# Collar Rots in Forests of Northwest Germany Affected by Ash Dieback

**GITTA LANGER***

*Department of Forest Protection, Northwest German Forest Research Institute (NW-FVA),*

*Goettingen, Lower Saxony, D-37079, Germany*

**For correspondence: gitta.langer@nw-fva.de, tel. +49 551 69401129*

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## *Abstract*

The formation of collar rots in association with ash dieback was studied under different site conditions. The fungal community associated with lesions, necroses and stem collar rots, especially the occurrence of *Hymenoscyphus fraxineus* at these symptomatic plant tissues, was investigated. Filamentous fungi and *Phytophthora* spp. were isolated from affected tissues of stem collar rots of various developmental stages. Tissue samples of collar rots were collected from 32 ash trees in seven different forest plots located in Northwest Germany. Obtained isolates were assigned to morphotypes and identified based on mycelial morphology and by molecular methods. Primary agents causing collar rots were identified and the influence of site conditions was derived. The studied stem collar rots were assigned to five symptomatic categories: (0) without collar rots or lesions, (1) emerging collar rots, (2) larger collar rots without visible wood decay, (3) advanced collar rots with visible wood decay and (4) collar rots, necroses or lesions associated with dark sap oozing. In most of the studied collar rots that were collected in Schleswig-Holstein and Lower Saxony, *H. fraxineus* was isolated and assumed to be the primary agent. From samples of category 0 neither *H. fraxineus* nor other fungi or *Phytophthora* species were isolated. From collar rots of the other symptomatic categories, varying quantities of endophytic, saprotrophic and pathogenic species had been isolated. Overall, the number of isolated species was higher in advanced stages of collar rot. Most common species were *H. fraxineus* and *Neonectria punicea*, followed by *Diaporthe eres*, *Botryosphaeria stevensii*, *Gibberella* sp., *Fusarium solani* and *Cadophora* sp. However, collar rots in early stages were only associated with *H. fraxineus* and *N. punicea*. *Armillaria* or *Phytophthora* species were only isolated from advanced collar rots or occurred under special side conditions.

**Keywords:** Ash dieback, *Fraxineus excelsior*, Collar rot, Wood decay, *Hymenoscyphus fraxineus*, *Neonectria punicea*, *Armillaria*, *Phytophthora*, Endophytes, Plant pathogens, Fungi

## Introduction

Meanwhile, ash dieback caused by *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya is present in all parts of Germany where common ash (*Fraxinus excelsior* L.) is growing. Since 2009 a dramatic increase in the number of infested stands and a severe disease progression has been obvious (Langer 2017). Main disease symptoms were necroses on leaves and twigs, which lead to crown dieback after several years of multiple infections (e.g. Bakys et al. 2009, Kirisits et al. 2009, Skovsgaard et al. 2010, Gross et al. 2014, Langer et al. 2015a). Tree mortality is increasing in older age classes and often connected to stem collar necroses associated e.g. with *H. fraxineus* or *Armillaria* root rot (Metzler 2012, Langer 2015b, Langer 2017). Disease progression and mortality in pole and timber size trees were intensified by increasing numbers of collar rots (Metzler and Herbstritt 2014). *H. fraxineus* is able to colonize the bases of ash stems and to infect stem tissues (Husson et al. 2012) and causes, in conjunction with secondary fungi, basal lesions, necrosis and collar rots (Lygis et al. 2005, Skovsgaard et al. 2010, Bakys et al. 2011, Husson et al. 2012, Enderle et al. 2013, Langer et al. 2015b). Collar rots or necroses associated with ash dieback had been monitored in Germany (e.g. Metzler 2012, Langer 2017) as well as in other European countries (e.g. Bakys et al. 2011, Chandelier 2015, Marçais et al. 2016, Muñoz et al. 2016). Of 60 adult ash dieback-diseased trees (92-148 years old) that were monitored in Schleswig-Holstein, 82 % exhibited collar rot in the year 2012 (Langer et al. 2015a). Chandelier (2015) reported that only 41 % of studied necroses at the collar base of diseased ash trees were infected

by *Armillaria* spp., while most of them (98 %) were associated with *H. fraxineus*.

Collar rots seem to be typical after-effects of ash dieback and are common in young ash as well as in older ash. They can occur on trees with or without crown symptoms of ash dieback (Enderle et al. 2013, Muñoz et al. 2016). Muñoz et al. (2016) showed that susceptibility towards collar rots seems to be genetically determined. Collar rots may be primarily induced by *H. fraxineus* itself, opportunistic wood decaying fungi or other pathogens (Bakys et al. 2011, Langer 2017, Metzler 2012). For example, in a 16-years-old ash stand in Lower Saxony, 96% of trees were infected by ash dieback and 6.8 % of the trees exhibited collar rot. These collar rots and tissue lesions were partly associated with *H. fraxineus* and other fungi, such as *Neonectria punicea* (J.C. Schmidt) Castl. et Rossman, *Fusarium solani* (Mart.) Sacc., and *Botryosphaeria stevensii* Shoemaker, but *Armillaria* species were not observed in the diseased tissues (Langer et al. 2015a, Langer 2017). In contrast, *Armillaria* spp. were assigned to be primary causal agents of collar rot in diseased trees, e.g. *Armillaria gallica* Marxm. et Romagn. in Germany (Metzler and Herbstritt 2014) and in Denmark (Skovsgaard et al. 2010), *A. cepistipes* Velen. in Lithuania (Bakys et al. 2011) and *A. gallica* and *A. cepistipes* in Belgium (Chandelier 2015). The association of *Phytophthora* species with root and collar rots of mature ash trees (*F. excelsior*) were reported from Poland and Denmark (Orlikowski et al. 2011). Collar rots seem to play a major role in disease progression and imperil the tree stability. Therefore, salvage cuttings have been necessary in Northwest Germany since 2009 (Langer 2017).

The German temperate seasonal climate is influenced by the oceanic western and the continental eastern European climate. A distinct climatic gradient from western to eastern parts exists, with more continental conditions and decreasing precipitation in the east. The northwestern and northern parts of Germany exhibit an oceanic climate with mild winters and warm summers. Northwest Germany, comprising the federal states Schleswig-Holstein (SH), Lower Saxony (LS), Saxony-Anhalt (ST) and Hesse (HE), is mainly characterized by lowland, which was essentially shaped by the Saale und Weichsel glacial periods. The latter led to spatially highly differentiated site conditions. The climate is euatlantic at the coasts and atlantic to subatlantic from the Northern parts of Lower Saxony and Schleswig-Holstein to the low mountain range bordering the area in the South. The potential natural zonal vegetation is characterized by beech forests (Fagion sylvaticae, Bohn and Neuhäusel 2000/2003). The Northwest German Highlands comprise areas of the low mountain range located north of the Main River. In the year 2012, 2.4% of the total forest area in Germany was covered by *F. excelsior* (Enderle et al. 2017).

To elucidate the formation of collar rots associated with diseased ash trees, different development stages of lesions, necroses and complex damaged tissues with wood rot were studied in stands with different site conditions. The presented study aimed to identify the primary invaders causing collar rot, and the influence of site conditions on the formation of collar rots and necroses. The main aim of the study was to characterize the fungal community associated with stem collar necroses of ash trees that were diseased by ash dieback, especially the occurrence of *H. fraxineus* at these symptomatic plant tissues.

## Materials and methods

### *Forest stands*

In total, seven different forest stands with ash trees (*F. excelsior*) were studied. The studied forest plots were located in three German federal states (SH, LS, HE). Except the forest stand in LS, which was a north-eastwards gently inclined slope in the foothill zone, all studied forest plots were flat and located in the planar zone. In all studied stands, *F. excelsior* was the dominant tree species, except Kuehkopf-Knoblochaue (HE), which was a mixed broad-leaved riparian forest dominated by *Fagus sylvatica* and *F. excelsior*. Ash dieback in SH was first observed in 2002 and laboratory-confirmed in 2005, in LS in 2006 and in HE in 2008. The presence of collar rots in the studied forest plots were recognized in different years: plots 1-4 in Satrup (SH): 2012, plot 5 in Nehmten (SH): 2007, plot 6 in Kuehkopf-Knoblochaue (HE): 2014 and plot 7 in Stroit (LS): 2007.

### *Studied material*

In total, stem collars of 32 ash trees that were diseased by ash dieback were sampled, most of them in SH at forest plots 1 and 2. Of interest were mainly ashes with characteristic stem collar rots or necroses and moderate ash dieback symptoms in the crowns. In addition, two ash trees without distinctly obvious stem collar rots or necroses, which were diseased by ash dieback, were sampled at forest plot 2 (trees No. 17 and 21) and investigated. Information about studied samples (i.e. *F. excelsior* trees) and locations and site conditions is listed below:

No. 1-8: 25 years old, forest plot 1, SH, Mohrkirch, 54°40'17"N, 09°41'20"E, eutrophic and weak changing moist to stagnant fresh site, leg. U. Harriehausen and I. Krischock, 06.02.2013.

No. 9-20: 21 year old, forest plot 2, SH, Boeklund, 54°36'30"N, 09°36'15"E, well mesotrophic and weak changing moist to stagnant fresh site, leg. U. Harriehausen and I. Krischock, 06.02.2013.

No. 21-22: 20 years old, forest plot 2, SH, Boeklund, 54°36'30"N, 09°36'15"E, eutrophic and fresh to stock fresh site, leg. U. Harriehausen, 04.01.2013.

No. 23-24: 20 years old, forest plot 3, SH, Obdrup, 54°41'32"N, 09°33'18"E, well mesotrophic and fresh to stock fresh site leg. U. Harriehausen, 04.01.2013.

No. 25: 65 years old, forest plot 4, SH, Obdrup, 54°41'25"N, 09°33'12"E, eutrophic and weak changing moist to stagnant fresh site, leg. U. Harriehausen, 04.01.2013.

No. 26-27: 20 years old, forest plot 5, SH, Nehmten, 54°05'15"N, 10°23'11"E, well mesotrophic and and fresh to stock fresh site, leg. I. Krischock, 07.02.2013.

No. 28-29: 15-20 years old, forest plot 6, HE, Kuehkopf-Knoblochaue, 49°50'15"N, 08°23'54"E, eutrophic and moist, alluvial site, leg. A. Noltensmeier, 03.06.2014.

No. 30-32: 15-20 years old, forest plot 7, LS, Stroit, 51°53'52"N, 09°51'42"E, eutrophic and fresh site, leg. P. Gawehn and M. Pfeffer, 21.01.2015.

***Sampling Method***

Trees were felled with a chainsaw. The affected stem base of each tree was collected and washed with running water. Stem parts with collar lesions, necroses and rots were dissected in longitudinal and cross-sections and the bark was removed. All stages of preparation were documented by photography.

***Classification of Stem collar rots***

The studied stem collar rots were assigned to the following symptomatic categories: (0) necrosis or collar rots not distinctly visible on the tree surface; no wood discolouration due to stem collar rot (Figure 1), (1) emerging collar rots, collar necroses or lesions distinctly visible on the tree surface but small, only several $cm^2$ in diameter; wood discolouration only visible as a small dark brownish, fan-like pattern in a cross section of the stem (Figure 2), (2) large collar rots, necroses and bark lesions (a single or several meshing necroses), were visible on the stem base surface; wood discolourations were visible as dark brownish, larger fan-like patterns and/ or other discolorations in a cross section of the stem (Figure 3), (3) advanced collar rots with very large necroses and bark lesions (a single or several meshing necroses); partly large portions of the wood and the root collar were discolourated and wood rot was visible (Figure 4), and (4) one or several collar necroses or lesions distinctly visible on the tree surface and associated with dark sap oozing from the margin of the necrosis; browning of the outer layer of the sapwood; wood discolourations were mainly peripheral, in cross-sections not visible as dark brownish fan-like patterns but diffuse or with greenish line of demarcation (Figure 5).

***Agar media***

Four different agar media (MEA. MYP, PDA, CJ) were used for the isolation in the detailed study of forest plots 1 and 2, while MYP was standard medium for all samples and CJ was standard medium for the isolation of *Phytophthora*.

Malt extract agar (MEA), modified according to Langer (1994): 20 g malt extract (Merck 1.05391.0500), 15 g agar (Fluka 05040-1KG), 1000 ml Aqua dest.

Malt yeast pepton (MYP), modified according to Langer (1994): 7 g malt extract (Merck 1.05391.0500), 0.5 g yeast extract (Fluka 70161-100G), 1 g pepton (Merck 1.07272.0500), 15 g agar (Fluka 05040-1KG), 1000 ml Aqua dest.

Potato dextrose agar (PDA): 39 g PDA FLUKA (70139-500G), 1000 ml Aqua dest.

Carrot juice agar (CJ): modified according to Kröber (1985): 50 ml cold pressed carrot juice, 18 g agar (Fluka 05040-1KG), 1000 ml Aqua dest.

***Isolation of filamentous fungi and Phytophthora***

For the isolation of fungi and *Phytophthora,* tissue pieces (wood chips) were extracted from discoloured areas in the wood. Per studied tree sample, 16 to 188 tissue pieces (77 tissue pieces on average) were extracted with sterile tools under sterile conditions. The number of tissue samples depended on the size of the collar rots and necroses. Tissue pieces were incubated on different agar media at room temperature (ca. 22°C) and daylight. Usually, three wood chips were placed on an agar medium in a 90 mm Petri dish. The Petri dishes were checked visually for developing colonies over a period of four weeks. Emerging mycelia were sub-cultured separately on MYP. Representative isolated fungal strains were kept in MYP slants at 4°C.

***Identification of isolated organisms***

Isolated strains were assigned to mycelial morphotypes (MT, Table 1) and identified by mircomorphological characters or based on DNA sequence similarities. For the identification of fungi, a ZEISS Axiostar plus microscope was used and standard procedures for fungi described in Lee and Langer (2012) were followed. In addition to standard literature recommended by Oertel (2003) for determination of fungi and forest diseases, the following literature was used: e.g. Booth (1971), Gerlach and Nirenberg (1982), Butin (2011), von Arx (1981) and Domsch et al. (1980). The names of fungal species follow Index Fungorum (www.indexfungorum.org) and Mycobank (www.mycobank.org).

If possible, at least one representative strain of each morphotype was used for molecular identification, involving DNA extraction from mycelium, polymerase chain reaction (PCR) amplification of ribosomal DNA, and DNA

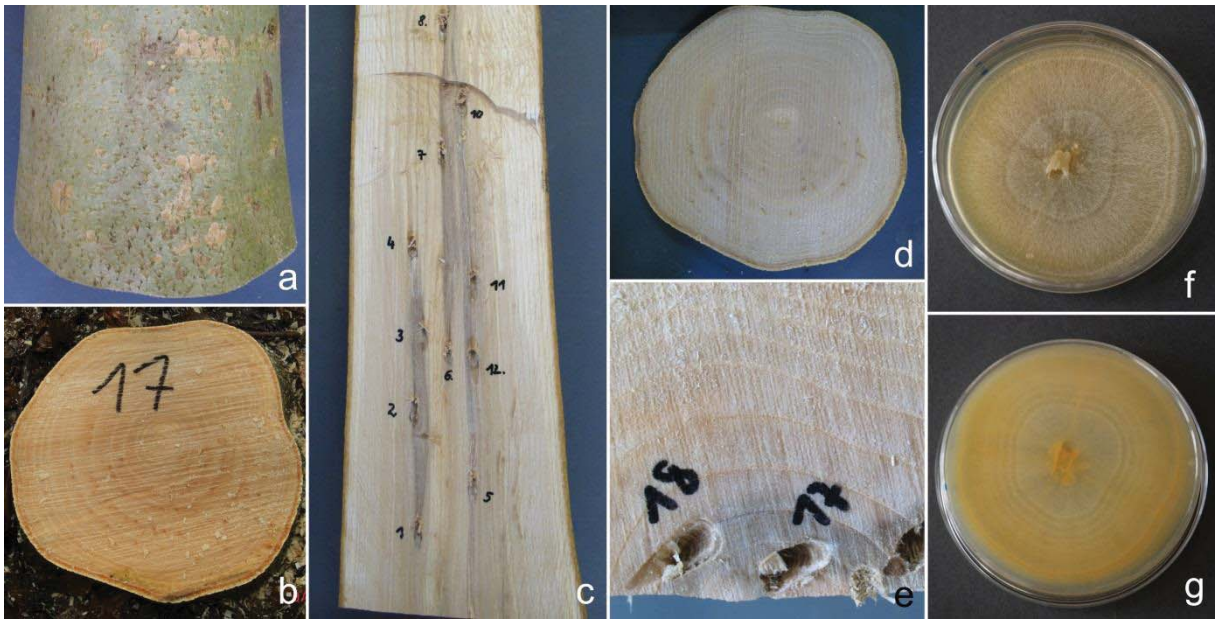


**Figure 1** Collar rot category 0: ash tree 17, a) stem base, b) stump surface, c) longitudinal-section of stem base, with numbered isolation loci, d) cross-section of the stem base without distinctly visible wood discolouration due to collar rots, e) central discolouration with numbered isolation loci, e) *Neonectria punicea*, isolated from tree 3, cultured three weeks on MYP

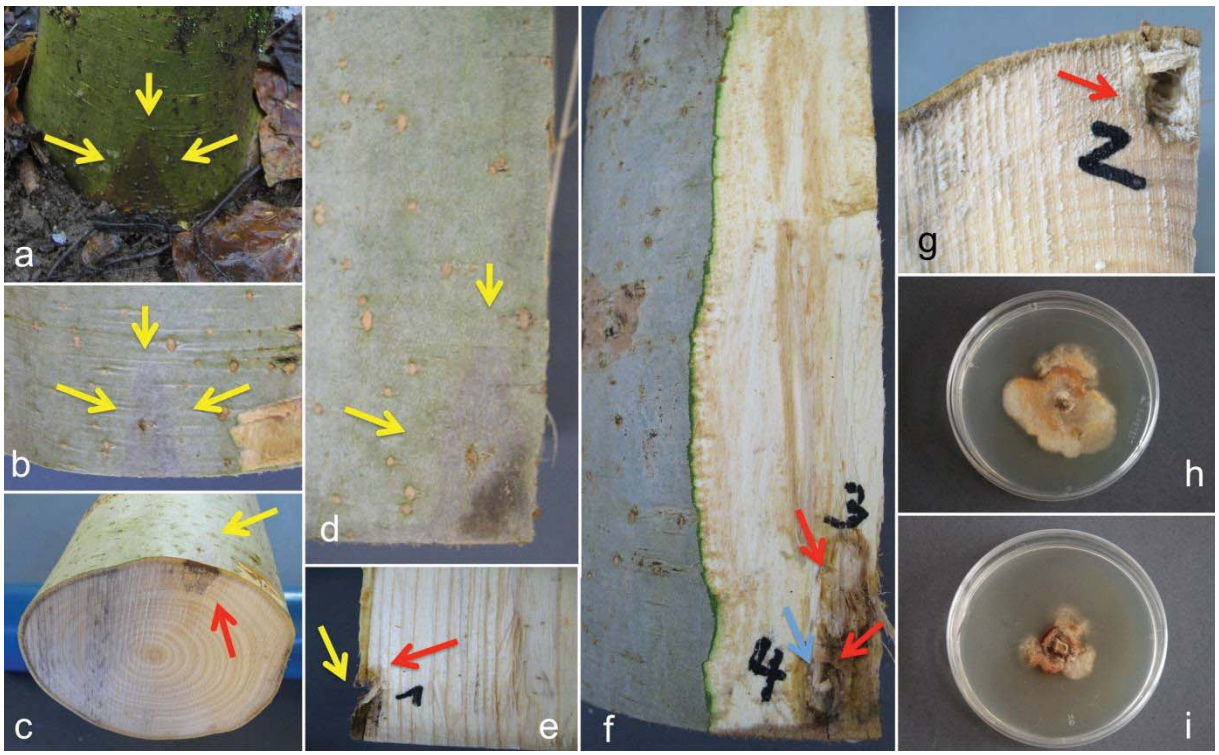


**Figure 2** Collar rot category 1: Ash tree 18, a) stem base of the living tree, b) stem surface with collar rot after washing, c) cross-section of the stem base with distinctly visible wood discolouration due to the collar rot d) part of stem base with distinctly visible wood discolouration due to collar rot, e) longitudinal-section of stem base, with numbered isolation locus, f) stem surface with removed bark and visible wood discolouration due to the collar rot, g) cross-section of the stem base with distinctly visible wood discolouration due to collar rot, h, i) *Hymenoscyphus faxineus*, cultured three weeks on MYP, h) isolated from tree 2 and i) isolated from tree 4. Yellow arrows indicate the collar rot; red arrows indicate isolation loci of *H. fraxineus*; blue arrow indicates an isolation locus of *Neonectria punicea*

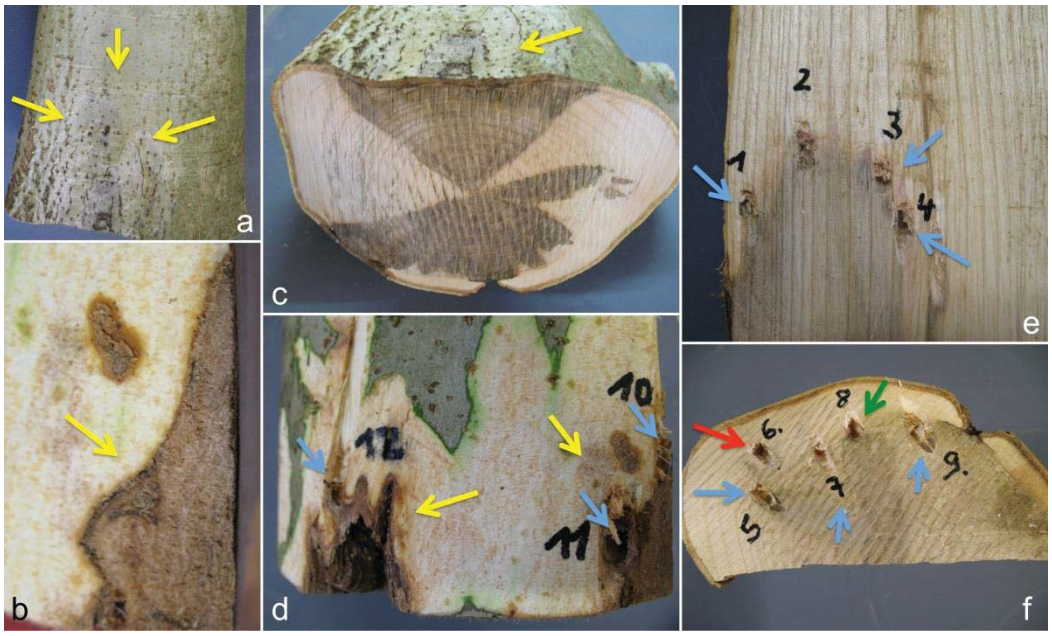


**Figure 3** Collar rot category 2: Ash tree 13, a) stem base after washing b) part of a stem collar with removed bark and visible wood discolouration due to the collar rot, c) cross-section of the stem base with distinctly visible, fan-like wood discolourations due to the collar rots d) part of the stem base with removed bark and visible wood discolouration and lesions due to the collar rots, e) longitudinal-section of stem base, with numbered isolation loci, f) cross-section of stem base halve with distinctly visible wood discolouration due to collar rot. Yellow arrows indicate the collar rot; red arrows indicate an isolation locus of *H. fraxineus*; blue arrows indicate isolation loci of *Neonectria punicea;* green arrow indicates the isolation locus of *Cadophora sp*.

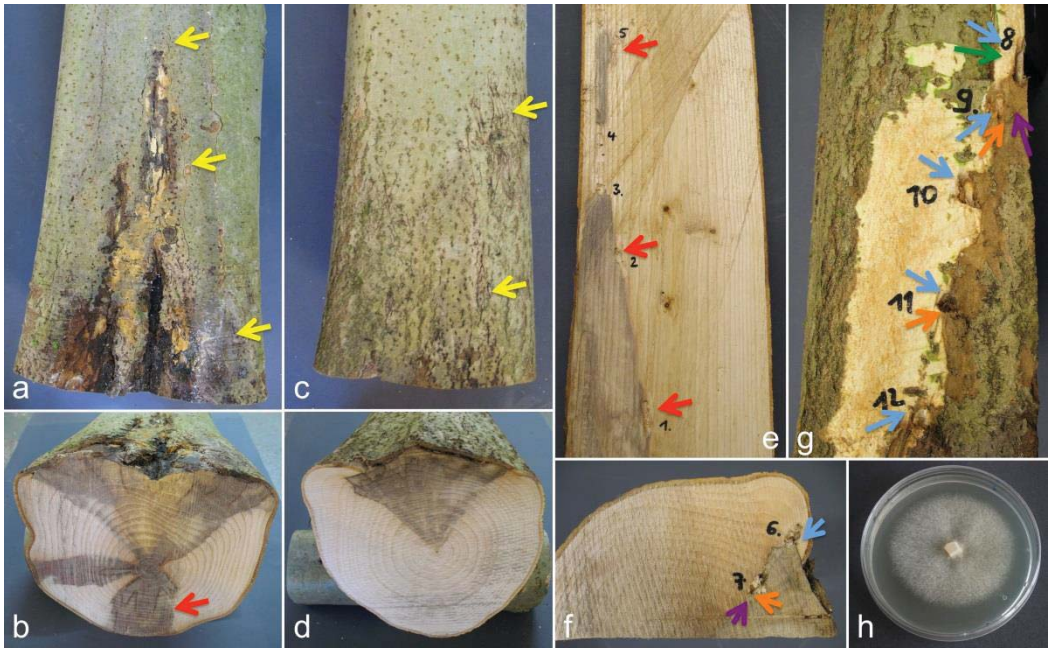


**Figure 4** Collar rot category 3: a-b) Ash tree 2, a) stem base after washing, b) cross-section of stem base with four, distinctly visible, fan-like wood discolourations due to collar rots; c-g) ash tree 5, c) stem base after washing d) cross-section of stem base with distinctly visible, fan-like wood discolouration due to collar rot, e) longitudinal-section of stem base, f) cross-section of stem parts with distinctly visible, fan-like wood discolouration due to collar rot g) stem surface with removed bark and visible wood discolouration due to the collar rot, h) *Flammulina velutipes*, (Ac. no: KU712226). Yellow arrows indicate the collar rot; red arrows indicate isolation loci of *H. fraxineus*; blue arrows indicate isolation loci of *Neonectria punicea*, green arrow indicates the isolation locus of *F. velutipes,* orange arrows indicate isolation loci of *Diaporthe eres*, violet arrows indicate isolation loci of *Botryosphaeria stevensii*

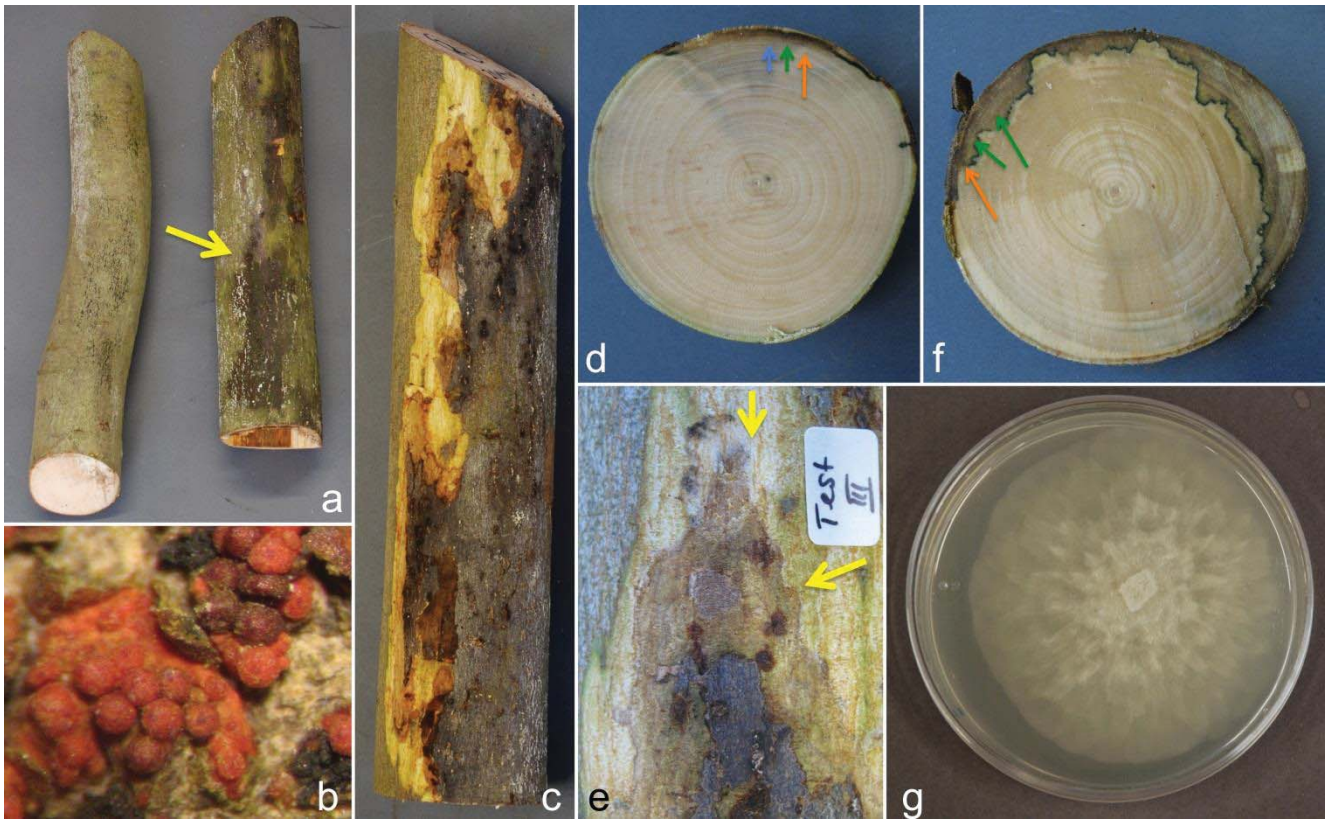


**Figure 5** Collar rot category 4: a) left: sample 28, right: sample 29. Figure b, c, f, e) sample 29: b) *Neonectria punicea*, ascocarps, c) collar rot with oozing sap and partly removed bark, e) brownish necrosis due to the *Phytophthora*-infection, and f) cross-section of stem base with wood discolouration and greenish line of demarcation. Figure d, g) sample 28: d) a cross-section of stem base with diffuse wood discolourations due the *Phytophthora*-infection and g) *P. plurivora*. Yellow arrows indicate the collar rot; blue arrow indicates isolation locus of *N. punicea;* green arrows indicate the isolation loci of *Gibberella sp.,* orange arrows indicate isolation loci of *Fusarium solani*

sequencing. The databases at GenBank (http://www.ncbi.nlm.nih.gov/genbank/, Altschul et al. 1997) were used to define the identity of sequences. Intraspecific ITS similarity for the sequenced fungi of 98-100% was used at species level.

To proof *H. fraxineus*, isolated suspicious strains were transferred to MYP medium. The mycelia were microscoped during the following days, in order to provide evidence of the typical conidiophores and conidia of *H. fraxineus*.

***Molecular methods***

DNA isolation, PCR and sequencing were performed by the laboratory of Prof. E. Langer, University Kassel by order and for account of the NW-FVA. From each morphotype, 1-2 mg culture tissue was suspended in 100 µl TE buffer in a 1.5 ml-tube. A microwave (600 W) was used twice for 1 min each, including a pause of 30 s, to break up cells. Tubes were cooled to -20°C for 20 min. and then centrifuged at 10000 rpm for 5 min. A 100 times diluted portion of the supernatant was used for PCR. Primer pairs for amplification of ITS1, 5.8S and ITS2 region were ITS1F/ITS4 or ITS1/ITS4 (Gardes and Bruns 1993, White et al. 1990). The PCR was performed with 45 µl Master mix from QIAGEN, Hilden, Germany and 5 µl of extracted DNA. PCR was carried out with the primer pairs with initial denaturation at 94°C for 3 min, followed by 29 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 60 s. Final elongation was performed at 72°C for 7 min. PCR products were separated on 1% agarose gel stained with GelRed fluorescence dye (Biotium, Hayward, CA, USA) followed by a cleaning with QIAquick PCR Purification Kit (QIAGEN). Sanger sequencing of purified products (Sanger et al. 1977) was commissioned at GATC Biotech (Cologne, Germany). Editing of DNA sequences and alignment were performed with MEGA6 (Tamura et al. 2013) followed by submission to GenBank (Table 1).

## Results

### *Stem collar rots*

Two of the 32 studied trees had no stem collar rots while 12.5% of trees showed stem collar rots of the symptomatic category 1, 37.5% exhibited stem collar rots of the symptomatic category 2, 37.5% showed stem collar rots of the symptomatic category 3, and 6.25% revealed stem collar rots of the symptomatic category 4. Stem collar rots of the symptomatic category 4 were only found at the moist riparian forest plot 6.

35 morphotypes and a few additional, nonrecurring taxa, which were not obtained as culture or not identified further, were isolated from the necroses and collar rots (Table 1). *H. fraxineus* (MT 35) was obtained on all tested agar media. Usually, anamorph-stages of this fungus were observed within one or two days after transfer of mycelia that was growing from wood chips in the original Petri dishes to MYP agar.

From collar rots of different symptomatic categories, varying numbers of species associated with wood discolouration, rot and lesions were isolated (Table 2). In the studied tissues of ash stems without distinctly visible necroses (category 0, Figure 1), neither *H. fraxineus* nor other fungi or *Phytophthora* species could be isolated. In the studied emerging collar rots (category 1), only *H. fraxineus* and / or *Neonectria punicea* had been detected and no wood decaying fungi nor *Phytophthora* species were present. In more advanced collar necrosis (category 2), *Botryosphaeria stevensi*, *Fusarium solani* (MT 4), *Gibberella* sp. (MT 5) and *Cadophora* sp. (MT 6) were species additional to *H. fraxineus* and *N. punicea*, which had been isolated at least in two of the studied trees. Advanced collar necroses or rots of category 3 were often associated with *B. stevensii, Diaporthe eres* Nitschke (MT 3)*, Gibberella sp.* (MT 5), the ascomycete MT 16 and *H. fraxineus* and *N. punicea*. The collar necroses of category 4 were caused by *Phytophthora plurivora* T. Jung & T.I. Burgess and only a few additional secondary pathogens like *N. punicea*, *F. solani* or *Gibberella* sp. were isolated.

### *Frequency of isolated organisms*

Most frequent species were *N. punicea* (MT 2, isolated from 77.4 % of 31 studied trees) and *H. fraxineus* (MT 35, 77.4 %) followed by *D. eres* (MT 3, 29 %), *B. stevensii* (MT 1, 25.8 %), *Fusarium solani* (MT 4, 22.8 %), *Gibberella* sp. (MT 5, 22.8 %), *Trichoderma* sp. (MT 27, 19.3 %) and *Cadophora* sp. (MT 6, 16.1 %). Other obtained species were only isolated from single up to 4 trees: *Diaporthe* sp. (MT 3.1), *Phomopsis* sp. (MT 3.2), *Xylaria polymorpha* (Pers.) Grev. (MT 7), *Flammulina velutipes* (Curtis) Singer (MT 8), Basidiomycete sp. (MTs 9 and 22) *Trichothecium roseum* (Pers.) Link (MT 10), *Ascocoryne* sp. (MT 14), *Alternaria* sp. (MT 20), Ascomycete sp. (MTs 13, 17, 24, 25, and 28), *Lophiostoma corticola* (Fuckel) E.C.Y. Liew, Aptroot & K.D. Hyde (MT 29), *Mortierella* sp. (MT 30), *Eurotium* sp. (MT 31), *Coprinellus micaceus* (Bull.) Vilgalys, Hopple & Jacq. Johnson (MT 32), and *Armillaria* sp. (MT 33) all with a frequency of 3.2 %; *Neofabraea* sp. (MT 12), Ascomycete sp. MT 19, *Ilyonectria* sp. (MT 23), *Epicoccum nigrum* Link (MT 26), and *Phytophthora plurivora* (MT 36) with a frequency of 6.5 %; *Valsa cypri* (Tul.) Tul. & C. Tul. (MT 11) with a frequency of 9.7 % and Ascomycete sp. (MT 16) with a frequency of 12.9 %.

## Discussion and conclusions

The presented study was the first research on ash dieback and stem collar rots in north-western Germany. A large number of young and adult ash trees that were diseased with ash dieback and associated with collar rots were observed. In most of the studied collar rots and necroses (67%, n = 30) that were collected in Schleswig-Holstein and Lower Saxony, *H. fraxineus* was isolated and assumed to be the first colonizer in most studied tissues. This assumption was motivated by the results that: 1) In the studied tissues of ash stems with emerging collar rots (category 1), only *H. fraxineus* and / or *N. punicea* had been detected, but wood decaying fungi, typical secondary fungi or *Phytophthora* species were not present. *N. punicea* and *H. fraxineus* were the most frequently isolated species. On average, the more advanced the collar rot was, the more species had colonized the tissue. 2) It was proven for *H. fraxineus* to cause symptomatic necroses of ash bark and cambium (Bakys et al. 2009) on stems and occasionally also on roots or root collars (Gross et al., 2014). Several studies confirmed a high pathogenicity of *H. fraxineus* towards *F. excelsior* (Bakys et al. 2009, Kowalski & Holdenrieder 2009, Husson et al. 2011, Kräutler et al. 2015, Gross and Sieber 2016, Kowalski et al. 2017). Moreover, it was proven that *H. fraxineus* is able to infect intact shoots of ash (Kräutler et al. 2015). 3) *Neonectria* species are known to be endophytes (Ceccarelli 2010, Sieber 2007). Nevertheless, they are also able to cause necroses and initialize complex diseases as secondary plant pathogens (Bressem 2002, Sieber 2007, Hirooka et al. 2013).

Hence, *H. fraxineus* is supposed to be the first colonizer of collar tissues, in which only a few other fungal species (typical secondary pathogens or endophytes like *N. punicea*) had been detected and in which no wood rotting fungi were present (categories 1-2). The hypothesis that the collar lesions and rots of ash trees affected by ash dieback are often caused primarily by *H. fraxineus* is supported by the presented results, Langer et al. (2015b and 2017) and by Chandelier (2015).

*N. punicea*, which is a species with *Cylindrocarpon* Wollenw. anamorphs, was the most common species

**Table 1** Isolated Morphotypes and NCBI GenBank Blasting information for isolated strains and the closest blast matches (Frequency = Frequency of occurrence in % of studied 31 trees; A = anamorph)

| MT | Frequency [% | Forest plot | Tree | Taxon name | NCBI GenBank BLAST check 05./08.02.2015 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | GenBank accession No. | GenBank taxon | Sequence ID | [%] ITS similarity | [%] Query coverage | Source |
| 1 | 25.8 | 1 | 6 | *Botryosphaeria stevensii,* A: *Diplodia mutila* | KU712211 | *Diplodia mutila* | KF766158 | 99 | 92 | Slippers et al. (2013) |
| 2 | 77.4 | 2 | 14 | *Neonectria punicea,* A: *Cylindrocarpon album,* syn. *Neonectria confusa* | KU712212 | *Neonectria punicea* | HM534901 | 99 | 99 | Jacklitsch and Voglmayr (2011) |
| | | 1 | 5 | | KU712213 | *N. punicea* | Jf268768 | 99 | 100 | Zhao et al. (2011) |
| | | | | | | *N. confusa* | KM515889 | 99 | 98 | Lombrad et al. (unpublished) |
| | | | | | | *Neonectria* sp. | JF268760 | 100 | 99 | Luo and Zhuang (2010) |
| | | | | | | *Neonectria* sp. | FJ560437 | 100 | 99 | |
| 3 | 29.0 | 1 | 5 | *Diaporthe* eres | KU712214 | *Diaporthe eres* | EU571099 | 100 | 97 | Kacergius and Jovaisiene (unp.) |
| | | 1 | 13 | | KU712215 | *D. cotoneastri* | KC843328 | 100 | 100 | Udayanga et al. (2014) |
| | | | | | | *D. eres* | JF430493 | 100 | 99 | Petrovic et al. (unp.) |
| | | 5 | 27 | | KU712216 | *D. cotoneastri* | KC843328 | 100 | 97 | Udayanga et al. 2014 |
| | | | | | | *D. eres* | JF430493 | 100 | 97 | Petrovic et al. (unp.) |
| | | 5 | 26 | | KU712250 | *Diaporthe cotoneastri* | KC145903 | 100 | 99 | Johnston and Park (unp.) |
| | | | | | | *D. eres* | JF430493 | 99 | 99 | Petrovic et al. (unp.) |
| 3.1 | 3.2 | 2 | 9 | *Diaporthe* sp. MT 3.1 | KU712217 | *Diaporthe viticola* | KC145904 | 99 | 99 | Johnston and Park (unp.) |
| | | | | | | *D. viticola* | KC145906 | 100 | 96 | |
| 3.2 | 3.2 | 5 | 26 | *Phomopsis sp.* MT 3.2 | KU712245 | *Phomopsis columnaris* | KC145883 | 99 | 99 | Johnston and Park (unp.) |
| | | | | | | *Phomopsis* sp. | KF428571 | 100 | 92 | Bonito et al. (unp.) |
| 4 | 22.6 | 1 | 4 | *Fusarium solani* | KU712218 | *Fusarium solani* | FJ478128 | 99 | 97 | Jiang et al. (unp.) |
| | | 5 | 26 | | KU712219 | *F. solani* | FJ478128. | 100 | 99 | |
| 5 | 22.6 | 5 | 10 | *Gibberella* sp. MT 5 | KU712220 | *Fusarium lateritium* | AF310980 | 100 | 100 | Schuett et al. (unp.) |
| 6 | 16.1 | 1 | 1 | *Cadophora* sp. MT 6 | KU712221 | *Cadophora luteo-olivacea* | HM116747 | 99 | 100 | Johnston et al. (2010) |
| | | 2 | 13 | | KU712222 | *Cadophora* sp. | HM116752 | 100 | 100 | Johnston et al. (unp.) |
| | | | | | | *C. malorum* | KT358982 | 93 | 100 | Diaz et al. (unp.) |
| | | 2 | 15 | | KU712223 | *C. luteo-olivacea* | HM116747 | 99 | 99 | Johnston et al. (2010) |
| 7 | 3.2 | 1 | 2 | *Xylaria polymorpha* | KU712224 | *Xylaria polymorpha* | KF897015 | 99 | 89 | Ma and Lei (unp.) |
| | | | | | | *X. polymorpha* | GU322460 | 98 | 93 | Hsieh et al. (2010) |
| | | 1 | 2 | | KU712228 | *X. polymorpha* | KF897015 | 99 | 89 | Ma and Lei (unp.) |

**Table 1.** (Continued)

| MT | Frequency [% | Forest plot | Tree | Taxon name | NCBI GenBank BLAST check 05./08.02.2015 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | GenBank accession No. | GenBank taxon | Sequence ID | [%] ITS similarity | [%] Query coverage | Source |
| 8 | 3.2 | 1 | 5 | *Flammulina velutipes* | KU712226 | *Flammulina velutipes* var. *longispora* | AF051700 | 99 | 96 | Hughes et al. (unp.) |
| | | | | | | *F. velutipes* | KM668876 | 99 | 93 | Senik et al. (2015) |
| 9 | 3.2 | 1 | 7 | Basidiomycete MT 9, not further identified , no ITS product | | | | | | |
| 10 | 3.2 | 1 | 2 | *Trichothecium roseum,* no ITS product | | | | | | |
| 11 | 9.7 | 1 | 8 | *Valsa cypri* A: *Cytospora pruinosa* | KU712230 | *Valsa cypri* | KT004557 | 99 | 89 | Kowalski et al. (unp.) |
| | | | | | | *Cytospora pruinosa* | EU552121 | 95 | 99 | Marincowitz et. al. (2008) |
| | | 2 | 10 | | KU712235 | *V. cypri* | KT004557 | 99 | 88 | Kowalski et al. (unp.) |
| | | | | | | *Cytospora pruinosa* | EU552121 | 95 | 100 | Marincowitz et. al. (2008) |
| 12 | 6.5 | 2 | 15 | *Neofabraea* sp. MT 12 | KU712232 | *Neofabraea malicorticis* | AF141189 | 99 | 98 | Abeln et. al. (2000) |
| | | | | | | *N. inaequalis* | KR859081 | 99 | 98 | Chen et. al. (2015) |
| | | | | | | *N. alba* | KJ396074 | 99 | 100 | Cameldi et al. (unp.) |
| | | 2 | 15 | | KU712233 | *N. malicorticis* | AF141189. | 99 | 99 | Abeln et. al. (2000) |
| | | | 12 | | KU712234 | *N. malicorticis* | AF141189 | 99 | 99 | |
| 13 | 3.2 | 1 | 8 | Ascomycet sp. MT 13, not amplified | | | | | | |
| 14 | 3.2 | 2 | 10 | *Ascocoryne sp.* MT 14 | KU712235 | *Ascocoryne sarcoides* | GQ411510 | 100 | 99 | Fukami et. al. (2010) |
| 15 | 3.2 | 2 | 15 | Basidiomycete MT 21 | KU712239 | *Phlebia* sp. | KP135360 | 100 | 93 | Floudas and Hibbett (2015) |
| | | 2 | 15 | | KU712238 | | | | | |
| 16 | 12.9 | 1 | 7 | Ascomycete MT 16 | KU712236 | Helotiales sp. | GU934595 | 99 | 99 | Bakys et al. (2011) |
| | | | | | | *Neobulgaria* sp. | KR072504 | 88 | 100 | Arhipova et al. (2011) |
| | | | | | | *Neobulgaria pura* | HM051080 | 88 | 98 | Wu et al. (unp.) |
| | | 2 | 27 | | KU712237 | Helotiales sp. | GU934595 | 99 | 100 | Bakys et al. (2011): |
| | | | | | | *N. pura* | HM051080 | 89 | 98 | Wu et al. (unp.) |
| 17 | 3.2 | 2 | 12 | Ascomycete MT 17 | KU712240 | *Phaeomollisia piceae* | LN714584 | 99 | 96 | Vetrovsky et al. (unp.) |
| | | | | | | *Phialocephala* sp. | FJ903362 | 99 | 88 | Arhipova et al. (2011) |
| | | | | | | *Phialoceph. fortinii* | AB671499 | 94 | 99 | Kiyuna et al. (2012) |
| 19 | 6.5 | 2 | 20 | Ascomycete sp. MT 19, *Sclerostagono-spora cycadis* | KU712246 | *Sclerostagonospora cycadis* | KR611890 | 99 | 99 | Crous et al. (2015) |
| 20 | 3.2 | 2 | 12 | *Alternaria* sp., not amplified | | | | | | |

**Table 1.** (Continued)

| MT | Frequency [% | Forest plot | Tree | Taxon name | NCBI GenBank BLAST check 05./08.02.2015 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | GenBank accession No. | GenBank taxon | Sequence ID | [%] ITS similarity | [%] Query coverage | Source |
| 22 | 3.2 | 5 | 27 | Basidiomycete MT 22, *Psathyrella panaeoloides* | KU712241 | *Psathyrella panaeoloides* | KC992894 | 99 | 97 | Larsson and Oerstadius (unp.) |
| 23 | 6.5 | 5 | 27 | *Ilyonectria* sp. MT 23 | KU712242 | *Ilyonectria robusta* | JF735264 | 100 | 99 | Groenewald et al. (2012) |
| | | 5 | 26 | | KU712243 | *I. europaea* | JF735294 | 100 | 99 | |
| | | | | | | *I. robusta* | JF735264 | 99 | 98 | |
| 24 | 3.2 | 7 | 32 | Ascomycete sp. MT 24, not amplified | | | | | | |
| 25 | 3.2 | 7 | 32 | Ascomycete sp. MT 25, not amplified | | | | | | |
| 26 | 6.5 | 2 | 19 | *Epicoccum nigrum,* not amplified | | | | | | |
| 27 | 19.3 | | | *Trichoderma* sp. MT 27, not obtained in culture, not amplified | | | | | | |
| 28 | 3.2 | 1 | 1 | Ascomycet sp. MT 28 | KU712244 | *Sydowia polyspora* | KF993419 | 100 | 93 | Garzoli (unp.) |
| | | | | | | *Pyrenochaeta acicola* | KT309815 | 100 | 88 | Johnston and Park (unp.) |
| 29 | 3.2 | 2 | 10 | *Lophiostoma corticola* | KU712227 | *Lophiostoma corticola* | KT004559 | 100 | 95 | Kowalski et al. (unp.) |
| 30 | 3.2 | 5 | 27 | *Mortierella* sp. MT 30 | KU712228 | *Mortierella verticillata* | KF944471 | 100 | 96 | Zhao (unp.) |
| 31 | 3.2 | 7 | 31 | *Eurotium sp.* MT 31 | KU712229 | *Eurotium* sp. | KF367488 | 100 | 100 | Oliveira et. al. (2015) |
| 32 | 3.2 | 1 | 6 | *Coprinellus micaceus* | KU712252 | *Coprinellus micaceus* | EU436684 | 99 | 100 | Miles et al. (2012) |
| 33 | 3.2 | 5 | 26 | *Armillaria* sp. MT 33 | KU712248 | *Armillaria gallica* | KP960530 | 99 | 99 | Tizzani et al. (unp.) |
| | | | | | | *A. cepistipes* | AB510862 | 99 | 99 | Hasegawa et al. (2010) |
| | | | | | KU712247 | *A. cepistipes* | GU934598 | 99 | 100 | Bakys et al. (2011) |
| | | | | | | *A. bulbosa* | EU784165 | 99 | 100 | Brock et al. (2012) |
| | | | | | | *A. gallica* | KP162313 | 99 | 100 | Guo (unp.) |
| 35 | 71 | 1 | 2 | *Hymenoscyphus fraxineus* | KU712251 | *Chalara fraxinea* | GU797159 | 100 | 99 | Rytkonen et al. (2011) |
| 36 | 6.5 | 6 | 28 | *Phytophthora plurivora*, two strains, identified by Marco Thines, Frankfurt via sequencing analysis | | | | | | |

associated with the studied collar necroses, except for *H. fraxineus*. It was isolated at all sampled forest plots, in 77.4 % of the collar rots and even if *H. fraxineus* was not found in the studied tissues. The native species *N. punicea* is distributed in Europe (Austria, France, Germany, Scotland, Slovakia and Switzerland), in North America (United States), and in Asia (China, Japan). So far, ascocarps were found on dead woody substrates of *Acer macrophyllum, Acer sp., Frangula alnus, Fagus grandifolia, F. sylvatica, Prunus* × *yedoensis, Quercus crispula, Rhamnus fallax, Rhamnus* sp., and *Ulmus* sp. (Hirooka et al. 2013). Until now, *N. punicea* was not rated as wood-inhabiting fungus in stems of *F. excelsior* (Lygis et al. 2005) and was only mentioned by Langer et al. (2015b) and Langer (2017) as species associated with stem collar rots in north-western Germany. *Neonectria* spp. have not been found to be associated with visually healthy shoots, necrotic shoots (Bakys et al. 2009) or with rotting roots (Bakys et al. 2011) of ash trees that were diseased by ash dieback in Lithuania. Moreover, *Neonectria* spp. have

**Table 2.** Isolated Organisms from stem collar rots (n = 32 studied *F. excelsior* trees)

| Collar rot category | Forest plot | Sample / tree | *H. fraxi-neus* | *Armillaria* | *Phy-tophthora* | Other fungi (Morphotypes) | Wood-decaying species | Species in total | Species on average |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 17 | - | - | - | - | 0 | 0 | 0 |
| 0 | 2 | 21 | - | - | - | - | 0 | 0 | |
| 1 | 2 | 11 | - | - | - | 2 | 0 | 1 | 1.8 |
| 1 | 2 | 14 | + | - | - | 2 | 0 | 2 | |
| 1 | 2 | 18 | + | - | - | 2 | 0 | 2 | |
| 1 | 3 | 24 | + | - | - | 2 | 0 | 2 | |
| 2 | 1 | 1 | + | - | - | 2, 6, 28 | 0 | 4 | 5.1 |
| 2 | 1 | 3 | + | - | - | 2 | 0 | 2 | |
| 2 | 1 | 4 | + | - | - | 1, 2, 4 | 0 | 4 | |
| 2 | 2 | 9 | + | - | - | 2, 3.1 | 0 | 3 | |
| 2 | 2 | 12[1] | + | - | - | 6, 12, 17, 19, 20, 1X | 0 | 7 | |
| 2 | 2 | 13 | + | - | - | 2, 6 | 0 | 3 | |
| 2 | 2 | 14 | + | - | - | 2, 6, 11, 3X | 0 | 7 | |
| 2 | 2 | 20 | + | - | - | 1, 2, 19, 27 | 0 | 5 | |
| 2 | 3 | 23 | + | - | - | 2, 5, 26, 27, 3X | 0 | 8 | |
| 2 | 2 | 22 | + | - | - | 2, 5, 3X | 0 | 6 | |
| 2 | 7 | 30 | + | - | - | 4, 4X | 0 | 6 | |
| 2 | 7 | 31 | - | - | - | 1, 3, 4, 31, 2X | 0 | 6 | |
| 3 | 1 | 2 | + | - | - | 2, 7, 10 | 1 | 4 | 6.6 |
| 3 | 1 | 5 | + | - | - | 1, 2, 3, 8, 27 | 1 | 6 | |
| 3 | 1 | 6[2] | + | - | - | 1, 2, 3, 27, 32 | 1 | 6 | |
| 3 | 1 | 7 | - | - | - | 1, 2, 3, 9, 16, 1X | 1 | 7 | |
| 3 | 1 | 8[2] | - | - | - | 1, 2, 3, 11, 13, 16 | 0 | 6 | |
| 3 | 2 | 19 | + | - | - | 2, 5, 16, 26, 27 | 0 | 6 | |
| 3 | 2 | 10 | + | - | - | 2, 3, 5, 11,14, 29 | 1 | 7 | |
| 3 | 2 | 15 | + | - | - | 2, 3, 5, 6, 12, 15, 2X | 1 | 9 | |
| 3 | 4 | 25 | + | + | - | na | 1 | na | |
| 3 | 5 | 26 | - | + | - | 1, 2, 3, 3.2, 23, 27, 1X | 1 | 8 | |
| 3 | 5 | 27 | - | - | - | 3, 4, 16, 22, 23, 30 | 2 | 6 | |
| 3 | 7 | 32 | + | - | - | 2[3], 4, 24, 25, 2X | 0 | 7 | |
| 4 | 6 | 28 | - | - | +[5] | 2, 4, 5 | 0 | 4 | 3,5 |
| 4 | 6 | 29[2,4] | - | - | +[5] | 4, 5 | 0 | 3 | |

+ = isolated; - = not isolated / detected; X = species unidentified or strains not obtained in pure culture; na = not applicable, or not tested; [1]) Stem base with an additional central discoloration of the wood beside the collar necrosis; [2]) stem collar had a secondary infestation with bark beetles; [3]) Ascocarps and anamorph-stages were visible on the tree surface; [4]) anamorph stages were visible on the tree surface; [5]) MT 36 = *Phytophthora plurivora*.

not been detected as foliar endophytes of *F. excelsior* in a floodplain forest in eastern Germany (Scholtysik et al. 2013) nor as endophyte of *F. excelsior* in New Zealand (Chen 2011).

In collar rots of advanced developmental stages that were not colonized by *H. fraxineus*, mainly wood decaying fungi (e. g. *X. polymorpha, F. velutipes, or Armillaria* sp.), *Ilyonetria* sp. or *Phytophthora* species have been suggested to be the causing agents. The infection with *Phytophthora* was considered to be a primary attack, because species of this genus can actively infect healthy plant tissue. *P. plurivora*, isolated from trees in Hesse, is an aggressive soil-borne plant pathogen, with worldwide distribution and a wide host range of tree species (Jung and Burgess 2009).

Except *P. plurivora* and *Armillaria* sp., most of the isolated fungi were assumed to be secondary colonizers of ash tissue necroses and were less frequent than *H. fraxineus* and *N. punicea*. Common species on dying stems and twigs of ash, such as *Cytospora pruinosa* (Fr.) Sacc. (teleomorph *Valsa cypri*), *Diaporthe eres*, *Diplodia mutila* (Fr.) Mont. (teleomorph *B. stevensii*), *Fusarium avenaceum* (Fr.) Sacc., *F. lateritium* Nees and *F. solani* (Kowalski et al. 2017) were frequently isolated, but only in advanced collar necroses or rots (categories 2-4).

The third common isolated species was *D. eres* with a frequency of 25.8%. Kowalski et al. (2017) suggested that this plant pathogen has significantly lower capacity than *H. fraxineus, B. stevensii* or *V. cypri* to cause necroses on *F. excelsior*. *D. eres* was often reported as common endophyte in symptomless stems and twigs of *F. excelsior*, but is considered as a weak pathogen (Kowalski et al. 2017). *D. eres* is known from more than 60 host species, e.g. *Fraxinus* in the Netherlands, in Scotland, and in Germany (Gomes et al. 2013). It is synonymizied with *Phomopsis cotoneastri* Punith. and its current name is *P. velata* (Sacc.) Traverso according to Index fungorum. Other *Phomopsis*-like species (MT 3.1 and 3.2) were associated with samples of category 1 necroses. *Diaporthe viticola* Nitschke, which is the closest blast match for the isolated MT 3.1, was isolated of initial necroses from shoots in declining ash (Bakys et al. 2009). It is known to colonize various hosts, especially grapevine, in which it causes the cane spot disease (Gomes et al. 2013). *Phomopsis* spp. were consistently found in healthy and necrotic shoots of declining ash in Lithuania (Bakys et al. 2009).

*B. stevensii* was isolated from the necrotic tissues of 25.8% of 31 studied trees (categories 1-3). According to studies of (Kowalski et al. 2017), *B. stevensii* was the second most pathogenic fungus, after *H. fraxineus*. It was one of the most frequently isolated species from healthy and necrotic ash shoots (Bakys et al. 2009), but not detected in rotting ash roots (Bakys et al. 2011). Furthermore, it was frequently associated with dieback and canker diseases of oak, but also occurred on several other deciduous or coniferous trees (Correia et al. 2004).

*F. solani* and *Gibberella* sp. were both isolated from the studied necrotic tissues (categories 2-4) with a frequency of 22.6%. *F. solani, F. avenaceum* and *F. latericium* were common species on dying stems and twigs of ash in Poland (Kowalski et al. 2017). However, in other studies, *F. solani* and *F. avenaceum* were not obtained from the root system of declining *F. excelsior* (Bakys et al. 2011), from healthy or necrotic shoots (Bakys et al. 2009) or from healthy leaves of ash (Scholtysik et al. 2013). *F. solani* and strains similar to MT 5 were demonstrated to be secondary pathogens on *Acer* (Langer et al. 2013). *F. latericium*, however, which is the closest blast match for the isolated *Gibberella* species (MT 5), was isolated in initial and advanced necroses from shoots in declining ash (Bakys et al. 2009) and as a foliar endophyte in ash (Scholtysik et al. 2013). *F. avenaceum* and *F. lateritium* are known as common endophytes in symptomless stems and twigs of *F. excelsior*. The intensity of induced necroses caused by *F. avenaceum* was low and those caused by *F. solani* or *F. lateritium* were statistically similar to the control (Kowalski et al. 2017).

*Valsa cypri* and *Neofabraea* sp. were associated with few samples of necroses of categories 2-3. *Valsa* Fr. species are linked to *Cytospora* Ehrenb. Fr.-anamorphs have a world-wide distribution and are saprobes or pathogens on various woody hosts (Spielman 1985). *V. cypri* is growing on Oleaceae, e.g. *Fraxinus* sp. (Spielman 1985). It was also isolated from visually healthy ash shoots (Bakys et al. 2009). According to Kowalski et al. (2017), *V. cypri* is the tested third most pathogenic species after *H. fraxineus*, to cause necrotic lesions on ash. Species of *Neofabraea* H.S. Jacks. are known to belong to the fungal community of *F. excelsior* (Chen 2011), but had not been isolated from healthy or necrotic ash tissues by Bakys et al. (2009, 2011). *Neofabraea vagabunda* (Desm.) Rossman is causing coin canker of ash (*Fraxinus* spp.) in North America (Putnam and Adams 2005).

Sporadically, various ascomycetes, e.g. *Cadophora* sp, *Epicoccum nigrum*, *Ascocoryne* sp., *Lophiostoma corticola* (Fuckel) E.C.Y. Liew, Aptroot & K.D. Hyde and *Ilyonectria* sp., were isolated from advanced collar rots. A species of the plant pathogenic genus *Cadophora* was found in roots of declining *F. excelsior*, too (Bakys et al. 2011). *E. nigrum* was among the most frequently isolated fungi from healthy and necrotic shoots of declining ash (Bakys et al. 2009) and healthy ash leaves (Scholtysik et al. 2013). *L. corticola* was also isolated from healthy ash shoots in New Zealand (Chen et al. 2011). *Ilyonectria radicicola* (Gerlach & L. Nilsson) P. Chaverri & Salgado was obtained from root rots of ash trees that were diseased by dieback (Bakys et al. 2011) and was among the most common isolated species in that study.

Moreover, wood decaying fungi, such as the ascomycetes *Xylaria polymorpha*, *Ascocoryne sp.* and MT 16 and some basidiomycetes, e.g. *Armillaria sp.*, *Flammulina velutipes*, *Coprinellus micaceus,* were obtained from advanced rots only. *Xylaria* species are belonging to the endophytic fungal community of *F. excelsior* (Chen 2011, Scholtysik et al. 2013) and were among the most common isolated species in the studied rotten ash roots in Lithuania (Bakys et al. 2011). *Armillaria* species were very often associated with root and collar rots of dieback diseased trees, as mentioned before (e.g. Bakys et al. 2011). Usually, as opportunistic pathogens, *A. gallica* and *A. cepistipes* are able to attack stressed trees (e.g. Entry et al. 1986, Lygis et al. 2005, Skovsgaard et al. 2010) and to cause white rot. In general, *A. cepistipes* has a pronounced saprophytic life cycle (Wahlstroem 1992), but it was apparently the most pathogenic of all tested fungi obtained from rotting ash roots in Lithuania (Bakys et al. 2011). *F. velutipes,* which causes white rot, is a typical stump-rotting basidiomycete and frequently occurs as a secondary damaging agent. *Coprinellus disseminatus* (Pers.) J.E. Lange and other white rotting basidiomycetes, such as *Phanerochaete* P. Karst. or *Pholiota* (Fr.) P. Kumm. species, were isolated by Bakys et al. from decaying ash roots (2011) and necrotic shoots (2009).

Summing up, collar necroses and collar rots are typical but not obligate after-effects of ash dieback. They can be caused by opportunistic wood decaying fungi on diseased trees or by primarily causing agents like *Phytophthora* species or the pathogen of ash dieback itself. In more advanced necroses and rots (categories 2-3), several species were isolated in addition to the causing agent *H. fraxineus,* such as typical bark and wood-decaying or wood-inhabiting fungi, which are known to belong to the fungal community of ash, e.g. *A. cepistipes, Coprinus disseminatus, Phlebia sp., Psathyrella candolleana* (Fr.) Maire, *Alternaria alternata* (Fr.) Keissl., *Ascocoryne sarcoides* (Jack.) Grov. & Wils., *B. stevensii, Diaporthe sp., E. nigrum, F. latericium, Gibberella avenacea* R. J. Cook, *Lophiostoma* sp., *Phialophora* species, *Phomopsis* sp., and *Xylaria* ssp. (Lygis et al. 2005, Chen 2011) or leaf-inhabiting endophytic fungi of *F. excelsior*, e.g. *Alternaria sp., E. nigrum* Link, *F. lateritium, L. corticola, Phomopsis* sp. and *Xylaria* sp. (Scholtysik et al. 2013).

Collar necroses on ash at different sites are caused by various pathogens, e.g. *H. fraxineus* in Schleswig-Holstein and Lower Saxony (ibid), *Armillaria gallica* in Germany (Baden-Wuerttemberg (Metzler and Herbstritt 2014) and in Denmark (Skovsgaard et al. 2010) and *A. gallica, A. cepistipes* and *Phytophthora* in Belgium (Chandelier 2015). An association of *Phytophthora* species with root and collar rots of mature ash trees, as documented in Hesse, was also described for Denmark and Poland, where the pathogen led to a decline of *F. excelsior* (Orlikowski et al. 2011). The studied stem collar necroses from Hesse were not associated with *H. fraxineus*. These affected trees grew in a floodplain forest, a riparian life zone of back water of the River Rhine with eutrophic, moist soils. The forest stands in north-western Germany, where *Armillaria* was isolated from collar rots in diseased ash trees, are characterized by eutrophic to well mesotrophic and weak changing moist to stagnant fresh or fresh to stock fresh soils. The association of collar rots with *Armillaria* spp. is not obligatory linked with an attack by *H. fraxineus* to the stem collar. Like other wood decaying fungi, *Armillaria* spp. can be soil-borne, opportunistic pathogens on ash trees that are weakened (e.g. by ash dieback).

For *H. fraxineus, A. alternata, B. stevensii, D. eres, E. nigrum, Phomopsis* sp. and *V. cypri,* the ability to cause symptomatic necroses in bark and cambium was proven (Bakys et al. 2009, Kowalski et al. 2017). It is also known that *A. cepistipes* can cause discolorations in functional sapwood of ash (Bakys et al. 2011). It remains unknown, if *N. punicea* itself is able to primarily cause wood discolourations or stem collar necroses. Further studies seem to be necessary to solve this problem.

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## References

**Abeln, E. C. A., de Pagter, M. A. and Verkley, G. J. M.** 2000. Phylogeny of *Pezicula, Dermea* and *Neofabraea* inferred from partial sequences of the nuclear ribosomal RNA gene cluster. *Mycologia* 92(4): 685-693.

**Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. 1997.** Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389-3402.

**Arhipova, N., Gaitnieks, T., Donis, J., Stenlid, J. and Vasaitis, R.** 2011. Butt rot incidence, causal fungi, and related yield loss in *Picea abies* stands of Latvia. *Canadian Journal of Forest Research* 41: 2337-2345.

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

**Bakys, R., Vasiliauskas, A., Ihrmark, K., Stenlid. J., Menkis, A. and Vasaitis, R.** 2011: Root rot, associated fungi and their impact on health condition of declining *Fraxinus ex-*

*celsior* stands in Lithuania. *Scandinavian Journal of Forest Research* 26: 128-135.

**Bohn, U. and Neuhäusel, R.** 2000/2003. Karte der natürlichen Vegetation Europas [Map of the Natural Vegetation of Europe]. BfN-Schriftenvertrieb im Landwirtschaftsverlag GmbH 48084 Münster.

**Booth, C.** 1971. The Genus *Fusarium*. Commonwealth Agricultural Bureaux. Commonwealth Mycological Institute, farnham Royal, Bucks, 237 pp.

**Bressem, U.** 2002. *Nectria*-Erkrankung an Bergahorn am Vogelsberg. [*Nectria*-diseases on sycamore at the Vogelsberg montains] *AFZ-Der Wald* 57: 368-370 (in German).

**Brock, P. M., Doering, H. and Bidartondo, M. I.** 2009. How to know unknown fungi: the role of a herbarium. *New Phytologist* 181 (3): 719-724.

**Butin, H.** 2011. Krankheiten der Wald- und Parkbäume. Diagnose, Biologie, Bekämpfung [Diseases of Forest and Park trees]. Ulmer Verlag, Stuttgart, 320 pp (in German).

**Ceccarelli, B.** 2011. Structure and diversity of "pathogenic" and "non-pathogenic" fungal endophyte community of *Fagus sylvatica* in the Mediterranean Basin, Thesis University of Tuscia Viterbo. 101 pp.

**Chandelier, A.** 2015: Collar necrosis in ash stands affected by *Hymenoscyphus fraxineus* in Belgium. COST Action FP1103 FRAXBACK 7th MC Meeting & Workshop, 12-16th of April 2015, Hotel "Cavtat", Dubrovnik, Croatia. Abstract.

**Chen, C., Verkley, G. J. M., Sun, G., Groenewald, J. Z. and Crous, P. W.** 2015. Redefining common endophytes and plant pathogens in *Neofabraea*, *Pezicula*, and related genera. *Fungal Biology* 30: 1-32.

**Chen, J.** 2011**.** Fungal Community Survey of Fungal Community Survey of *Fraxinus excelsior* in New Zealand. Thesis Master's programme, Swedish University of Agricultural Sciences (SLU), Department of Forest Mycology and Pathology. Uppsala, 33 pp.

**Correia, A., Luque, J. and Phillips, A.** 2004. *Botryosphaeria corticola*, sp. nov. on *Quercus* species, with notes and description of *Botryosphaeria stevensii* and its anamorph, *Diplodia mutila*. *Mycologia* 96(3): 598-613.

**Crous, P. W., Schumacher, R. K., Wingfield, M. J., Lombard, L., Giraldo, A., Christensen, M., Gardiennet, A., Nakashima, C., Pereira, O. L., Smith, A. J. and Groenewald, J. Z.** 2015. Fungal Systematics and Evolution: FUSE 1. *Sydowia* 67: 81-118.

**Domsch, K. H., Gams, W., Anderson, T. H.** 1980: Compendium of Soil Fungi - Vol. 1 & 2. Academic Press, 1264 pp.

**Enderle, R., Fussi, B., Lenz, H. D., Langer, G., Nagel, R., Metzler, B.** 2017. Ash dieback in Germany: research on disease development, resistance and management options. In: R. Vasaitis and R. Enderle (Editors), Dieback of European Ash (*Fraxinus* spp.) – Consequences and Guidelines for Sustainable Management. Swedish University of Agricultural Sciences, Uppsala, p. 89-105.

**Enderle, R., Peters, F., Nakou, A. and Metzler, B.** 2013. Temporal development of ash dieback symptoms and spatial distribution of collar rots in a provenance trial of *Fraxinus excelsior*. *European Journal of Forest Research* 132(5-6): 865-876.

**Entry, J. A., Martin, N. E., Cromack, K. Jr. and Stafford, S. G.** 1986. Light and nutrient limitation in *Pinus monticola*: Seedling susceptibility to *Armillaria* infection. *Forest Ecology and Management* 17: 189-198.

**Floudas, D. and Hibbett, D. S.** 2015. Revisiting the taxonomy of *Phanerochaete* (Polyporales, Basidiomycota) using a four gene dataset and extensive ITS sampling. *Fungal Biology* 119(8): 679-719.

**Fukami, T., Dickie, I. A., Paula Wilkie, J., Paulus, B. C., Park, D., Roberts, A., Buchanan, P. K. and Allen, R. B.** 2010. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. *Ecology Letters* 13(6): 675-684.

**Gardes, M. and Bruns, T. D.** 1993. ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113-118.

**Gerlach, W., Nirenberg, H.I.** 1982. The Genus *Fusarium* - a Pictorial atlas. In: G. Hagedorn, M. Burhenne, H.I. Nirenberg (Editors.), *Mitteilungen Biologische Bundesanstalt für Land- und Forstwirtschaft*, 406 pp.

**Gomes, R. R., Glienke, C., Videira, S. I. R., Lombard, L., Groenewald, J. Z. and Crous, P. W.** 2013. *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia* 31: 1-41.

**Groenewald, J. Z., Rego, C., Oliveira, H. and Crous, P. W.** 2012. *Cylindrocarpon* root rot: multigene analysis reveals novel species within the *Ilyonectria radicicola* species complex. *Mycological Progress* 11(3): 655-688.

**Gross, A., Holdenrieder, O., Pautasso, M., Queloz, V. and Sieber, T. N.** 2014. *Hymenoscyphus pseudoalbidus*, the causal agent of European ash dieback. *Molecular Plant Pathology* 15: 5-21.

**Gross, A. and Sieber. T. N.** 2016. Virulence of *Hymenoscyphus albidus* and native and introduced *Hymenoscyphus fraxineus* on *Fraxinus excelsior* and *Fraxinus pennsylvanica*. *Plant Pathology* 65: 655-63.

**Hasegawa, E., Ota, Y., Hattori, T. and Kikuchi, T.** 2010. Sequence-based identification of Japanese *Armillaria* species using the elongation factor-1 alpha gene. *Mycologia* 102(4): 898-910.

**Hirooka, Y., Rossman, M. J., Zhuang, W.-Y., Salgado-Zalazar, C. and Charverri, P.** 2013. Species delimitation for *Neonectria coccinea* group including the causal agents of beech bark disease in Asia, Europe, and North America. *Mycosystema* 32(3): 485-517.

**Hsieh, H. M., Lin, C. R., Fang, M. J., Rogers, J. D., Fournier, J., Lechat, C. and Ju, Y. M.** 2010. Phylogenetic status of *Xylaria* subgenus *Pseudoxylaria* among taxa of the subfamily Xylarioideae (Xylariaceae) and phylogeny of the taxa involved in the subfamily. *Molecular Phylogenetics and Evolution* 54(3): 957-969.

**Husson, C., Caël, O., Grandjean, J. P., Nageleisen, L. M. and Marçais, B.** 2012. Occurrence of *Hymenoscyphus pseudoalbidus* on infected ash logs. *Plant Pathology* 61: 889-895.

**Husson, C., Scala, B., Cael. O. Frey, P., Feau, N., Ioos, R. and Marçais, B.** 2011. *Chalara fraxinea* is an invasive pathogen in France. *European Journal of Plant Pathology* 130: 311-324.

**Jaklitsch, W. M. and Voglmayr, H.** 2011. *Nectria eustromatica* sp. nov., an exceptional species with a hypocreaceous stroma. *Mycologia* 103(1): 209-218.

**Johnston, P. R., Park, D. and Manning, M. A.** 2010. *Neobulgaria alba* sp. nov. and its *Phialophora*-like anamorph in native forests and kiwifruit orchards in New Zealand. *Mycotaxon* 113: 385-396.

**Jung, T. and Burgess, T. I.** 2009: Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia* 22: 95-110.

**Kirisits, T., Matlakova, M., Mottinger-Kroupa, S., Cech, T. and Halmschlager, E.** 2009. The current situation of ash dieback caused by *Chalara fraxinea* in Austria. In: T. Dogmus-Lehtijärvi (Editor) Proceedings of the IUFRO Working Party 7.02.02, Egirdir, Turkey, p. 97-119.

**Kiyuna, T., An, K., Kigawa, R., Sano, C., Miura, S. and Sugiyama, J.** 2012. Bristle-like fungal colonizers on the stone walls of the Kitora and Takamatsuzuka Tumuli are identified as *Kendrickiella phycomyces*. *Mycoscience* 53: 446-459.

**Kowalski, T. and Holdenrieder, O.** 2009. Pathogenicity of *Chalara fraxinea*. *Forest Pathology* 39: 1-7.

**Kowalski, T., Bilański, P. and Kraj, W. 2017**. Pathogenicity of fungi associated with ash dieback towards *Fraxinus excelsior*. *Plant Pathology*, doi:10.1111/ppa.12667

**Kräutler, K, Treitler, R. and Kirisits. T.** 2015. *Hymenoscyphus fraxineus* can directly infect intact current-year shoots of *Fraxinus excelsior* and artificially exposed leaf scars. *Forest Pathology* 45: 274-80.

**Kröber, H.** 1985. Erfahrungen mit *Phytophthora* de Bary und *Pythium* Pfringsheim [Experiences with *Phytophthora* de Bary and *Pythium* Pfringsheim]. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* 225: 1-175 (in German).

**Langer, G.** 1994. Die Gattung *Botryobasidium* Donk (Corticiaceae, Basidiomycetes) [The genus *Botryobasidium* Donk (Corticiaceae, Basidiomycetes)]. *Bibliotheca Mycologica* 158: 459 pp. (in German).

**Langer, G. J.** 2017. Current forest pathogenic fungi in Northwest Germany. In: P. Planz, P. Döbbeler, M. Grube, H. Hertel, and F. Oberwinkler (Editors), Ecology of fungi, Lichens and Moses. Kerner von Marilaun Workshop 2015 in memory of Josef Poelt. Biosyst. Ecol. Ser., Wien: ÖAW (accepted).

**Langer, G., Bressem, U. and Habermann, M.** 2013. Vermehrt Pilzkrankheiten an Bergahorn in Nordwestdeutschland [Increased fungal diseases on sycamore in Northwest Germany]. *AFZ-Der Wald* 68(6): 22-26 (in German).

**Langer, G., Harriehausen, U. and Bressem, U.** 2015a. Eschentriebsterben und Folgeerscheinungen [Ash dieback and after-effects]. *AFZ/Der Wald* 70(20): 22-28 (in German).

**Langer, G., Harriehausen, U. and Bressem, U.** 2015b. Stammfußnekrosen bei Esche [Collar rots on Ash]. *AFZ-Der Wald* 70(20): 29-31 (in German).

**Lee, I. and Langer, E.** 2012: New records of *Hyphodontia* species from Taiwan. *Nova Hedwigia* 94: 239-244.

**Luo, J. and Zhuang W.-J.** 2010. Three new species of *Neonectria* (Nectriaceae, Hypocreales) with notes on their phylogenetic positions. *Mycologia* 102(1): 142–152.

**Lygis, V., Vasiliauskas, R., Larsson, K. H. and Stenlid, J.** 2005. Wood-inhabiting fungi in stems of *Fraxinus excelsior* in declining ash stands of northern Lithuania, with particular reference to *Armillaria cepistipes*. *Scandinavian Journal of Forest Research* 20: 337–346.

**Marçais, B. Husson, C., Godart, L. and Caël, O.** 2016. Influence of site and stand factors on *Hymenoscyphus fraxineus* induced basal lesion. *Plant Pathology* 65: 1452-1461.

**Marincowitz, S., Crous, P. W., Groenewald, J. Z. and Wingfield, M. J.** 2008. Microfungi occuring on Proteaceae in the fynbos. CBS Biodiversity series 7, 166 pp.

**Metzler, B.** 2012. Eschentriebsterben: Schadensintensivierung durch Stammfußnekrosen [Ash dieback: Disease progression due to collar rots]. Waldschutz-INFO 3/2012, FVA Baden-Württemberg, Freiburg, 4 pp. (in German)

**Metzler, B. and Herbstritt, S.** 2014. Sicherheitsrisiko durch Stammfußnekrosen an Eschen, insbesondere auf Nassstandorten [Safety hazards by collar rots affecting ash, particularly on wet sites]. Waldschutz-INFO 1/2014, FVA Baden-Württemberg, Freiburg, 4 pp. (in German).

**Miles, L. A., Lopera, C. A., Gonzalez, A., Cepero de Garcia, M. C., Franco, A. E. and Restrepo, S.** 2012. Exploring the biocontrol potential of fungal endophytes from an Andean Colombian Paramo ecosystem. *Biocontrol* (Dordrecht) 57(5): 697-710.

**Muñoz, F., Marçais, B., Dufour, J. and Dowkiw, A.** 2016. Rising out of the ashes: additive genetic variation for susceptibility to *Hymenoscyphus fraxineus* in *Fraxinus excelsior*. *Phytopathology* 106(12): 1535-1543.

**Oertel, B.** 2003: Bibliographische Recherchen in der Mykologie. Ein Leitfaden für Leser im deutschsprachigen Raum und darüber hinaus [Bibliography in mycology. A guideline for readership in the German-speaking area and beyond]. *Zeitschrift für Mykologie* 69: 3-42 (in German)

**Oliveira, B. R., Barreto Crespo, M. T., San Romao, M. V., Benoliel, M. J., Samson, R. A. and Pereira, V. J.** 2013. New insights concerning the occurrence of fungi in water sources and their potential pathogenicity. *Water Research* 47(16): 6338-6347.

**Orlikowski, L. B., Ptaszek, M., Rodziewicz, A., Nechwatal, J., Thinggaard, K. and Jung, T.** 2011: *Phytophthora* root and collar rot of mature *Fraxinus excelsior* in forest stands in Poland and Denmark. *Forest Pathology* 41: 510-519.

**Putnam, M. L. and Adams, G. C.** 2005. *Phlyctema vagabunda* Causes Coin Canker of Ash (*Fraxinus* spp.) in North America. *Plant Disease* 89(7): 773.

**Rytkonen, A., Lilja, A., Drenkhan , R., Gaitnieks, T. and Hantula, J.** 2011. First record of *Chalara fraxinea* in Finland and genetic variation among isolates sampled from Åland, mainland Finland, Estonia and Latvia. *Forest Pathology* 41(3): 169-174.

**Sanger. F., Nicklen, S., Coulson, A. R**. 1977. DNA sequencing with chainterminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* 74(12): 5463–5467.

**Scholtysik, A., Unterseher, M., Scholtysik, P. and Wirth, C.** 2013. Spatio-temporal dynamics of endophyte diversity in the canopy of European ash (*Fraxinus excelsior*). *Mycological Progress* 12(2): 291–304.

**Senik, S. V., Maloshenok, L. G., Kotlova, E. R., Shavarda, A. L., Moiseenko, K. V., Bruskin, S. A., Koroleva, O. V. and Psurtseva, N. V.** 2015. Diacylglyceryltrimethylhomoserine content and gene expression changes triggered

by phosphate deprivation in the mycelium of the basidiomycete *Flammulina velutipes*. *Phytochemistry* 117: 34-42.

**Sieber, T. N.** 2007. Endophytic fungi in forest trees: are they mutualists? *Fungal Biology Reviews* 21: 75-89.

**Skovsgaard, J. P., Thomsen, I. M., Skovgaard, I. M. and Martinussen, T.** 2010. Associations between symptoms of dieback in even-aged stands of ash (*Fraxinus excelsior* L.). *Forest Pathology* 40: 7-18.

**Slippers, B., Boissin, E., Phillips, A. J. L., Groenewald, E. J. Z., Lombard, L., Wingfield, M. J., Postma, A., Burgess, T. and Crous, P. W.** 2013. Phylogenetic lineages in the Botryosphaeriales: a systematic andevolutionary framework. *Studies in Mycology* 76 (1): 31-49.

**Spielman, L. J.** 1985. A monograph of *Valsa* on hardwoods in North America. *Canadian Journal of Botany* 63: 1355-1378.

**Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S.** 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2725-2729.

**Udayanga, D., Castlebury, L. A., Rossman, A. Y. and Hyde, K. D.** 2014. Species limits in *Diaporthe*: molecular reassessment of *D. citri*, *D. cytosporella*, *D. foeniculina* and *D. rudis*. *Persoonia* 32: 83-101.

**von Arx, J. A.** 1981. The Genera of Fungi Sporulating in Pure Culture. Third, fully revised edition. Vaduz, J. Cramer Verlag, 424 pp.

**Wahlstroem, K. T.** 1992: Infection biology of *Armillaria* species: In vitro pectinolytic activity, infection strategy, field distribution and host responses, Doctoral dissertation, Swedish University of Agricultural Sciences, Uppsala, 46 pp.

**White, H. T., Bruns, T .D., Lee, A., and Taylor, J. W.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M.A. Innis, D.H. Gelefand, J.J. Snisky and T.J. White (Editors), PCR protocols, London, Academic, p. 315-322.

**Zhao, P., Luo, J., Zhuang, W., Liu, X. and Wu, B.** 2011. DNA barcoding of the fungal genus *Neonectria* and the discovery of two new species. *Science China Life Sciences* 54(7): 664-674.