wood and spore traps from the same sampled host plant. Other species, such as *Diplodia seriata*, *Collophora africana* and *Leucostoma personii* also were obtained from wood and traps, but were dispersed over different host species.

Impact on vineyards: Based on studies worldwide (see above), the following species revealed in our study may be considered as related to GTDs: *Cytospora chrysosperma* (prior to our study reported from grapevine in Spain, Iran, U.S.A.), *Cadophora novi-eboraci* (Italy, U.S.A.), *Diaporthe eres* (Croatia, Italy, Switzerland), *D. foeniculina* (California, South Africa), *Diplodia mutila* (worldwide, including Germany), *D. seriata* (worldwide,

including Germany), *Dothiorella iberica* (Spain, California), *Eutypa lata* (worldwide, including Germany), *Phaeoacremonium angustius* (Italy, Portugal, Spain, U.S.A.), and *Stereum hirsutum* (worldwide, including Germany). Up to now no findings from grapevine in Germany exist for *Cytospora chrysosperma, Diaporthe eres, D. foeniculina, Dothiorella iberica*, and *Phaeoacremonium angustius*; data on *Cadophora malorum* and *C. novi-eboraci* are incomplete and need further confirmation (Haag and Fischer, in prep.). Whether the mentioned species exist at all in German vineyards and, if yes if *Vitis* has to be seen as their main host remains unknown at the moment. As has been speculated before (Cloete *et al*. 2011, Moyo *et al*. 2016) the existence of GTD fungi on other hosts than *Vitis* may result in extra infection pressure coming into the vineyards from different inoculum sources in the surrounding area.

Our respective studies (Fischer *et al*. 2016) have shown that surprisingly little is known about the biodiversity of fungi, including possible pathogens, in German vineyards and neighboring areas. Consequently, additional research on this topic, using a wider variety of sampling and identification methods, is presently under way. Special emphasis lies on i) assessment of sporulation period of putative pathogens (see reference studies by Larignon and Dubos 2000, Eskalen and Gubler 2001, Kuntzmann *et al*. 2009, van Niekerk *et al*. 2011), and possible interrelation to pruning period of vines. With our data, existence of airborne inoculum was shown only for a limited number of species, and no information was obtained about the distribution during winter time; ii) frequency of the particular fungi in the field over time, both within and outside of vineyards; and, iii) possible co-existence with widespread GTD pathogens such as *Phaeomoniella*, *Phaeoacremonium* and *Fomitiporia* in the host.

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