# 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.

Key words: 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 adapta-

tion 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, ÚR-BEZ-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 Fisch-ER 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**

Wood samples and culturing: 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 <i>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·μL<sup>-1</sup> 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<sup>TM</sup> 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  $\mu L$  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 <b>bold letters</b> discussed as pathogens in GTDs)
Malus domestica	apple	Cadophora malorum (W¹) 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) <b>Diaporthe foeniculina</b> (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) <b>Stereum hirsutum</b> (W)
Prunus persica	peach	Collophora paarla (W) Leucostoma persoonii (W)
Prunus cerasus	cherry	Collophora paarla (W)
Juglans regia <sup>2</sup>	walnut	Paraconiothyrium fuckelii (W)
Corylus avellana	hazelnut	Dothiorella iberica (S)
Pyrus communis	pear	Aureobasidium pullulans (S, W)

<sup>&</sup>lt;sup>1</sup> origin of isolate: W = wood sample; S = spore trap

# 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

<sup>&</sup>lt;sup>2</sup> removed branch lying on the ground next to tree of Juglans regia

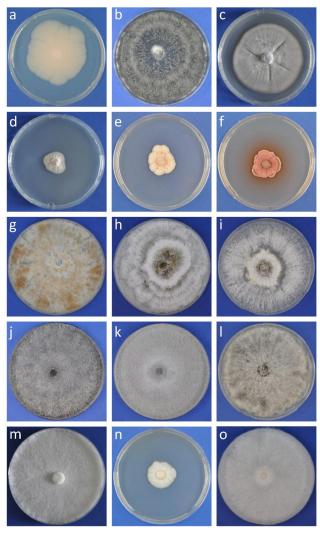


Figure: Fungi derived from non-Vitis hosts in Germany. Species in **bold letters** are discussed as related to grapevine trunk diseases. Cultures are on PDA after 14 d of incubation at approx. 23 °C under daylight conditions. a: Aureobasidium pullulans; b: Botryosphaeria sarmentorum; c: Cadophora malorum; 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 *Bo*tryosphaeria 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 (ÚR-BEZ-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. 1. 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 *Dothiorel*-

*la 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 FISCH-ER, 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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