

# Current forest pathogenic fungi in Northwest Germany

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**Abstract:** Three fungal pathogens occurring in Northwest Germany actually causing diseases on a great scale and relevant for forestry were presented: *Heterobasidion annosum*, *Sphaeropsis sapinea* and *Hymenoscyphus fraxineus*. *Heterobasidion* root and butt rot, *Sphaeropsis* blight and ash dieback in Northwest Germany were envisioned. Disease patterns, distribution of the causal agents in Schleswig-Holstein, Lower Saxony, Saxony-Anhalt and Hesse plus after-effects were contributed. Only the native species *H. annosum* s.str. and *H. parviporum* were observed in the investigated area and severe forestal losses were mainly ascertained in the Northwest German lowland dominated by pine stands. Noticeable losses due to *H. annosum* were observed in spruce stands and in advanced planted Douglas fir and Northern red oak in pine stands. Primary causal agents of collar rot and lesions associated with ash trees affected by ash dieback and following fungal species were identified and discussed. Regarding the primary causing fungus three different types of collar rots were detected: initiated by *H. fraxineus* as invader of bark and wood at the stem base, wood rooting fungi as opportunistic fungal pathogens in ash trees weakened by ash dieback and primary root and collar rot caused by *Phytophthora*.

## 1. Introduction

The Northwest German lowland is geologically mainly characterized by the Saale und Weichsel glacial periods which lead to spatially highly differentiated site conditions. This landscape is bordered by the coasts of the North and Baltic sea in the North and the Central European mountainous threshold in the South. 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. A distinct climatic gradient is existing from West to Easterns parts, traceable by becoming subcontinental and decreasing precipitations. The potential natural zonal vegetation is characterized by beech forests (*Fagion sylvaticae*, TÜXEN 1956, ELLENBERG 1996, BOHN & NEUHÄUSL 2000/2003). Regionally, rural areas developed to different man-made landscapes. Today, there are still several peasant ecosystems due to heath and moorland agriculture (e.g. Luneburg Heath) remaining after the last 200 years of land use. Especially the deforestation and the removal of sods in heathers and forests led to an extensive eluviation (Podsolierung) of soils and new formation of dunes. In the 19<sup>th</sup> century afforestation took place on a large scale at former peat, heath and farmland. Preferentially, Scots pine (*Pinus sylvestris* L.) was used to establish forest stands. The Northwest German Highlands are comprising areas of the low mountain range located north of the Main, e.g. parts of the Rheinische Schiefergebirge, the Harz, the mountainous areas of Lower Saxony including the Weser hills, and the mountainous area of Hesse including parts of the Rhön.

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Since 2006, the scientific supervision of forests in most parts of Northwest Germany is assigned to the Northwest German Forest Research Station, a joint department for the German federal states Schleswig-Holstein, Lower Saxony, Saxony-Anhalt and Hesse. The latter four federal states are covering ca. 2.7 million hectare forest. The Northwest German Forest Research Station is responsible for practical forest research and advising the different types of forest owners in the participating states for the benefit of the forest estate and its owners. The task of the section Mycology and Complex Diseases is the investigation of harmful fungal organisms and complex diseases in forests. Due to the different climate and geographical conditions and different vegetation types there are varying fungal pathogens causing destructive diseases in the investigated forests.

*Heterobasidion annosum* (FR.) BREF. (Bondarzewiaceae, Russulales, Basidiomycota) is very common in Germany (KRIEGLSTEINER 1991, METZLER et al. 2013b) and it is among the most destructive, necrotrophic fungi on conifers in the northern hemisphere (GONTHIER & THOR 2013). In the Northwest German lowland *H. annosum* is latently distributed in all coniferous stands with a variable degree of damage. Tentative estimations in Lower Saxony show more than 100,000 ha of infested forest stands (METZLER et al. 2013b). Especially forest stands with Scots pine in the Lüneburg Heath, reforested farmland in the Northwest German lowland, and spruce stands (*Picea* spp.) also on higher altitudes are affected. The genus *Heterobasidion* previously included five taxonomic species: *H. annosum* (Fr.) BREF., *H. araucariae* P.K. BUCHANAN, *H. insulare* (MURRILL) RYVARDEN, *H. pahangense* CORNER and *H. rutilantiforme* (MURRILL) STALPERS (GARBELOTTO & GONTHIER 2013). But species number has been risen by reason that *H. annosum* and *H. insulare* have been shown to be species complexes (KORHONEN 1978, KORHONEN et al. 1998, MITCHELSON & KORHONEN 1998, NIEMELÄ & KORHONEN 1998, DAI et al. 2002, DAI & KORHONEN 2009). The *H. annosum* species complex (*H. annosum* s. l.) comprises the Eurasian intersterility groups with the species *H. annosum* (Fr.) BREF. s. str., *H. parviporum* NIEMELÄ & KORHONEN and *H. abietinum* NIEMELÄ & KORHONEN as well as both North American groups *H. irregulare* (UNDERW.) GARBEL. & OTROSINA and *H. occidentale* OTROSINA & GARBEL. Aside from the *H. annosum* species complex most of the *Heterobasidion* species are strictly saprotrophic or show an uncertain pathogenicity (NIEMELÄ & KORHONEN 1998, DAI & KORHONEN 2009).

*H. annosum* s.str. has a wide host range. It mostly attacks pines, in particular Scots pine, but it is often associated with various other conifers including spruce and some broad-leaved trees (KORHONEN & STENLID 1998, NIEMELÄ & KORHONEN 1998). *H. parviporum* seem to be strictly associated with Norway spruce (*Picea abies* (L.) KARST.). Both species occur in Northwest Germany and lead to severe damages in stands with Norway spruce and Scots pine. *H. abietinum* is mainly distributed in the Mediterranean area and seems to be less

virulent or often saprotrophic northerly the European Alps. It is usually associated with Silver fir (*Abies alba* MILL.) or other species of the genus *Abies* and rarely occurs on larch (*Larix* spp.), pine, beech and Douglas fir (*Pseudotsuga menziesii* (MIRBEL) FRANCO (KORHONEN & STENLID 1998). Summarized, Eurasian *Heterobasidion* species were responsible for losses estimated at 790 millions Euros per year in the European Forestry caused by the root and butt rot of several conifer species (WOODWARD et al. 1998).

The North American species *H. irregulare*, the sister taxon of *H. annosum* (GARBELOTTO & GONTHIER 2013), affects species of *Pinus*, *Juniperus* and *Calocedrus decurrens* (TORR.) FLORIN (GARBELOTTO & GONTHIER 2013). It is an invasive species in Central Italy, where it was introduced presumably during World War II (GONTHIER et al. 2004). It is spreading in natural pine stands, pine plantations, in urban parks, in oak-pine mixed woodlands and in natural oak woodlands (GONTHIER et al. 2007, 2012, 2014). After GONTHIER et al. (2014) *H. irregulare* is pathogenic on both Aleppo (*P. halepensis* MILL.) and Italian stone pine (*P. pinea* L.), while it was presumed to be mostly saprobic on oak wood (GONTHIER et al. 2012). Inoculations experiments have shown that Scots pine is rather susceptible to *H. irregulare* (GARBELOTTO et al. 2010). *H. irregulare* and *H. annosum* are both pine-associated but they diverged in allopatry (DALMAN et al. 2010). Their mating systems have persisted largely compatible (STENLID & KARLSSON 1991) and therefore interspecific hybrids between both have been reported in Italy (GONTHIER et al. 2007, GONTHIER & GARBELOTTO 2011). The second North American species *H. occidentale* displays a wider host range and it occurs on several genera like *Abies*, *Picea*, *Tsuga*, *Pseudotsuga* and *Sequoiadendron* (GARBELOTTO & GONTHIER 2013).

*Sphaeropsis* blight or previously known as *Diplodia* tip blight is a disease that affects mainly pines in all stages, worldwide (STANOSZ et al. 1996). Dead, brown needles at the tips of pine branches are usually the first disease symptoms. It is caused by the anamorphic species *Sphaeropsis sapinea* (FR.) DYKO & B. SUTTON (syn. *Diplodia pinea* (DESM.) J. KICKX f., Botryosphaeriaceae, Botryosphaeriales, Ascomycota). *S. sapinea* causes tip blight shoot on several conifers trees (LANGER et al. 2011, LUCHI et al. 2007) that are growing under stressful conditions. Additionally, *S. sapinea* has been associated with crown wilt, damping-off and collar rot of seedlings, bluestain of sapwood, stem canker and root disease (SWART & WINGFIELD 1991). Most commonly, *Sphaeropsis* blight affects Austrian (*P. nigra* J.F. ARNOLD), Scots and Red (*P. resinosa* AITON) pine trees. *S. sapinea* is proofed to be an endophyte in pine (LANGER et al. 2011) because it is able to live inside the host tissue (mainly in twigs) in latent phase, without any visible symptoms. It switches to a parasitic phase when climatic and environmental factors induce the fungus to invade host cells and cause death of the tissues (STANOSZ et al. 2001). Black pycnidia occur saprotrophically on pine needles or twigs and it is frequently sporulating on dead shoots and mature seed cones (PALMER et al. 1988, FLOWERS

et al. 2001). Conidia are usually rain-splash dispersed throughout the growing season (PALMER et al. 1988), but occasionally vectored by insects (e.g. *Tomicus* sp.) on pine (LUCHI et al. 2007). The fungus is able to penetrate the host directly (BROOKHOUSER & PETERSON 1971, CHOU 1978) or through wounds (MUNCK & STANOSZ 2010). *S. sapinea* may outlast in or on asymptomatic hosts or the disease development may proceed rapidly (FLOWERS et al. 2001, SMITH et al. 2002, STANOSZ et al. 2005). Studies on the environmental factors enhancing the outbreak of *Sphaeropsis* blight in pine plantations reveal the influence of water stress on the host susceptibility (STANOSZ et al. 2001, PAOLETTI et al. 2001). Other identified disease and infection triggering factors are e.g. hail injury (SWART & WINGFIELD 1991), drought (SWART et al. 1987), insect damage (SWART et al. 1987) and defoliation by pine sawflies (LANGER et al. 2011) or other species of the pine feeding guild. Higher temperatures facilitate the infection and the colonization with *S. sapinea* of the pine tissue (SWART & WINGFIELD 1991). The fungus weakens the tree so that secondary insects and diseases may attack them. *Sphaeropsis* blight is able to disfigure significantly the tree and to kill current-year shoots, major branches, and, ultimately, entire trees. First reports of serious economic damages in Central Europe due to *S. sapinea* were from the Netherlands, in 1982 and 1985 on *P. nigra* and *P. sylvestris* (SWART & WINGFIELD 1991). Financial losses in South African forestry caused by hail injury and following *Sphaeropsis* blight in Pine plantations were estimated as approximately 3.8 million US \$ annually (ZWOLINSKY et al. 1990). In the Northwest German lowland severe losses caused by *Sphaeropsis* blight after defoliation by pine sawflies (mainly *Diprion pini* L.) were reported from pine stands with poor and sandy soils and low precipitation in the climate sensitive region of the Colbitz-Letzlinger Heath, Saxony-Anhalt (LANGER et al. 2011).

Recently, a new invasive fungal pathogen (*Hymenoscyphus fraxineus* (T. KOWALSKI) BARAL, QUELOZ & HOSOYA, Helotiaceae, Helotiales, Ascomycota) affecting native ash (*Fraxinus excelsior* L. and *Fraxinus angustifolia* VAHL) has emerged as a serious forest health problem in Europe, named Ash dieback (GROSS et al. 2014). *H. fraxineus* is believed to be native in Eastern Asia, where it is acting as an endophyte in respectively saprobiont on *F. mandshurica* RUPR. and *F. rhynchophylla* HANCE (HOSOYA et al. 1993, ZHAO et al. 2012, BARAL et al. 2014, DRENKHAN et al. 2015). The European ash dieback was first detected in 1995, and later the causing pathogen was described as *Chalara fraxinea* T. KOWALSKI from Poland (KOWALSKI 2006), which was initially miss connected with a native teleomorph *Hymenoscyphus albidus* (GILLET) W. PHILLIPS (KOWALSKI & HOLDENRIEDER 2009). Meanwhile, ash dieback is observed in most European countries. *H. fraxineus* causes leaf loss, crown dieback and bark lesions, girdling of twigs and branches, shoot and collar necroses, and discoloration of wood in affected trees. Occasionally, uneven flushing and premature leaf-shedding were observed. Ascocarps occur predominantly on

dead pseudosclerotial ash leaf petioles and rachises in the leaf litter and rarely on woody parts of ash (KIRISITS et al. 2014). If the infections are multiple and repeated annually, the disease is usually fatal either directly, or indirectly by weakening the tree to the point where it succumbs the infestation by following pests or pathogens, especially *Armillaria* species, other wood-rotting fungi, or barkbeetle like e.g. *Hylesinus fraxini* PANZER or *H. crenatus* FABRICIUS.

Common ash (*F. excelsior*) covers approximately 214,000 ha, i.e. 2.0 % of the forest area in Germany (METZLER et al. 2013a). In Northwest Germany, ash is found in private, governmental and communal or state-owned forests. State-owned forests have the following portions of ash stands (with at least 1 % ash trees as main or mixed tree species): 4.4 % (2,060 ha) Schleswig-Holstein, 5.1 % (16,600 ha) Lower Saxony and 2.4 % (2,219 ha) Saxony-Anhalt. In Hesse the ash stands with at least 20 % ash as main or mixed tree species have a portion about 0.5 % (2,990 ha) of the communal and state-owned forests. Ash dieback was first observed in Germany in 2002, the *C. fraxinea* primarily recorded in 2007 in northeastern Germany (SCHUMACHER et al. 2007). Meanwhile, the disease is distributed in all German federal states.

## 2. Materials and methods

Since 2006, complex forest diseases and diseases of forest trees caused by fungi were studied in field or laboratory either routinely or in response to request by foresters. Species were identified in the field, if possible, and noted as field records. Samples not possible to identify ad hoc were investigated using a ZEISS Axiostar plus microscope using standard procedures for fungi described in LEE & LANGER (2012). In addition to standard literature recommended by OERTEL (2003) for determination of fungi and forest diseases, the following literature was used: BOOTH 1971, GERLACH & NIRENBERG 1982, BUTIN 2011, HARTMANN et al. 2007, VON ARX 1981, DOMSCH et al. 1980). To detect disease causing fungal pathogens in tree tissues isolations were performed on different culture media in Petri dishes. As standard culture medium malt yeast pepton agar (MYP) beside malt extract agar (MEA) was used (LANGER 1994). Isolated strains were assigned to morphotypes and identified by micromorphological characters or on the basis of DNA sequence similarities of the amplified ribosomal ITS region with ITS 1 and ITS 4 (WHITE et al. 1990). Isolated fungal strains were representatively kept in MYP-slants at 4°C.

The presence of *Heterobasidion annosum* s.l. in trees showing disease symptoms was confirmed by the isolation of the fungi on malt yeast pepton agar (MYP, LANGER 1994), the record of basidocarps on the affected host tree or the evidence of the anamorph *Spiniger meineckellus* (A.J. OLSON) STALPERS on the host tissue after an incubation in a moist chamber. Species differen-



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tiation was based on host specificity, pore size and ITS sequence with primers described by WHITE et al. (1990). Survey area: Northwest Germany.

The occurrence of *Sphaeropsis sapinea* in specimens showing disease symptoms was verified by the isolation of the fungi on MYP agar or the formation of pycnidia after incubation of the material in a moist chamber (LANGER et al. 2011). Survey areas: Northwest Germany – Saxony-Anhalt, private Scots pine stands in area of the Colbitz-Letzlinger Heath; Lower Saxony, private pine stands in area of Gartow-Prezelle; Hesse, communal forests with Scots pine close to Wetzlar.

To prove *Hymenoscyphus fraxineus* as causal agent in ash tissue with symptoms of ash dieback, the fungus was isolated in pure culture and the typical anamorph stage *Chalara fraxinea* was observed. Annual surveys on severity of tree damage and progression of ash dieback had been performed since 2009 in mixed stands with old common ash (2009: 89–145 years old) in Schleswig-Holstein close die Baltic see coast at the plot Horstkoppel in the forestry district Satrup. The evaluation of ash dieback followed the forest damage classification based on declining crowns by LANGER et al. (2015a): damage classification “0” = vital, not infected or no visible infection; “1” = slightly reduced crown with minor infestation; “2” = slightly reduced crown with obvious ash dieback symptoms; “3” = highly reduced crown, beginning of the infestation with secondary insects and wood-rotting fungi; “4” = severe crown damages, infestation with secondary insects and wood-rotting fungi; “5” = dead tree, deceasing tree or tree felled because of traffic security responsibilities. Mortality and infection rates due to ash dieback were determined in selected stands of different age-classes. Since 2013 the prevalence and development of collar necrosis in common ashes were studied by isolation and determination of the causing fungi. Studied material: NW-FVA 2013-3\_1-8: 25 year old *F. excelsior*, Germany, Schleswig-Holstein, Mohrkirch, forest district Satrup Abt. 3350b, leg. U. Harriehausen and I. Krischok, 06.02.2013. NW-FVA 2013-3\_9-20: 21 year old *F. excelsior*, Germany, Schleswig-Holstein, Böklund, forest district Satrup Abt. 3410a, leg. U. Harriehausen and I. Krischok, 06.02.2013. NW-FVA 2013-9 and 10: *F. excelsior*, brush stage, Germany, Schleswig-Holstein, Nehmten, leg. I. Krischok, 06.02.2013. NW-FVA 2014-11: 15-20 year old common ashes, (*F. excelsior*), Germany, Hesse, Forest office Groß-Gerau, forest district Kühkopf-Knoblochäue, Abt. 430, leg. A. Noltensmeier, 03.06.2014. NW-FVA 2015-2\_1-3: common ashes, (*F. excelsior*), Germany, Lower-Saxony, forest district Hils-Vogler-Ost, Stroit, leg. P. Gawehn and M. Pfeffer, 21.01.2015. Survey areas: Germany – Schleswig-Holstein, forest district Satrup: Abt. 3350b (Mohrkirch), 3410a, (Böklund), Abt. 4050a, 4050b, 4049b and 4049c (Horstkoppel), privat forest in Nehmten near Plön; Lower Saxony, forest district Hils-Vogler-Ost (Stroit).

Names of fungal species follow Index of Fungi ([www.indexfungorum.org](http://www.indexfungorum.org)) and Mycobank ([www.mycobank.org](http://www.mycobank.org)). Names of insects follow Fauna Europaea (<http://www.faunaeur.org>).

Maps in figures 1–3 are based on the source GeoBasis-DE/BKG 2010 and were created by the Northwest German Forest Research Station, Dep. Forest Protection B4. Damage classification in figure 4 is based on declining crowns by LANGER et al. (2015a).

### 3. Results: Local distribution and frequency of

#### *Heterobasidion annosum* – Root and butt rot

368 studied *Heterobasidion* collections partly associated with cases of damage were mapped in Northwest Germany since 2006 until 2015 (Fig. 1). Only *H. annosum* s. str. and *H. parviporum* were detected. 84 % of all studied specimens were collected or isolated from Scots pine. The 12 (ca. 3 %) identified specimens of *H. parviporum* were collected or isolated from Norway spruce. There were no specimens of *H. abietinum* or of the non-native species *H. irregulare* and *H. occidentale* associated with losses of conifers in Schleswig-Holstein, Lower Saxony, Saxony-Anhalt and Hesse found. *H. annosum* s. str. caused die offs of trees in different age classes, mainly Scots pine. Additional identified host species/substrate of *H. annosum* s.str. and collections only identified as *H. annosum* s.l. in Northwest Germany were: *Abies grandis* (DOUGLAS ex D. DON) LINDL., *A. nobilis* (DOUGLAS ex D. DON) LINDL., *A. nordmaniana* (STEVEN) SPACH, *Betula* sp., *Carpinus betulus* L., *Fagus sylvatica*, *Larix decidua* MILL., *Picea abies*, *P. sitchensis* (BONG.) CARRIÈRE, *Pinus* sp., *P. strobus* L., *P. sylvestris*, *Pseudotsuga menziesii*, *Quercus rubra* L. and unidentified coniferous wood. In adult Scots pine stands losses of single trees as well as gaps and disintegration of forest stands due to *Heterobasidion* root and butt rot were observed mainly in the Luneburg Heath. The evidence of *H. annosum* in the butt of living Scots pine or Norway spruce trees without typical disease symptoms was very high in the Northwest German lowland e.g. in several pine stands in Lower Saxony: 94 % in the forest district Uchte (64 years old, n = 35), 98 % in the forest district Karrenbusch (41 years old, n = 285), 96 in the forest district Lintzel (43 years old, n = 108) and 88 % in the forest district Miele (58–63 years old, n = 150). In a studied Norway spruce stand in the forest district Bobenwald, *H. annosum* s.l. (*H. annosum* s.str. mixed with *H. parviporum*) could be detected in 76 % of the 237 studied trees (36, 45, 58 years old). In contrast, no *H. annosum* was traced in studied beech trees in the forest district Lintzel (88 years old, n = 20). Very often the perishing of young ca. 2–15 years old trees caused by *Heterobasidion* root rot in advanced artificial reproduction in infested pine or spruce forest stands was observed. Very susceptible were *P. menziesii* and *Q. rubra* while pines and

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*F. sylvatica* show more field resistance. Progression of the *Heterobasidion* root and butt rot was realized when harmful forest insects or other forest fungal pathogens attacked the trees, e.g. *Sphaeropsis sapinea*.

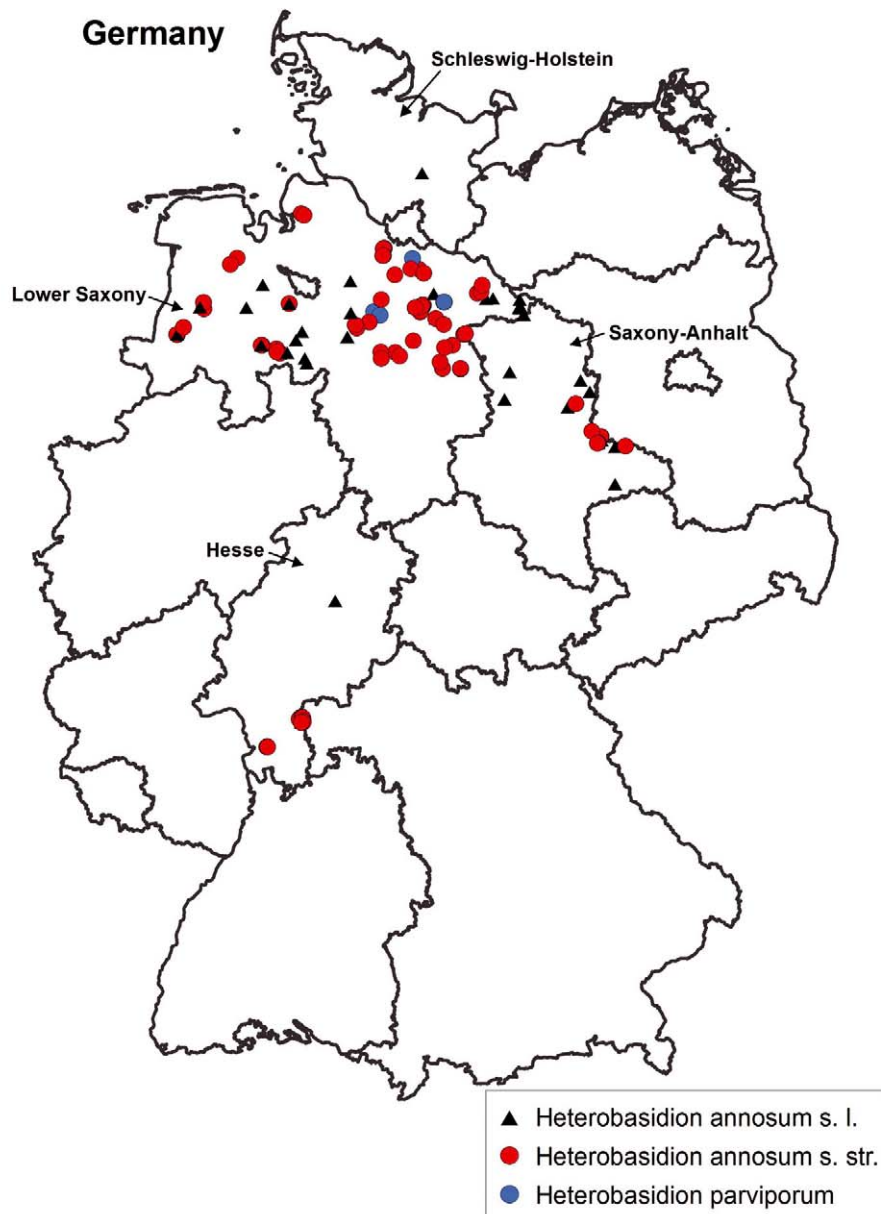


Fig. 1: Occurrence of *Heterobasidion* species from 2006 to 2015 in the German Regional States marked. Details in the text.



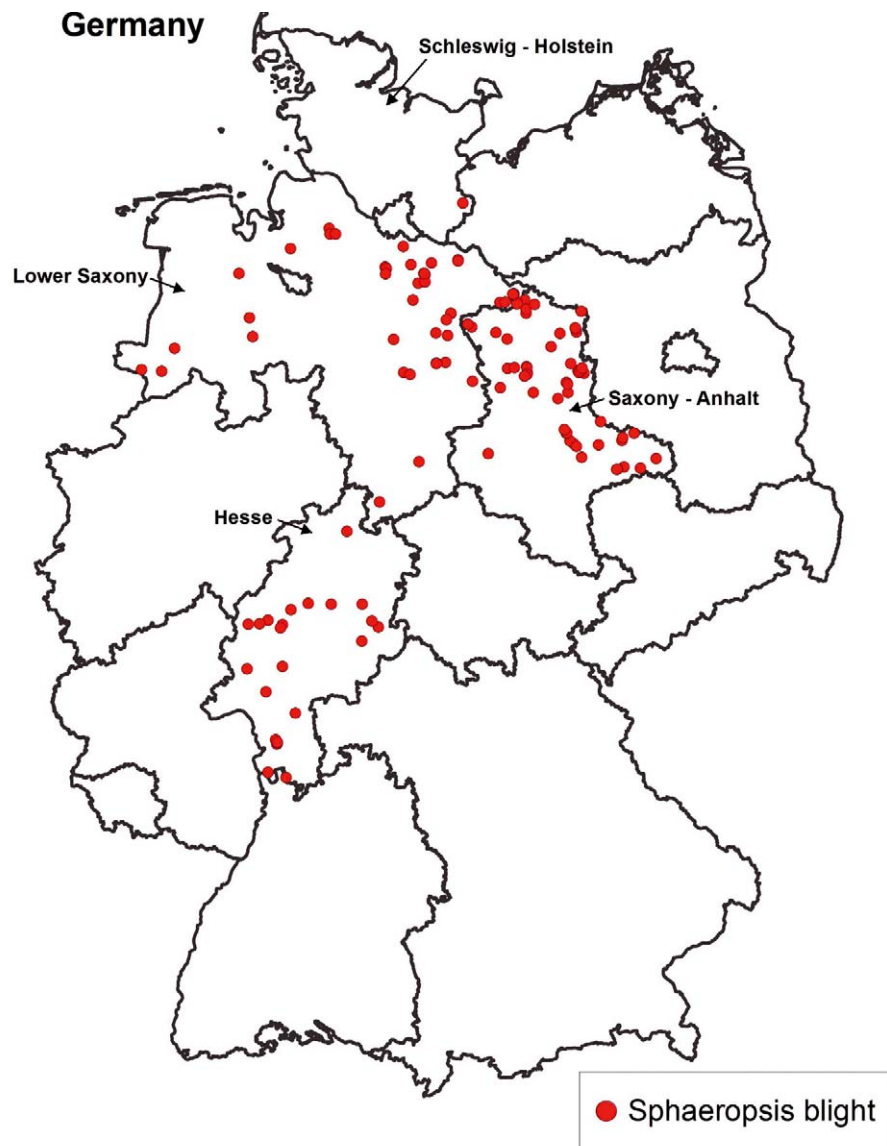


Fig. 2: *Sphaeropsis sapinea* (*Sphaeropsis* blight) from 2006 to 2014 in the German Regional States marked. Details in the text.

### *Sphaeropsis sapinea* – *Sphaeropsis* blight or *Diplodia* tip blight

The studied 115 *Sphaeropsis* blight damage events mapped in Northwest Germany since 2006 until 2014 are presented in Fig. 2. Damages due to *Sphaeropsis sapinea* were identified in all age classes of *P. sylvestris* (50 %). Additionally rechecked notifications were made sporadically from *P. nigra*, *P. mugo* TURRA, *P. strobus*, *P. ponderosa* DOUGLAS ex C. LAWSON, *P. cembra* L., *Abies*

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*concolor* (GORDON) LINDL. ex HILDEBR., *A. grandis* and *Larix decidua*, but often from *Pseudotsuga menziesii* (21 %), mainly in juvenile or brush stage. The pine stands of the Colbitz-Letzlinger Heath (Saxony-Anhalt) which had been highly defoliated in 2009 (< 70 % of the needle foliage) by pine sawflies were clear cutted and directly afforested with Scots pine. In 2014, several losses of juvenile pines due to *S. sapinea* were visible. In 2013 and following years, a defoliation of Scots pine caused by a severe outbreak of the Pine Moth (*Dendrolimus pini* L.) population in Gartow-Prezelle (Lower Saxony), a member of the pine feeding guild, took place. In the years afterwards it came to die offs by strongly defoliated pines associated with *S. sapinea* blight and *H. annosum* rot. After several weeks of drought in the summer of 2013, Scots pine (138–145 years old) were perishing in autumn the same year in the area of Wetzlar, forest districts Lemp and Hohenahr. The dying of the pines was associated with brownish, drying crowns and needle loss. After salvage cuttings and a following mild winter without heavy frost but with precipitation deficiency, the decline of pines increased in the early spring 2014. The decline was caused by *S. sapinea* partly associated with an *Armillaria* root and stem rot. The ultimate cause of the outbreak of the *Sphaeropsis* blight and the *Armillaria* rot was the drought and the predisposition of the locality. The latter is characterized by a summit to south slope position, poor sandy soils which are permeable to water and a low water supply with 600–700 mm precipitation annually.

### *Hymenoscyphus fraxineus* – Ash dieback

In Northwest Germany ash dieback was discovered in the years 2002 in Saxony-Anhalt, 2005 in Schleswig-Holstein, 2006 in Lower Saxony and 2008 in Hesse while *H. fraxineus* was proved to be the causal agent in 2007, 2006, 2006 and 2008, respectively. Since 2009, a dramatic increase in the number of infested stands and a severe disease progression was obvious. Until 2012, the occurrence of ash dieback was mapped in the forest in responsibility of the NW-FVA (Fig. 3). In 2013, ash dieback was present in all parts of Northwest Germany where common ash is growing. Strong infestations of young and old ash stands, natural regenerations and in urban greens with typical ash dieback symptoms resulting in die offs are wide spread. In Schleswig-Holstein, tree mortality is increasing in the older age classes and often connected with stem collar necrosis associated with *H. fraxineus* and *Armillaria* root rot. Therefore, salvage cuttings have been necessary since 2009. Since 2012, noticeable removal of damaged or dead trees had been mandatory, especially because of traffic security responsibilities. The progression of ash dieback in stands of adult ash was observed in the mixed stands in Horstkoppel, forest district Satriup, Schleswig-Holstein since 2009 until 2015. In the year 2009, neither healthy (“0”) nor dead trees (“5”) were assessed within the 60 selected adult

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ash trees of the infested observation plot Horstkoppel. In 2015, the portion of minor infested trees with lightly reduced crowns (damage class 1) decreased from 25 % in 2009 to 3 % in 2015. The mortality rate in year 2015 was 30 % (n = 60, Fig. 4). The mortality in an ash afforestation (n = 157 ash plants) close to Stroit, in Lower Saxony) with a high infection pressure was observed since 2009. The young ash planted in 2009 showed an infection rate of 80 % of within one year and 100 % after five years. The mortality rate increased from 0 % in 2009 to 73 % in 2015.

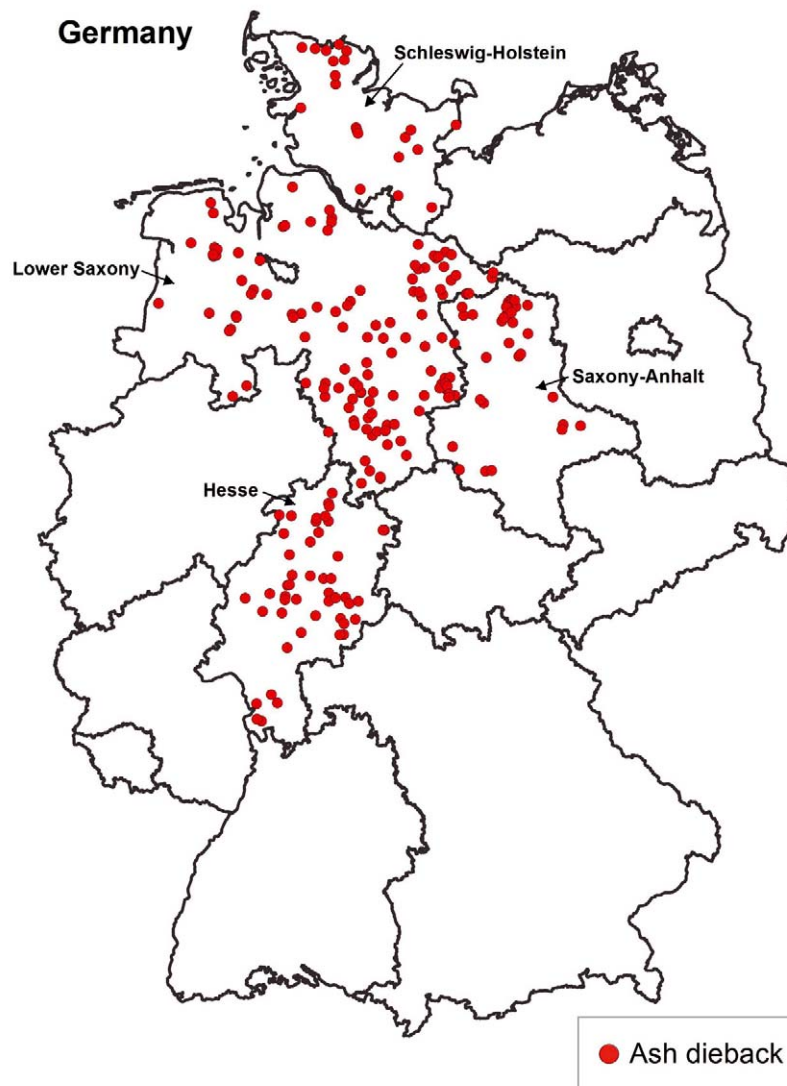


Fig. 3: Occurrence of ash dieback caused by *Hymenoscyphus fraxineus* from 2006 to 2012 in the German Regional States marked. Details in the text.

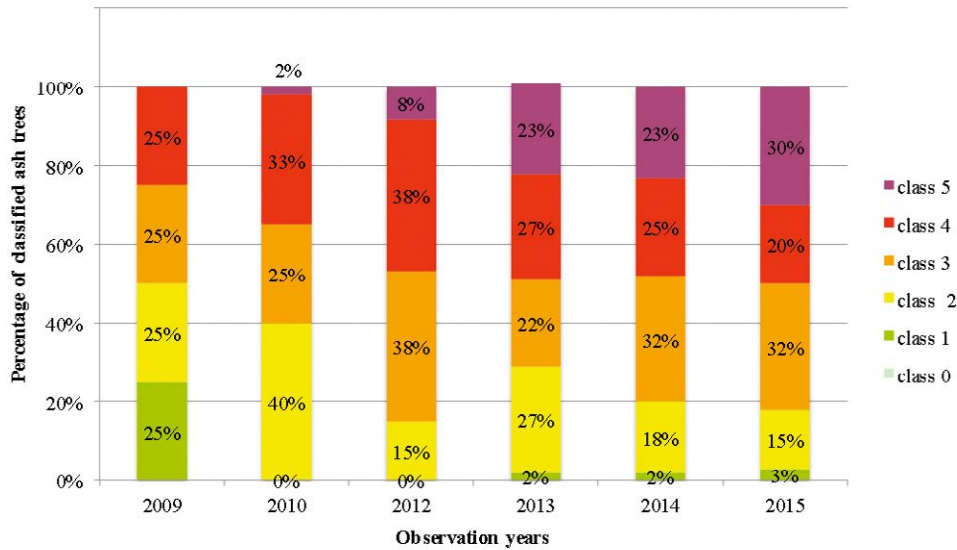


Fig. 4: The progression of ash dieback observed in the mixed stand (with year old ash trees) in Horstkoppel, Schleswig-Holstein since 2009 (ash trees 89, 96, or 145 year old) until 2015. Damage classification: class “0” = vital, not infected or no visible infection; “1” = slightly reduced crown with minor infestation; “2” = slightly reduced crown with obvious ash dieback symptoms; “3” = highly reduced crown, beginning of the infestation with secondary insects and wood-rotting fungi; “4” = severe crown damages, infestation with secondary insects and wood-rotting fungi; “5” = dead tree, deceasing tree or tree felled because of traffic security responsibilities. Details in the text.

Collar necroses were common in young ash as well as in older ashes but the primary causing fungi were different. 2014, e.g. the ash dieback affected thickend stage of common ash (16 years old) exhibited an infection rate of 96 % (n = 446) and a portion of 6.8 % (n = 347 standing ash trees) with collar rots in an afforestation close to Stroitt. Two of the three studied tissues allied with emerging collar rots collected in Stroitt were associated with *H. fraxineus*. *Neonectria* sp. could be isolated from two collar rots and its ascocarps were found on one collar lesion. *Fusarium solani* (MART.) SACC. was isolated in all three collar rot tissues and *Botryosphaeria stevensii* SHOEMAKER in one, respectively. Only a few other fungal species were sporadically isolated in addition. The DNA sequence obtained with the Primers ITS1 and ITS4 of the isolated *Neonectria* sp. show an 100 % ITS similarity with *Neonectria* sp. JL-2009a GenBank: FJ560437.1 and 99 % with *N. punicea* strain CBS 124262. Therefore the morphotype *Neonectria* sp. was referred as *N. punicea* (J.C. SCHMIDT) CASTL. & ROSSMAN.

In 2012, the 60 adult ash trees monitored in Satrup hold 82 % trees (ash dieback damage classes 2–5) with collar rots. An extra survey on the isolation of causal fungi of collar rot exhibited on 21 respectively 25 year old common ash (n = 19, forest district Satrup, plots Mohrkirch and Böklund) was conducted

in 2013. It results in 84 % collar necrosis associated with *H. fraxineus*. Collar rots of different development stages were studied. The more advanced the rot was, the more additional fungal species could be traced. E.g. only two fungal species, *H. fraxineus* and *N. punicea* were associated with the emerging collar rot of the investigated tree 2013-3\_18. *N. punicea* was isolated from 89.5 % of the studied 19 collar rots. Advanced collar rots exhibited up to seven associated fungi, e.g. tree 2013-3\_10 or 2013-3\_5 with 5 isolated species: *H. fraxineus*, *N. punicea*, *B. stevensii*, *Diaporthe eres* NITSCHKE and *Flammulina velutipes* (CURTIS) SINGER. *B. stevensii* and *Diaporthe* spp. were isolated from 31.6 % of the collar rots. Infrequently, species like *Gibberella* sp., *F. solani*, *Cytospora* sp., *Xylaria polymorpha* (PERS.) GREV. amongst other ascomycetes and basidiomycetes, but no *Armillaria* species had been isolated. Often the collar rot associated with *F. fraxineus* was connected with an fan-like brownish discoloration of the wood. In contrast, there were no rot and wood discoloration visible, nor isolation of *H. fraxineus* or other fungi was successful when studied an ash without collar necrosis (2013-3\_17). Advanced collar rots studied from other ash dieback affected trees or locations displayed *Armillaria* sp. with or without the association of *H. fraxineus* in the collar rot. Other secondary fungi like *B. stevensii*, *D. eres*, *F. solani*, *Gibberella* sp. and *Ilyonectria radicola* (GERLACH & L. NILSSON) P. CHAVERRI & C. SALGADO were also found. Case studies in 2014 on collar rots exhibited by 15–20 year old common ash trees in riparian, periodically overflowed forests (Hesse, forest district Kühkopf-Knoblochaue) showed that *Phytophthora plurivora* T. JUNG & T.I. Burgess was the cause of the rot. In that case, bark beetles (*Xylosandrus germanus* BLANDFORD) as well as the fungi *F. solani* and *Gibberella* sp. were detected as secondary invaders. There were no *Armillaria* sp. nor *H. fraxineus* specimens isolated.

#### 4. Discussion on proliferation and on ecological and economical implications

Only *H. annosum* s. str. and *H. parviporum* were identified in Northwest Germany, what goes well together with the distribution presented by GARBELOTTO & GONTHIER (2013). All studied losses due to *Heterobasidion* rot in *Abies* were caused by *H. annosum* s. str., in contrast to South Germany where *H. abietinum* is naturally distributed (METZLER et al. 2013b, GARBELOTTO & GONTHIER 2013). There were no non-native species found like in Italy (GONTHIER et al. 2004). Severe damages caused by *H. annosum* s. str. and *H. parviporum* were only recorded in the Northwest German lowland from Western Lower Saxony to Northeastern Saxony-Anhalt. *H. annosum* played a minor role as forest pathogen in the hills of low mountain range in Lower Saxony, Hesse and as well in Southwest Saxony-Anh. In the South of Hesse, especially in the Upper Rhine Rift, were losses due to *H. annosum* s. str. obvious in as-



sociation with precipitation deficiency and *Sphaeropsis* blight. Based on the high rates of *Heterobasidion* proved in living, healthy looking trees, the NW-FVA does not recommend the use of stump treatment with the control agent *Phlebiopsis gigantea* (Fr.) JÜLICH (GONTHIER & THOR 2013) in infested pine or spruce stands in the Northwest German lowlands as it is suggested in several other countries. The selection of tree species for preliminary planting under infested pine or spruce stands should consider that e.g. *P. menziesii* and *Q. rubra* are susceptible to *H. annosum* s. str. in their juvenils stages. *P. menziesii* had been tentatively classified as “moderately damaged” by *H. annosum* s. str. (GARBELOTTO & GONTHIER 2013).

The native, thermophile species *Sphaeropsis sapinea* did not appear as serious forest pathogen until the 1990s in North Germany (HEYDECK & DAHMS 2012). *S. sapinea* mainly occurred on Scots pine in Northwest Germany. It was published earlier by LUCHI et al. (2007) that it grows also in other conifer trees. In this survey, *S. sapinea* was recorded from *P. nigra*, *P. mugo*, *P. strobus*, *P. ponderosa*, *P. cembra*, *Abies concolor*, *A. grandis*, *Larix decidua* and *Pseudotsuga menziesii*. It seems to be present in an endophytic stage in all investigated areas with pine. Since 1990 the accumulation of extreme weather conditions including hails and droughts may improve the conditions necessary for the growth of *S. sapinea* (HEYDECK & DAHMS 2012) and the outbreak of *Sphaeropsis* blight. This assumption could be affirmed by the results of the presented study. Additionally, the predisposition of forest stands like exposition (summit to south slope), poor sandy soils which are permeable to water, and low water supply or infestation with other pests, like feeding insects or root rotting fungi are triggering or intensifying the disease. E.g. as after-effects of a mass gradation of pine needle feeding insects like pine sawflies in the Colbitz-Letzlinger Heath (LANGER et al. 2011) or the Pine Moth in Gartow-Prezelle several hundred hectare pine forests perished or had been clear cutted. *Sphaeropsis* blight is most severe on old or weakened trees, or juvenil trees planted at infested stands. The infested trees exposed to unsuitable growing conditions, previous mechanical injury or damage by insects or fungi may be killed.

Ash dieback spread very quickly in Northwest Germany as it is known from other countries in Europe (KOWALSKI & HOLDENRIEDER 2009, VASAITIS & LYGIS 2008, TIMMERMANN et al. 2011). It is present in all parts of Northwest Germany where common ash is growing. A dramatic increase in the number of infested stands and a severe disease progression was obvious since 2009. High infection and mortality rates as well as a large percentage of intensely diseased adult ash trees associated with collar rots were observed. Collar rots or lesions associated with ash dieback had been often reported (e.g. METZLER 2012, CHANDELIER 2015, METZLER & ENDERLE 2015). Disease progress and mortality in pole and timber size trees is boosted by increasing numbers of collar rots (METZLER & ENDERLE 2015). To the author’s mind, collar rots are typical after-

effects of ash dieback and may be primary induced by *H. fraxineus* itself, opportunistic wood rotting fungi, or *Phytophthora* sp. Under different site conditions, the prevalence of collar rots in ash is caused by different pathogens, e.g. *Armillaria gallica* MARXM. & ROMAGN. in Baden-Württemberg (METZLER & ENDERLE 2015) and *A. gallica* and *A. cepistipes* VELEN. in Belgium (CHANDELIER 2015) or *Flammulina velutipes* (ibid.). *H. fraxineus* is assumed to be the first invader of the collar tissue in the studied emerging collar rots were only a few other fungal species had been detected and no wood rotting fungi were present, because most of the additional species e.g. *Neonectria punicea*, *Botryosphaeria stevensii*, *Diaporthe eres* are usually secondary pests after injuries or known as endophytes (BRESSEM 2002, SIEBER 2007). The opinion, that the collar lesion and rot of ash trees affected by ash dieback often caused primarily by *H. fraxineus* is supported by the results of LANGER et al. (2015b) and CHANDELIER (2015). CHANDELIER reported that only 41 % of studied necrosis at the collar base were infected with *Armillaria* spp. while most of them (98 %) were infected with *H. fraxineus*.

The association of *Phytophthora* species with root and collar rots of mature ash trees was also described from Denmark and Poland, where it led to a decline of *F. excelsior* (ORLIKOWSKI et al. 2011). The most common morphotype isolated from ash collar roots, *Neonectria* sp. showed 99–100 % ITS similarity with the strain *Neonectria* sp. JL-2009a GenBank: FJ560437.1., respectively 99 % with several strains of *N. punicea*, e.g. CBS 124262. The specimen linked with GenBank accession number FJ560437 was assigned to the newly described species *Neonectria confusa* J. LUO & W.Y. ZHUANG from China (LUO & ZHUANG 2010). The latter was synonymized with the native species *N. punicea* (HIROOKA et al. 2013).

Until recently, the native *Hymenoscyphus albidus* was the only known close relative of the ash dieback pathogen *H. fraxineus* (GROSS & QUELOZ 2015). Meanwhile, four closely related species were additionally described from Asia: *H. albidoides* H.-D. ZHENG & W.-Y. ZHUANG from *Picrasma quas-sioides* in China (ZHENG & ZHUANG 2014), *H. linearis* HOSOYA, GROSS & BARAL from *Fraxinus platypoda* OLIV. in Japan, *H. koreanus* GROSS & J.G. HAN and *H. occultus* GROSS & J.G. HAN, both from *Fraxinus chinensis* ROXB. subsp. *rhynchophylla* (HANCE) A.E. MURRAY in Korea (GROSS & QUELOZ 2015). Without regard to the lack of croziers at the ascus base, the teleomorphs of the latter three species are almost indistinguishable from that of *H. fraxineus*. But anamorphs and pseudosclerotial structures differ (GROSS & QUELOZ 2015). An unsettled species complex seems to be existing in which *H. koreanus* forms a sister species to *H. albidus* (GROSS & QUELOZ 2015).

In conclusion, the question comes up whether or not there is an arising threat to *F. excelsior* and other native forest tree species, by global trade of forest products and potential hybridization of sibling species linked with an increase virulence or other non-native invading fungal species.

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