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ORIGINAL ARTICLE

Fungi associated with woody tissues of *Acer pseudoplatanus* in forest stands with different health status concerning sooty bark disease (*Cryptostroma corticale*)

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Abstract
From 2018 to 2020, Germany experienced periods of exceptional weather conditions. Extremely high summer temperatures and precipitation deficits induced stress and mortality in forest trees. *Acer pseudoplatanus* (sycamore) was one of the affected tree species. Symptoms of sooty bark disease (SBD) and severe damage of entire stands, both caused by the fungal species *Cryptostroma corticale*, were reported more frequently. To explore the non-symptomatic distribution of *C. corticale*, wood cores from visibly healthy sycamore stems were sampled and all outgrowing fungi were identified and recorded. In total, 50 trees, aged 30–65 years, were sampled at five different forest stands, from which 91 endophytic filamentous morphotypes could be isolated. The fungal endophytic community in the woody tissue of the sycamore trees varied greatly at the different sites and between the trees. The number of isolated morphotypes at the different sites ranged from 13 to 44 and no morphotype was found at all sites. At 1.20-m stem height, 3.3 fungi could be isolated from woody tissue per tree on average. The most abundant species isolated from visibly healthy sycamore in regard to both occurrence at the studied sites and continuity was *C. corticale*. It was recorded at four of the studied forest stands, from 26% of all studied sycamore trees, and had a frequency of 7.85% relative to the 293 isolated filamentous strains that were isolated. The second most abundant species was *Xylaria longipes* followed by *Lopadostoma turgidum*. In this study clear evidence for the endophytic lifestyle of *C. corticale* is presented which thus appears to be spread further than expected based on visible SBD symptoms.

Keywords *Acer pseudoplatanus* · *Cryptostroma corticale* · Endophytic fungi · Fungal community · Sooty bark disease

Introduction

Sycamore (*Acer pseudoplatanus* L., *Sapindaceae*) is a deciduous tree that can be found throughout large parts of Europe (EUFORGEN 2022). In Germany, besides *A. pseudoplatanus*, two other native *Acer* species occur, namely *Acer platanoides* L. and *Acer campestre* L. Pure maple stands, if found in Germany, are the result of active management, since no natural pure *Acer* stands exist in this region. According to the phytosociological assignment and syntaxonomical treatment following Runge (1994), maple occurs in subalpine sycamore-beech forests (*Aceri-Fagion*) or in lime-maple mixed forests (*Tilio-Acerion*). In German forests, maple is often found in mixed stands, on calcareous soil with good nutrient and good water supply. *Acer pseudoplatanus* can be found in various forest communities, mainly paired with beech and highly valuable hardwoods (Schmidt 2009). The timber is very durable and thus often used for furniture and floors. Additionally, the occasionally wavy grained wood is used for the manufacture of musical instruments, making sycamore a valuable timber species (EUFORGEN 2022). Sycamore is a very valuable urban tree species as well.

Even though many fungi are reported from *A. pseudoplatanus* (244 different fungal genera according to the USDA website, Farr and Rossman (2022)), only a few studies focus specifically on endophytes of sycamore or fungi associated with the living woody tissue of sycamore (Kowalski and Kehr 1992; Kelnarová et al. 2017). The available data on fungi associated with *A. pseudoplatanus* mainly refers to dead wood (Butin and

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Kowalski 1986; Chlebicki 1988; Unterseher et al. 2005; Brglez et al. 2020a) or to leaves (Schlegel et al. 2018).

According to Petrini (1991), Saikkonen et al. (1998), Arnold and Lutzoni (2007), and Sieber (2007), we consider those fungi as endophytes that spend a significant amount of their life cycle within the host plant tissue without causing any symptoms there. A change of environmental conditions can cause a change in the lifestyle of the fungus from endophytic to pathogenic (Sieber 2007) or to saprotrophic when the host tissue dies (Sun et al. 2011). Some fungi can be endophytic in one tree species and pathogenic in another tree species (Petrini 1991; Saikkonen et al. 2010; Sanz-Ros et al. 2015). In other cases, endophytes can change to a pathogenic lifestyle under the right circumstances, such as stress in the host tree and high summer temperatures (Ragazzi et al. 2003; Hyde and Soytong 2008). Many wood-decay fungi appear to have a transient endophytic lifestyle, which may be in preparation for their saprotrophic life stage (Boddy and Rayner 1983; Parfitt et al. 2010).

As a result of the very warm and dry summers and mild winters in the years 2018–2020, many forest trees started showing signs of stress and mortality. In Germany, these years were characterised by mean daily temperatures during the meteorological summer months (June, July, and August) averaging from 19.3 °C in 2018 (2.2 °C above average of the reference period from 1981 to 2010), to 19.2 °C in 2019 (+2.1 °C above average) and 18.2 °C in 2020 (+1.1 °C above average (DWD 2018, 2019, 2020)). The precipitation sum for the three summer months was 130 l/m^2 in 2018, 175 l/m^2 in 2019, and 230 l/m^2 in 2020, while the required average precipitation for the reference period is 239 l/m^2 (DWD 2018, 2019, 2020). As a consequence, German forests developed a soil water deficit resulting in signs of stress in the trees (NW-FVA 2020, 2021). Following these weather extremes, an extraordinary outbreak of sooty bark disease (SBD), caused by the invasive fungus *Cryptostroma corticale* (Ellis & Everh.) P.H. Greg. & S. Waller (Ellis and Everhart, 1889; Gregory and Waller 1951), was observed more frequently in several regions in Germany (Bork 2018; Rohde et al. 2019; Wenzel et al. 2019; Delb et al. 2019). Symptoms included wilting and dieback in the crown in earlier stages, as well as the production of masses of black conidia under the outer layer of the bark in later stages of the disease (Enderle et al. 2020; Schlößer and Langer 2021).

Cryptostroma corticale is presumed to be opportunistic with endophytic, pathogenic, and saprophytic life stages that react to stress, and it is known to have an optimal growing temperature of 25 °C (Dickenson 1980; Enderle et al. 2020). Currently, there is no published evidence for an endophytic/latent lifestyle of the pathogen, despite occasional isolations of *C. corticale* from symptomless tissue (e.g. Kelnarová et al. (2017); Tropf (2020)). However, in these cases, the symptomless tissue samples originate from trees already showing SBD symptoms (wood discolouration, defoliation, etc.) in other parts of the tree. As the distance of investigated symptomless samples to symptomatic tissue in no study is communicated, the fungal growth could have simply extended beyond the recognizable necrotic tissue; and therefore, a true endophytic/latent phase expressed by this species is questionable. It is assumed that *C. corticale* primarily infects the tree through fresh wounds (Townrow 1953; Dickenson 1980) and causes a soft rot in infested tissues of the tree, like many other closely related fungi from the *Xylariales* (Worrall et al. 1997; Schwarze 2018). The original description of *C. corticale* by Ellis and Everhart (1889) originated from Canada. The first record in Germany reported by the plant protection office in Berlin dates back to 1964 (Plate and Schneider 1965). In this case, spores of *C. corticale* were detected on infested firewood originating from trees of the Berlin Tiergarten park area and stored in a basement. Triggered by extremely warm weather and precipitation deficits in the year 2003, several incidences of SBD occurred in this and the following years throughout Germany and Europe (Cech 2004; Engesser et al. 2004; Metzler 2006; Robeck et al. 2008; Langer et al. 2013; Bencheva 2014; Koukol et al. 2014). The recently observed cases of disease from 2018 to 2021 constitute the biggest outbreaks of SBD in Germany yet. Since the data are recorded by each forest protection office in Germany for their respective regions, there is a gap in compiled data about the actual distribution of SBD in German forests. A preliminary distribution map regarding German forests was published by Schlößer and Langer (2021); the updated version is presented in this paper.

The main goals of this research were to undertake a better assessment of (1) fungi associated with woody tissues of *Acer pseudoplatanus* paired with an investigation into the spread of *C. corticale* in its endophytic stage, and (2) the current status and distribution of *C. corticale* as well as the potential risks of SBD in Germany. Therefore, the distribution of SBD in German forests was mapped based on compiled data from forest protection offices in Germany and fungi associated with living sycamore woody stem tissue from forest stands with different health status in respect to SBD were studied. In order to explore fungi associated with living woody tissue of sycamore, as well as the non-symptomatic distribution of *C. corticale* in visibly healthy trees, a study was conducted examining stem wood cores, following an adjusted version of the method used by Kelnarová et al. (2017). The results of this study are relevant regarding the risk assessment of potential disease outbreaks especially in light of the ongoing climate change.

Materials and methods

Mapping of the sooty bark disease

In order to map the distribution of SBD cases in German forests, three different approaches were perused: (1) infestation data from the federal forest protection institutions of

Germany were compiled (deadline 31.05.2021); (2) from May 2020 to September 2020, 123 forest stands with *A. pseudoplatanus* (aged 20–100 years, with sycamore as a dominant tree species) in Hesse were evaluated with regard to visible symptoms of sooty bark disease; (3) data on cases of SBD in Schleswig-Holstein, Lower Saxony, Hesse, and Saxony-Anhalt were retrieved from the 'Waldschutzmeldeportal' Forest Protection Reporting Portal (WSMP) of the Northwest German Forest Research Institute (NW-FVA); and (4) cases of SBD directly reported by forest owners or foresters and checked by the authors between May 2020 and March 2022. Forest owners and foresters can register georeferenced forest damage in the online WSMP. QGIS (v. 3.22.3, www.qgis.org) was used to create a combined map based on a preliminary distribution map (Schlößer and Langer 2021).

Sampling sites

Wood samples were taken from five forest stands (Table 1) containing sycamore, located in two federal states of Germany. Four of the sites are located in Hesse in the middle of Germany and one, Nehmten, in Schleswig-Holstein, in the very north of Germany. The four Hessian sites were located in the forest departments of Melsungen, Nidda, Fulda, and Beerfelden. Two of the five stands, in Fulda and in Nidda, were visibly affected by SBD at the time of sampling, and several maple trees exhibited black conidia underneath the ruptured bark. In the studied stands in Melsungen, Beerfelden, and Nehmten, no visible signs of SBD were observed. The forest stands without symptoms of SBD were located at different distances from the nearest known infestation point with *C. corticale*. The studied site in Beerfelden was 200 m away from the next SBD-affected trees, while in Melsungen there was more than 30 km of aerial distance to the closest infested stand. In Schleswig-Holstein, no case of SBD in forests had been reported until June 2022. Forest stands of different health status were chosen in order to check for occurrence of *C. corticale* in stands without visible signs of SBD.

Isolation of fungi

At each site, ten living and obviously healthy trees, aged 30–65 years and without visible symptoms of SBD, were sampled using increment borers (Haglöf Increment Borer Mora-Coretax, three-edged, 300 mm drilling depth, 5.15 mm diameter). Two 20 cm increments of stem tissue per tree were taken at a 1.20 m stem height above ground following the method of Kelnarová et al. (2017). In Melsungen, the first sampled site, three increments were taken per tree. The bark of the sample trees was sprayed with 70% ethanol at sampling height and wiped with a paper towel. Two borers were used alternately

Table 1 Sampling site conditions; apart from Nehmten (mesotrophic), all other studied forest sites are eutrophic. *UTM*, Universal Transverse Mercator (Grid)

Forest site (coordinates UTM)	Date of sampling	Elevation above sea level (m)	Exposition	Inclination	Climate	Soil water supply	Bedrock	Stand tree components	Visible signs of SBD	*C. corticale* isolated
Beerfelden (32 U 485720 5492792)	09.09–10.09.2021	450–500	North and south	Moderately inclined (0°–18°)	Strongly subatlantic	Slightly moist/moist	Granite with loamy loess	Maple, sycamore, European beech	No	Yes
Fulda (32 U 546727 5586419)	04.05.2021	410–450	West	Moderately inclined (0°–18°)	Weakly subatlantic	Slightly moist/moist	Basalt with loamy loess	Hornbeam, European beech, maple	Yes	No
Melsungen (32 U 539938 5674066)	10.03.2021	470–490	Hilltop	Slightly inclined (0°–9°)	Moderately subatlantic	Moist/changing water content	Basalt with loamy loess	Maple, European beech, European ash	No	Yes
Nehmten (32 U 589629 5993467)	30.09.–31.09.2021	66	South-west	Moderate inclination (0°–9°)	Subatlantic	Moist/high water storing capacity	Diluvial sand. Boulder clay	Sycamore	No	Yes
Nidda (32 U 502557 5581999)	13.07.2021	290–330	North	Moderate to slight inclination (0°–18°)	Weakly subatlantic	Slightly dry/ slightly moist	Basalt	European ash, sycamore	Yes	Yes

and sterilised by flame shortly before every use. The outer layer of the bark was carefully scraped and the exposed tissue was disinfected with ethanol again. Increments, taken on the vertical axis, were carefully removed from the extractor by disinfected hands and placed into clean and marked plastic tubes. The borer, extractor, and knife were cleaned each time before taking a new sample by spraying with ethanol and wiping away the excess plant material which was followed by flame sterilisation. Samples were cooled during transportation to the laboratory. Sampling was performed from March to September 2021. For each tree, diameter at breast height (1.30 m) as well as the following were recorded: crown vitality, the number of epicormic shoots as an indicator for vitality as well as visible signs of infestation with *C. corticale* or *Stegonsporium pyriforme* (Hoffm.) Corda (Online Resource 1).

In the laboratory, each increment was surface sterilised by rinsing the sample with 70% ethanol and left to dry for a few minutes. Increments were cut into 5 mm-long segments and three pieces were placed on each 90 mm petri dish containing malt yeast peptone (MYP) agar, modified according to Langer (1994) containing 0.7% malt extract (Merck, Darmstadt, Germany), 0.05% yeast extract (Fluka, Seelze, Germany), 0.1% peptone (Merck, Darmstadt, Germany), and 1.5% agar (Fluka, Seelze, Germany). From the sampling sites in Fulda, Beerfelden, Nidda, and Nehmten, the increment segments with visible wood discolouration or signs of rot were observed and counted. The petri dishes were incubated at room temperature with ambient daylight for 4 weeks. The cultures were monitored every second day in the first 2 weeks and twice a week in the third and fourth weeks. Emerging mycelia were sub-cultured into pure cultures. The pure cultures were tentatively grouped into morphotypes (MTs) based on morphological observation following the method of Schulthess and Faeth (1998). At least one representative culture for each MT was stored on MYP slants at 4 °C in the fungal culture collection of the NW-FVA.

Frequency of isolated taxa, defined as proportion of isolated strains in relation to the total number of isolated filamentous strains, was calculated. Continuity of isolated taxa, defined as the number of trees from which the fungus was isolated in relation to the total number of trees, was calculated.

Molecular analysis

As a rule, one representative strain per MT was used for genetic analysis and species identification. Mycelium was placed in 1.5 ml Eppendorf tubes with five glass beads (3 mm) and 150 µl of TE buffer (10 ml 1 mmol Tris HCl (pH 0.8), 2 ml 0.5 mmol EDTA; Carl Roth, Karlsruhe, Germany). The mycelium was crushed in a Mixer Mill MM 200 (Retsch, Haan, Germany) with 25 vibrations per second for 90 s. Subsequently, genomic DNA was extracted following the protocol of Izumitsu et al. (2012).

For all strains, the 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers ITS-1 and ITS-2 (ITS) was amplified using the primer pair ITS-1F (Gardes and Bruns 1993) + ITS-4 (White et al. 1990). For strains belonging to *Neonectria*, the actin gene (*ACT*) and portion of the β-tubulin gene (*TUB*) were additionally amplified using primer pairs T*act*1 + T*act*2 (Samuels et al. 2006) and T1 (O'Donnell and Cigelnik 1997) + Bt-2b (Glass and Donaldson 1995), respectively. The PCR mixture consisted of 1 µl of DNA and 19 µl mastermix which contained 2.5 µl 10× PCR reaction buffer (with 20 mM $MgCl_2$, Carl Roth, Karlsruhe, Germany), 1 µl of each primer (10 mmol), 2.5 µl $MgCl_2$ (25 mmol), 0.1 µl Roti®-Pol Taq HY Taq polymerase (Carl Roth, Karlsruhe, Germany), and 2.5 µl of 2 mmol dNTPs (Biozym Scientific GmbH, Hessisch Oldendorf, Germany). Each reaction was topped up to a volume of 20 µl by adding sterile water.

A StepOnePlus™ PCR System (Applied Biosystems, Waltham, Massachusetts, USA) was used to carry out the DNA amplifications. The PCR conditions for the amplification of the ITS region were set according to Bien et al. (2020). The amplification conditions for the primer pair T*act*1 + T*act*2 were as follows: initial denaturation at 94 °C for 10 min; followed by 30 cycles of denaturation at 94 °C for 35 s, annealing at 48 °C for 30 s and extension at 72 °C for 80 s; and a final extension step of 10 min at 72 °C. The amplification conditions for the primer pair T1 + Bt-2b were set according to Cabral et al. (2012b) with the exception of a 60 °C annealing temperature. A 1% agarose gel was used to visualise the PCR products. The products were sent to Eurofins Scientific Laboratory (Ebersberg, Germany) for sequencing. Initially, PCR samples of the ITS region were sequenced using the forward reaction (primer ITS-1F). In case of imprecise results, reverse reactions (primer ITS-4) were sequenced in addition. All other DNA sequence regions were sequenced by the respective forward and reverse reactions. By using BioEdit Sequence Alignment Editor (v. 7.2.5; Hall (1999)), all sequences were visually checked, and defective sequence beginnings and ends trimmed. In case of forward and reverse sequences available, consensus sequences were generated using BioEdit and further processed in the same way. Sequences were submitted to GenBank (Table 2).

Identification of fungi

Morphotypes were assigned to a taxonomic level by molecular analysis of representative strains of each morphotypic group following the method of Guo et al. (2000). The BLAST algorithm (http://www.ncbi.nlm.nih.gov/genbank, Altschul et al. (1997)) was used for fungal taxon determination. The results were re-checked against literature and known cultures for confirmation. BLAST results below a threshold of 98% identity were not trusted to be accurate enough for final

Table 2 List of isolated fungi sorted alphabetically within division: A, *Ascomycota*; B, *Basidiomycota*; M, *Mucoromycota*; I.s., Incertae sedis; occurrence at sites (Melsungen, Fulda, Beerfelden, Nidda, and Nehmten): 0, not isolated; 1, isolated. Basis of identification: '-' indicates that no NCBI Blast result was a close match for identification or that DNA extraction was unsuccessful. Blast conducted: 21.02.2022

Species	Division	Order	NW-FVA ID	Accession No.[1]	n isolates	Frequency (%)	Continuity (%)	Sites isolated from
cf. *Akanthomyces*	A	Hypocreales	6247	-	1	0.34	2	1
Angustimassarina sp.	A	*Pleosporales*	6253	ON710906[I]	5	1.71	4	2
Apiognomonia sp.	A	*Diaporthales*	6272	ON710918[I]	1	0.34	2	1
Arthrinium cf. *marii*	A	I.s.	7004	ON710953[I]	5	1.71	4	1
Arthrinium rasikravindrae	A	I.s.	6999	ON710949[I]	4	1.37	6	1
Arthrinium sp.	A	I.s.	7713	-	1	0.34	2	1
Aureobasidium sp.	A	*Dothideales*	6586	ON710936[I]	1	0.34	2	1
Beauveria cf. *bassiana*	A	*Hypocreales*	6581	ON710933[I]	1	0.34	2	1
Biscogniauxia nummularia	A	*Xylariales*	6227	ON710888[I]	5	1.71	6	2
Boeremia exigua	A	*Pleosporales*	6430	ON710928[I]	1	0.34	2	1
Cadophora prunicola	A	*Helotiales*	6596	ON710939[I]	3	1.02	6	3
Calosporella innesii	A	*Diaporthales*	6573	ON786729[I]	3	1.02	2	1
Capronia sp.	A	*Chaetothyriales*	6262	ON710913[I]	2	0.68	4	1
Cladosporium sp. 1	A	*Capnodiales*	6597	ON710940[I]	2	0.68	4	2
Cladosporium sp. 2	A	*Capnodiales*	7195	ON710963[I]	1	0.34	2	1
Cladosporium sp. 3	A	*Capnodiales*	6259	ON710910[I]	1	0.34	2	1
Cladosporium sp. 4	A	*Capnodiales*	6598	ON710941[I]	2	0.68	2	1
Cladosporium sp. 5	A	*Capnodiales*	6991	ON710944[I]	1	0.34	2	1
Cladosporium sphaerospermum	A	*Capnodiales*	7706	ON710965[I]	1	0.34	2	1
Clonostachys rosea	A	*Hypocreales*	6243	ON710898[I]	1	0.34	2	1
Cryptostroma corticale	A	*Xylariales*	7001	ON710951[I]	23	7.85	26	4
Cytospora cf. *populina*	A	*Diaporthales*	6237	ON710895[I]	19	6.48	8	1
Cytospora cf. *rodophila*	A	*Diaporthales*	6232	ON710892[I]	15	5.12	8	1
Cytospora sp.	A	*Diaporthales*	6249	ON710902[I]	1	0.34	2	1
Diaporthe cf. *eres*	A	*Diaporthales*	7714	ON710970[I]	1	0.34	2	1
Diaporthe cf. *rudis*	A	*Diaporthales*	7002	ON710952[I]	4	1.37	4	1
Diaporthe pustulata	A	*Diaporthales*	6228	ON710889[I]	3	1.02	6	2
Didymella macrostoma	A	*Pleosporales*	6244	ON710899[I]	6	2.05	6	2
Didymellaceae sp.	A	*Pleosporales*	6998	ON710948[I]	1	0.34	2	1
Dothideomycetes sp.	A	I.s.	6429	ON710927[I]	1	0.34	2	1
Dothiorella spp.	A	*Botryosphaeriales*	6230	ON710891[I]	1	0.34	2	1
Eutypa cf. *petrakii* var. *hederae*	A	*Xylariales*	6267	ON710915[I]	1	0.34	2	1
Eutypa maura	A	*Xylariales*	6245	ON710900[I]	6	2.05	4	1
Eutypella quaternata	A	*Xylariales*	6238	ON710896[I]	3	1.02	4	1
Exophiala cf. *pisciphila*	A	*Chaetothyriales*	6423	ON710924[I]	1	0.34	2	1
Furcasterigmium furcatum	A	*Glomerellales*	7014	ON710955[I]	1	0.34	2	1
Hypoxylon fragiforme	A	*Xylariales*	7707	ON710966[I]	2	0.68	4	1
Hypoxylon rubiginosum	A	*Xylariales*	7711	ON710968[I]	1	0.34	2	1
Jackrogersella cohaerens	A	*Xylariales*	7020	ON710957[I]	1	0.34	2	1
cf. *Leptodontidium* sp.	A	*Helotiales*	6276	ON710921[I]	1	0.34	2	1
Leptosillia muelleri	A	*Xylariales*	6576	ON710931[I]	6	2.05	12	2
Lopadostoma turgidum	A	*Xylariales*	6575	ON710930[I]	10	3.41	10	3
Lophiostoma carpini	A	*Pleosporales*	6993	ON710946[I]	2	0.68	2	1
Lophium arboricola	A	*Mytilinidiales*	6260	ON710911[I]	1	0.34	2	1

Table 2 (continued)

Species	Division	Order	NW-FVA ID	Accession No.[1]	n isolates	Frequency (%)	Continuity (%)	Sites isolated from
Melanomma populicola	A	*Pleosporales*	6274	ON710920[I]	1	0.34	2	1
Mycoarthris sp.	A	*Helotiales*	6426	ON710925[I]	1	0.34	2	1
Nectria cinnabarina	A	*Hypocreales*	6236	ON710894[I]	9	3.07	4	1
Neocucurbitaria acerina	A	*Pleosporales*	6258	ON710909[I]	2	0.68	4	1
Neodidymelliopsis sp.	A	*Pleosporales*	6261	ON710912[I]	2	0.68	4	2
Neoleptosphaeria rubefaciens	A	*Pleosporales*	6255	ON710907[I]	1	0.34	2	1
Neonectria cf. *ramulariae*	A	*Hypocreales*	6264	ON710914[I] ON803475[Act] ON803478[T]	3	0.68	4	1
Neonectria punicea	A	*Hypocreales*	7203	ON710964[I] ON803476[Act] ON803479[T]	2	0.68	2	1
Neonectria sp.	A	*Hypocreales*	6582	ON710934[I] ON803477[Act] ON803480[T]	1	0.34	2	1
Neosetophoma cf. *italica*	A	*Pleosporales*	6250	ON710903[I]	4	1.37	6	1
Neosetophoma cf. *samarorum*	A	*Pleosporales*	6252	ON710905[I]	2	1.02	2	1
Nigrograna cf. *norvegica*	A	*Pleosporales*	7189	ON710960[I]	1	0.34	2	1
Nigrograna mycophila	A	*Pleosporales*	6990	ON710943[I]	1	0.34	2	1
Paracamarosporium cf. *fagi*	A	*Pleosporales*	6420	ON710923[I]	1	0.34	2	1
Penicillium cf. *tularense*	A	*Eurotiales*	7021	ON710958[I]	1	0.34	2	1
Penicillium sp.	A	*Eurotiales*	6231	-	9	3.07	12	1
Petrakia irregularis	A	*Pleosporales*	6580	ON710932[I]	2	0.68	2	1
Pezicula sporulosa	A	*Helotiales*	6992	ON710945[I]	1	0.34	2	1
Phoma sp.	A	*Pleosporales*	6248	ON710901[I]	6	2.05	6	1
Pleosporales sp. 1	A	*Pleosporales*	6278	ON710922[I]	1	0.34	2	1
Pleosporales sp. 2	A	*Pleosporales*	6989	ON710942[I]	1	0.34	2	1
Pleosporales sp., cf. *Splanchnonema*	A	*Pleosporales*	6590	ON710937[I]	1	0.34	2	1
Preussia cf. *aemulans*	A	*Pleosporales*	6585	ON710935[I]	1	0.34	2	1
Pseudogymnoascus cf. *pannorum*	A	*Thelebolales*	6428	ON710926[I]	1	0.34	2	1
Querciphoma carteri	A	*Pleosporales*	6594	ON710938[I]	1	0.34	2	1
Ramularia collo-cygni	A	*Mycosphaerellales*	7023	ON710959[I]	1	0.34	2	1
Sordariomycetes sp. 1	A		7712	ON710969[I]	1	0.34	2	1
Sordariomycetes sp. 2	A		6572	ON710929[I]	1	0.34	2	1
cf. *Thyridiaceae*	A	*Thyridiales*	6256	ON710908[I]	2	0.68	2	1
Thyridium vestitum	A	*Thyridiales*	6251	ON710904[I]	14	4.78	8	1
Tolypocladium sp.	A	*Hypocreales*	7191	ON710961[I]	1	0.34	2	1
cf. *Tolypocladium* sp.	A	*Hypocreales*	7010	ON710954[I]	1	0.34	2	1
Trichoderma sp.	A	*Hypocreales*	7705	-	3	1.02	6	3
Xenocylindrosporium sp.	A	I.s.	6592	ON786728[I]	1	0.34	2	1
Xylaria longipes	A	*Xylariales*	6229	ON710890[I]	9	3.07	14	4
Agaricales sp.	B	*Agaricales*	7192	ON710962[I]	2	0.68	4	1
Coprinellus micaceus	B	*Agaricales*	6233	ON710893[I]	2	0.68	4	1
Coprinellus sp.	B	*Agaricales*	7000	ON710950[I]	1	0.34	2	1

Table 2 (continued)

Species	Division	Order	NW-FVA ID	Accession No.[1]	n isolates	Frequency (%)	Continuity (%)	Sites isolated from
Hymenochaetaceae sp.	B	*Hymenochaetales*	7019	ON710956[I]	31	10.58	4	2
Hypholoma fasciculare	B	*Agaricales*	6241	ON710897[I]	2	0.68	2	1
Porostereum spadiceum	B	*Polyporales*	6997	ON710947[I]	1	0.34	2	1
Serpula himantioides	B	*Boletales*	7710	ON710967[I]	1	0.34	2	1
Stereum cf. *hirsutum*	B	*Russulales*	6270	ON710916[I]	1	0.34	2	1
Trametes versicolor	B	*Polyporales*	6271	ON710917[I]	3	1.02	6	1
Mucoromycota sp.	M		7022	-	1	0.34	2	1
coelomycete			7709	-	1	0.34	2	1
Fungus sp.			7196	-	2	0.68	4	2

Species	Sampling sites					ITS NCBI Blast results			
	Melsungen	Fulda	Beerfelden	Nidda	Nehmten	Basis of identification	ID	Identity (%)	Reference
cf. *Akanthomyces*	1	0	0	0	0	DNA extraction failed	-	-	-
Angustimassarina sp.	1	1	0	0	0	*Lophiostoma corticola*	KU712227.1	99.46	Langer (2017)
Apiognomonia sp.	1	0	0	0	0	*Apiognomonia pseudohystrix*	MT177938.1	97.81	Li et al. (2020)
Arthrinium cf. *marii*	0	0	1	0	0	*Arthrinium marii*	NR_166043.1	99.82	Vu et al. (2019)
Arthrinium rasikravindrae	0	0	1	0	0	*Arthrinium rasikravindrae*	MT487807.1	100	Limsivilai and Yurayart unpublished
Arthrinium sp.	0	0	0	0	1	morphologically determined	-	-	-
Aureobasidium sp.	0	1	0	0	0	*Aureobasidium* sp.	MN378414.1	100	Bueno et al. (2020)
Beauveria cf. *bassiana*	0	1	0	0	0	*Beauveria bassiana*	MH859798.1	98.82	Vu et al. (2019)
Biscogniauxia nummularia	1	0	0	1	0	*Biscogniauxia nummularia*	MH860015.1	100	Vu et al. (2019)
Boeremia exigua	1	0	0	0	0	*Boeremia exigua*	MN077467.1	100	Johnston unpublished
Cadophora prunicola	0	1	1	1	0	*Cadophora prunicola*	MN232951.1	100	Bien and Damm (2020a)
Calosporella innesii	0	1	0	0	0	*Prosthecium innesii*	JF681965.1	100	Kruys and Castlebury 2012
Capronia sp.	1	0	0	0	0	*Capronia pulcherrima*	AF050256.1	99.15	Untereiner and Naveau (1999)
Cladosporium sp. 1	0	1	0	0	1	*Cladosporium allicinum*	MT573471.1	100	Wysoczański et al. (2021)
Cladosporium sp. 2	0	0	0	1	0	*Cladosporium herbarum*	MH864860.1	100	Vu et al. (2019)
Cladosporium sp. 3	1	0	0	0	0	*Cladosporium perangustum*	MT645919.1	100	Cambon et al. unpublished
Cladosporium sp. 4	0	1	0	0	0	*Cladosporium* sp.	LC586224.1	99.80	Itagaki and Hosoya (2021)
Cladosporium sp. 5	0	0	1	0	0	*Cladosporium* sp.	MN853869.1	100	Haenzi et al. (2021)
Cladosporium sphaerospermum	0	0	0	0	1	*Cladosporium sphaerospermum*	MF473261.1	99.80	Bensch et al. (2018)
Clonostachys rosea	1	0	0	0	0	*Clonostachys rosea*	MH865141.1	100	Vu et al. (2019)
Cryptostroma corticale	1	0	1	1	1	*Cryptostroma corticale*	MH857008.1	100	Vu et al. (2019)
Cytospora cf. *populina*	1	0	0	0	0	*Valsa ambiens*	MH862828.1	100	Vu et al. (2019)
Cytospora cf. *rodophila*	1	0	0	0	0	*Cytospora rhodophila*	KY051928.1	100	Jami et al. Unpublished

Table 2 (continued)

Species	Sampling sites					ITS NCBI Blast results			
	Melsungen	Fulda	Beerfelden	Nidda	Nehmten	Basis of identification	ID	Identity (%)	Reference
Cytospora sp.	1	0	0	0	0	*Cytospora* sp.	KU516449.1	100	Jankowiak (2005)
Diaporthe cf. *eres*	0	0	0	0	1	*Diaporthe eres*	MK442579.1	99.09	Crous et al. (2019)
Diaporthe cf. *rudis*	0	0	1	1	0	*Diaporthe rudis*	KC343232.1	100	Gomes et al. (2013)
Diaporthe pustulata	1	1	0	0	0	*Diaporthe pustulata*	KC343187.1	100	Gomes et al. (2013)
Didymella macrostoma	1	1	0	0	0	*Didymella macrostroma*	MH858090.1	99.58	Vu et al. (2019)
Didymellaceae sp.	0	0	1	0	0	*Didymella pomorum*	MH861278.1	100	Vu et al. (2019)
Dothideomycetes sp.	1	0	0	0	0	*Nematostoma parasiticum*	MT547819.1	97.04	Bilanski et al. unpublished
Dothiorella spp.	1	0	0	0	0	*Dothiorella vimadera*	MT587416	100	Zhang et al. 2021
Eutypa cf. *petrakii* var. *hederae*	1	0	0	0	0	*Eutypa* cf. *petrakii* var. *hederae*	MH862077	99.46	Vu et al. (2019)
Eutypa maura	1	0	0	0	0	*Eutypa maura*	AY684224.1	97.65	Trouillas and Gubler (2004)
Eutypella quaternata	1	0	0	0	0	*Eutypella quaternata*	MN698987.1	99.84	Bußkamp and Langer unpublished
Exophiala cf. *pisciphila*	1	0	0	0	0	*Exophiala pisciphila*	MH859072.1	99.84	Vu et al. (2019)
Furcasterigmium furcatum	0	0	1	0	0	*Furcasterigmium furcatum*	MH859660.1	99.8	Vu et al. (2019)
Hypoxylon fragiforme	0	0	0	0	1	*Hypoxylon fragiforme*	MH855287.1	100	Vu et al. (2019)
Hypoxylon rubiginosum	0	0	0	0	1	*Hypoxylon rubiginosum*	KC968929.1	98.04	Kuhnert et al. (2014)
Jackrogersella cohaerens	0	0	0	1	0	*Annulohypoxylon cohaerens*	KU516435.1	99.81	Jankowiak et al. (2016)
cf. *Leptodontidium* sp.	1	0	0	0	0	*Leptodontidium elatius*	AY787713.2	100	Lygis et al. (2005)
Leptosillia muelleri	0	1	1	0	0	*Leptosillia muelleri*	NR_164065.1	100	Voglmayr et al. (2019)
Lopadostoma turgidum	0	1	1	1	0	*Lopadostoma turgidum*	KC774617.1	98.21	Jaklitsch et al. (2014)
Lophiostoma carpini	0	0	1	0	0	*Lophiostoma carpini*	NR_173000.1	99.80	Andreasen et al. (2021)
Lophium arboricola	1	0	0	0	0	*Lophium arboricola*	NR_153447.1	99.39	Bills et al. (1999)
Melanomma populicola	1	0	0	0	0	*Melanomma populicola*	MT223816.1	100	Crous et al. (2020)
Mycoarthris sp.	1	0	0	0	0	*Mycoarthris* sp.	MZ493003.1	100	Kowalski and Bilański (2021)
Nectria cinnabarina	1	0	0	0	0	*Nectria cinnabarina*	MH856245.1	100	Vu et al. (2019)
Neocucurbitaria acerina	1	0	0	0	0	*Neocucurbitaria acerina*	NR_154254.1	98.91	Wanasinghe et al. (2017)
Neodidymelliopsis sp.	1	1	0	0	0	*Neodidymelliopsis polemonii*	NR_158233.1	98.56	Aveskamp et al. (2010)
Neoleptosphaeria rubefaciens	1	0	0	0	0	*Neoleptosphaeria rubefaciens*	MT153724.1	100	Bien and Damm (2020b)
Neonectria cf. *ramulariae*	1	0	0	0	0	*Neonectria candida*	JF735314.1	99.82	Cabral et al., 2012a)
Neonectria punicea	0	0	1	0	0	*Neonectria faginata*	HQ840385.1	100	Zhao et al. (2011)
Neonectria sp.	0	1	0	0	0	*Neonectria candida*	OK338557.1	100	Dove et al. unpublished
Neosetophoma cf. *italica*	1	0	0	0	0	*Neosetophoma italica*	LC206635.1	99.5	Hosoya et al. (2018)
Neosetophoma cf. *samarorum*	1	0	0	0	0	*Neosetophoma samarorum*	KF251162.1	98.42	Quaedvlieg et al. (2013)

Table 2 (continued)

Species	Sampling sites					ITS NCBI Blast results			
	Melsungen	Fulda	Beerfelden	Nidda	Nehmten	Basis of identification	ID	Identity (%)	Reference
Nigrograna cf. *norvegica*	0	0	0	1	0	*Nigrograna norvegica*	NR_147655.1	97.87	Jaklitsch and Voglmayr (2016)
Nigrograna mycophila	0	0	1	0	0	*Nigrograna mycophila*	NR_147654.1	98.11	Jaklitsch and Voglmayr (2016)
Paracamarosporium cf. *fagi*	1	0	0	0	0	*Paracamarosporium fagi*	NR_154318.1	100	Crous et al. (2015)
Penicillium cf. *tularense*	0	0	0	1	0	*Penicillium tularense*	JX313166.1	94.85	Frisvad et al. (2013)
Penicillium sp.	1	0	0	0	0	DNA extraction failed	-	-	-
Petrakia irregularis	0	1	0	0	0	*Petrakia irregularis*	NR_164281.1	99.60	Vu et al. (2019)
Pezicula sporulosa	0	0	1	0	0	*Pezicula sporulosa*	MH862573.1	100	Vu et al. (2019)
Phoma sp.	1	0	0	0	0	*Phoma* sp.	MK066907.1	100	Bußkamp et al. (2020)
Pleosporales sp. 1	1	0	0	0	0	*Pleosporales* sp.	MH063651.1	97.81	Glynou et al. (2018)
Pleosporales sp. 2	0	0	1	0	0	*Parapyrenochaeta* sp.	MK441755.1	98.13	Liu and Xu unpublished
Pleosporales sp., cf. *Splanchnonema*	0	1	0	0	0	*Splanchnonema pupula*	MN251065	100	Brglez et al., 2020a)
Preussia cf. *aemulans*	0	1	0	0	0	*Preussia aemulans*	MH858745.1	99.59	Vu et al. (2019)
Pseudogymnoascus cf. *pannorum*	1	0	0	0	0	*Pseudogymnoascus pannorum*	MH864775.1	100	Vu et al. (2019)
Querciphoma carteri	0	1	0	0	0	*Querciphoma carteri*	KF251209.1	100	Quaedvlieg et al. (2013)
Ramularia collo-cygni	0	0	0	1	0	*Ramularia collo-cygni*	NR_154944.1	100	Videira et al. (2016)
Sordariomycetes sp. 1	0	0	0	0	1	*Phomatospora biseriata*	NR_154640.1	98.69	Senanayake et al. (2016)
Sordariomycetes sp. 2	0	1	0	0	0	*Sordariomycetes* sp.	KR909162.1	98.14	Travadon et al. (2015)
cf. *Thyridiaceae*	1	0	0	0	0	*Parathyridaria* sp.	MN244551.1	100	Brglez et al. (2020b)
Thyridium vestitum	1	0	0	0	0	*Thyridium vestitum*	MH863721.1	99.82	Vu et al. (2019)
Tolypocladium sp.	0	0	0	1	0	*Tolypocladium* sp.	MT153684.1	99.81	Bien and Damm (2020b)
cf. *Tolypocladium* sp.	0	0	1	0	0	*Tolypocladium* sp.	MW064161.1	98.00	Utermann et al. (2020)
Trichoderma sp.	1	1	0	0	1	DNA extraction failed	-	-	-
Xenocylindrosporium sp.	0	1	0	0		*Xenocylindrosporium* sp.	MT791078.1	98.79	Spies et al. (2020)
Xylaria longipes	1	1	0	1	1	*Xylaria longipes*	MG098261.1	100	Bußkamp et al. (2020)
Agaricales sp.	0	0	0	1	0	*Hemipholiota populnea*	MG735315.1	99.85	Matheny et al. unpublished
Coprinellus micaceus	1	0	0	0	0	*Coprinellus micaceus*	KU712252.1	100	Langer (2017)
Coprinellus sp.	0	0	1	0	0	*Coprinellus ellisii*	MH858016.1	100	Vu et al. (2019)
Hymenochaetaceae sp.	0	0	0	1	1	*Inonotus cuticularis*	KF446595.1	93.31	Zhou et al. (2014)
Hypholoma fasciculare	1	0	0	0	0	*Hypholoma fasciculare*	MZ493083.1	100	Kowalski and Bilański (2021)
Porostereum spadiceum	0	0	1	0	0	*Porostereum spadiceum*	MH856439.1	98.15	Vu et al. (2019)
Serpula himantioides	0	0	0	0	1	*Serpula himantioides*	MH855789	99.3	Vu et al. (2019)
Stereum cf. *hirsutum*	1	0	0	0	0	*Stereum hirsutum*	LN714607.1	99.84	Větrovský et al. (2016)

Table 2 (continued)

Species	Sampling sites					ITS NCBI Blast results			
	Melsungen	Fulda	Beerfelden	Nidda	Nehmten	Basis of identification	ID	Identity (%)	Reference
Trametes versicolor	1	0	0	0	0	*Trametes versicolor*	AM084699.1	99.49	Anderson and Parkin (2007)
Mucoromycota sp.	0	0	0	1	0	DNA extraction failed	-	-	-
coelomycete	0	0	0	0	1	DNA extraction failed	-	-	-
Fungus sp.	0	0	1	1	0	DNA extraction failed	-	-	-

[1] *I*, internal transcribed spacers and intervening 5.8S nrDNA; *T*, β-tubulin gene; *Act*, actin gene

determination. Each identification was critically interpreted with emphasis on well-curated culture collections such as the Westerdijk Fungal Biodiversity Collection (CBS). In case no definite affiliation was possible to a specific taxonomic level, the identification was marked by cf. (confer) to indicate uncertainties.

Since BLAST results for different *Neonectria* and *Eutypa* strains were inconclusive, additional comprehensive analyses were performed. For strains belonging to *Neonectria*, final determination of the genus and species was based on two separate comprehensive phylogenetic analyses using the *ACT* and *TUB* gene regions, respectively, including reference sequences retrieved from GenBank (data not shown). Similarly, for the final determination of different *Eutypa* strains, a phylogenetic analysis based on the ITS results was accomplished.

Analysis with R

Analysis of the fungal diversity found in this study was conducted using RStudio V 4.1.2 (R Core Team 2021). The package tidyverse (Wickham et al. 2019) was used, where a distribution chart was created using the function 'pie'. The packages ggplot 2 (Wickham 2016) and ggVennDiagram (Gao 2021) were used to analyse the overlap between the fungi found at each site. Here the function 'ggVennDiagram' was used.

It was further checked manually and using RStudio (R Core Team 2021) whether the presence of *C. corticale* influenced the fungal community using the 'plot' function as well as the 'ddply' function of the plyr package (Wickham 2011) and a distance matrix using the function 'vegdist' from the package vegan (Oksanen et al. 2022) was generated. Additionally, it was analysed whether the third increment taken at Melsungen had a significant influence on the diversity of isolated morphotypes at that site using the 'plot' function as well.

Results

Distribution of SBD in German forests

By 31.05.2021, we had received reports of SBD from forests all over Germany with the exception of the federal state of Schleswig-Holstein, where no outbreak has been recorded so far. In total, 403 stands with obvious symptoms of SBD were reported (Fig. 1). Of the 123 evaluated stands in Hesse, 31.71% showed visible symptoms of SBD. At the beginning of 2022, a new collection of SBD reports were registered in the WSMP for Hesse and added to the map.

Isolated fungi

In total, 4124 segments of stem tissue increments originating from 50 sycamore trees were incubated. From these, 379

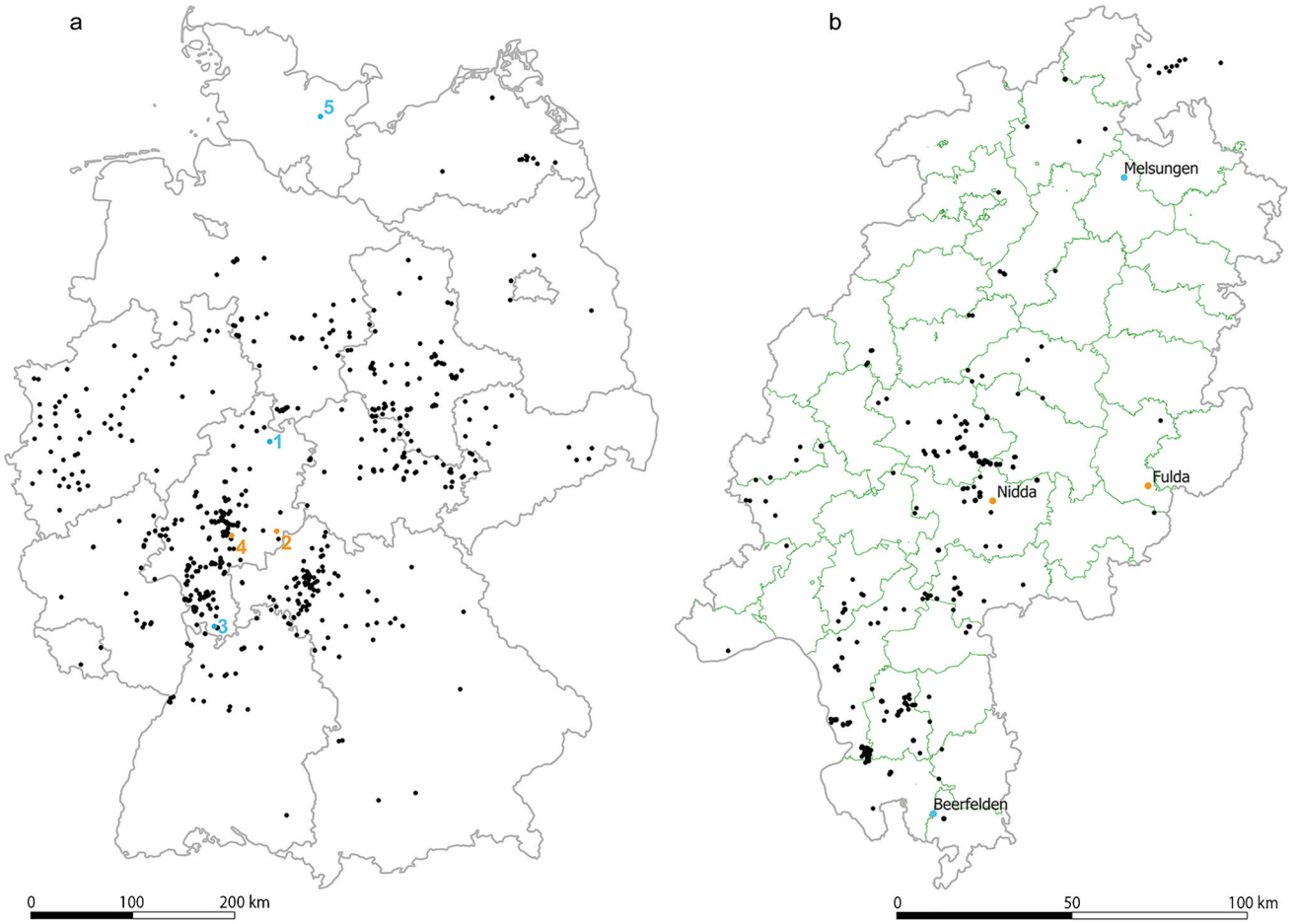

Fig. 1 **a**: Distribution of SBD cases in Germany, reported by 31.05.2021, last updated for Hesse: 31.03.2022, 1 = Melsungen, 2 = Fulda, 3 = Beerfelden, 4 = Nidda, 5 = Nehmten; **b**: detailed view of the cases in the federal state of Hesse; © GeoBasis-DE / BKG (2021), © HessenForst 2020, last updated: 31.03.2022 (QGIS Desktop 3.22.3); ● sampling sites without sooty bark disease, ● sampling sites with sooty bark disease, ● reported stands with visible sooty bark disease, ▭ hessian forest department borders, ▭ borders of the federal states.

mycelial outgrows were observed and 292 of them were transferred to pure cultures (Online Resource 2). Most fungi grew out between weeks 1 and 3 after incubation of the increment segment. When the original plates were discarded, only a few outgrowths had been observed in the preceding week. Some of the 379 outgrows were omitted due to obvious repetitions or contaminations. The resulting pure culture isolates were assigned to 91 morphotypes and all but 14 could be assigned to genus or species level (Table 2, Fig. 2). The majority of the wood tissue increments studied (93.8%, n = 3163 studied increment segments) had no visible discolouration or even signs of rotting. From sampling sites in Fulda, Beerfelden, Nidda, and Nehmten, 28 of the 80 increments showed visible signs of infection, resulting in 195 increment segments with wood discolouration or rot (6.2%, n = 3163). From those segments, yeast grew out in 78 cases and mycelia in 43 cases (nine morphotypes), and no growth was observed in 78 cases. Seven of the isolated morphotypes from discoloured or decayed wood were assigned to genus or species level, namely *Biscogniauxia nummularia* (Bull.) Kuntze, *C. corticale*, *Diaporthe* sp., *Furcasterigmium furcatum* (C. Moreau & Moreau ex W. Gams) Giraldo López & Crous, *Jackrogersella cohaerens* (Pers.) L. Wendt, Kuhnert & M. Stadler, *Leptosillia muelleri* (Duby) Voglmayr & Jaklitsch, and *Neonectria* sp. The remaining two morphotypes could be assigned to the order of *Agaricales* (NW-FVA 7192) and the family of *Hymenochaetaceae* (cf. *Inonotus* sp. NW-FVA 7019), respectively.

The majority of the isolated filamentous fungi from all samples were *Ascomycota* (79 taxa, 86.81%), nine taxa (9.89%) belonged to the division of *Basidiomycota*, and one morphotype was determined to be *Mucoromycota* (1.1%). The remaining two taxa (2.19%) could not be classified due to unsuccessful DNA extraction, one presumably being a coelomycete fungus. Within the *Ascomycota*, the most frequently observed orders (Fig. 3) were *Pleosporales* (26.58%), followed by *Xylariales* (13.92%) and *Hypocreales* (12.66%). The *Basidiomycota* morphotypes were assigned to *Agaricales* sp. (NW-FVA 7192), *Coprinellus micaceus* (Bull.) Vilgalys, Hopple & Jacq. Johnson, *Coprinellus* sp. (NW-FVA 7000), *Hymenochaetaceae* sp. (NW-FVA 7019), *Hypholoma fasciculare* (Huds.) P.

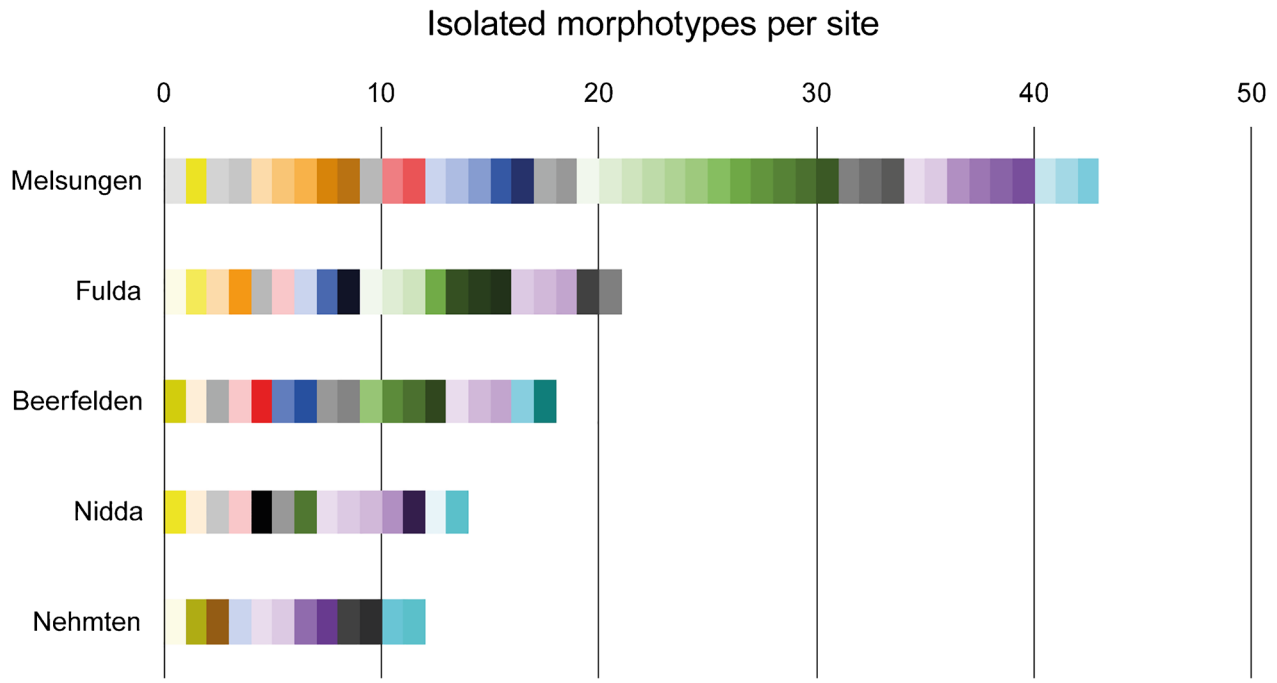

Fig. 2 Overview of isolated morphotypes per site visualized by a stacked bar chart, sorted alphabetically by order within the Ascomycota (*Capnoidales*, yellow; *Diaporthales*, orange; *Helotiales*, red; *Hypocreales*, blue; *Pleosporales*, green; *Xylariales*, purple; remaining orders of the *Ascomycota*, grey, and *Basidiomycota*, turquoise)

Kumm., *Porostereum spadiceum* (Pers.) Hjortstam & Ryvarden, *Serpula himantioides* (Fr.) P. Karst., *Stereum* cf. *hirsutum* (NW-FVA 6270), and *Trametes versicolor* (L.) Lloyd.

Of the 91 isolated morphotypes detected, 41 (45%) were isolated more than once and only six morphotypes were obtained ten or more times. The remaining 50 morphotypes (55%) were only isolated once. Between 0 and 19 different morphotypes were found in the studied woody tissue per tree. On average, 3.3 morphotypes were recorded on each tree. From four trees, no isolations could be made. None of the isolated morphotypes was found at all sites and merely 15 out of the 91 morphotypes were found at more than one site. In total, 84.62% of the isolated

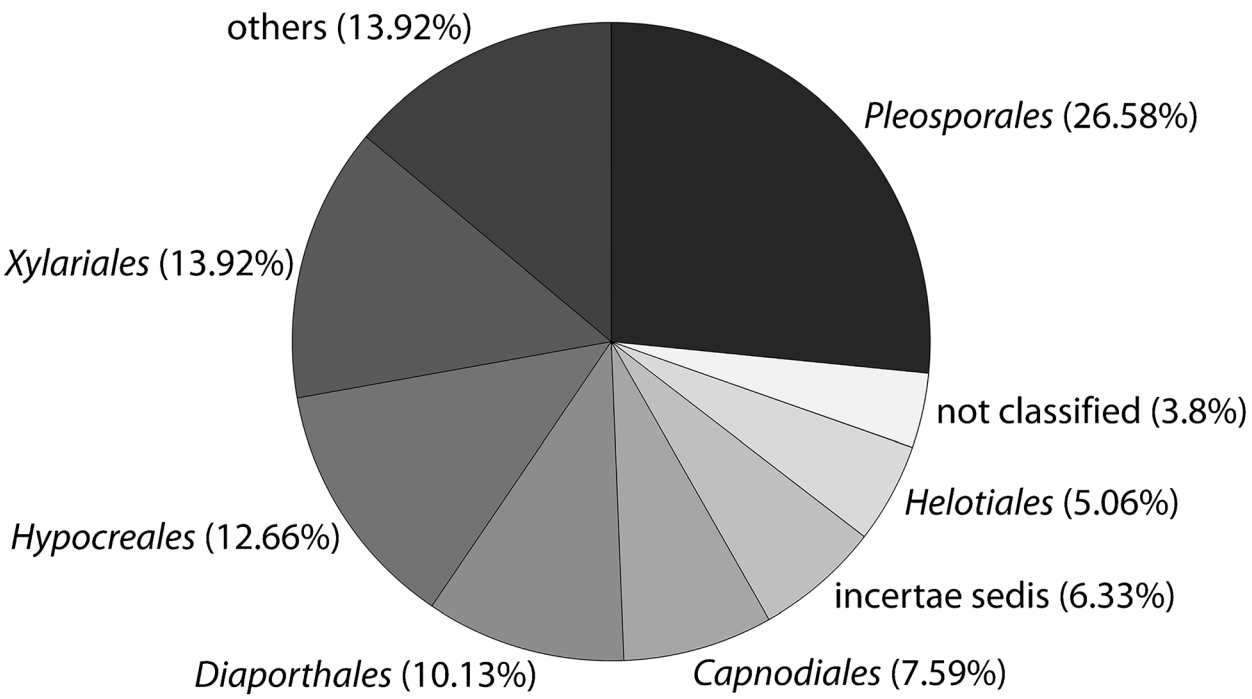

Fig. 3 Isolated orders of the *Ascomycota*, *n* = 79 of the isolated species belonging to the *Ascomycota* (RStudio 4.1.2).

morphotypes were found solely at one site while 63.74% of all isolated morphotypes were isolated from a single tree, respectively. The top ten morphotypes with the highest frequency were *Hymenochaetaceae* sp. (10.6%), *C. corticale* (7.85%), *Cytospora* cf. *populina* (6.48%), *Cytospora* cf. *rodophila* (5.12%), *Thyridium vestitum* (Fr.) Fuckel (4.78%), *Lopadostoma turgidum* (Pers.) Traverso (3.41%), *Xylaria longipes* Nitschke (3.07%), *Penicillium* sp. (3.07%), and *Nectria cinnabarina* (Tode) Fr. (3.07%).

The most abundant morphotypes, in regard to both occurrence at the studied sites and continuity, were *C. corticale* (4 sites, 26% continuity, 7.85% frequency), *X. longipes* (4, 14%, 3.07%), *Lo. turgidum* (3, 10%, 3.41%), *Cadophora prunicola* Damm & S. Bien (3, 6%, 1.02%), *Trichoderma* sp. (3, 6%, 1.02%), *L. muelleri* (2, 12%, 2.05%), *Diaporthe pustulata* Sacc. (2, 6%, 1.02%), *Didymella macrostoma* (Mont.) Qian Chen & L. Cai (2, 6%, 2.05%), and *B. nummularia* (2, 6%, 1.71%).

The number of morphotypes isolated at the different sites ranged from 13 to 44. Hence, the fungal communities at the sites studied differed in their species composition and diversity (Fig. 4). In Melsungen, 44 morphotypes (48.35% of all isolated taxa) were found and 36 (39.56%) of those occurred exclusively at this site. The most common isolated morphotypes in Melsungen, sorted by continuity, were *C. corticale*, *Penicillium* sp., *Cytospora* cf. *rodophila*, *Cytospora* cf. *populina*, and *T. vestitum*. *Cryptostroma corticale* was isolated from 60% of the trees studied at this site. Twenty-one morphotypes (23.07%) were found in the samples from the stand in Fulda, eleven of which (12.01%) were only found there. The most common species at that site were *X. longipes*, *L. muelleri*, *Lo. turgidum*, *D. pustulata*, and *Neonectria* sp. *Cryptostroma corticale* was not isolated from any tree in Fulda. At the Beerfelden study site, 19 morphotypes (20.9%) were found, 13 of which (14.29%) were found exclusively at that site. The five most frequently found species were *Arthrinium rasikravindrae* Shiv M. Singh, L.S. Yadav, P.N. Singh, Rah. Sharma & S.K. Singh, *Arthrinium* cf. *marii*, *L. muelleri*, *C. corticale*, and *Lophiostoma carpini* Andreasen, Jaklitsch & Voglmayr. *Cryptostroma corticale* was found in one of the trees studied (10%). Fifteen morphotypes were found in the Nidda samples (16.5%), eight of which (8.8%) were exclusive to that site, namely *C. corticale*, *B. nummularia*, *Lo. turgidum*, *Hymenochaetaceae* sp., and an unidentified species (NW-FVA 7196). *Cryptostroma corticale* was isolated from

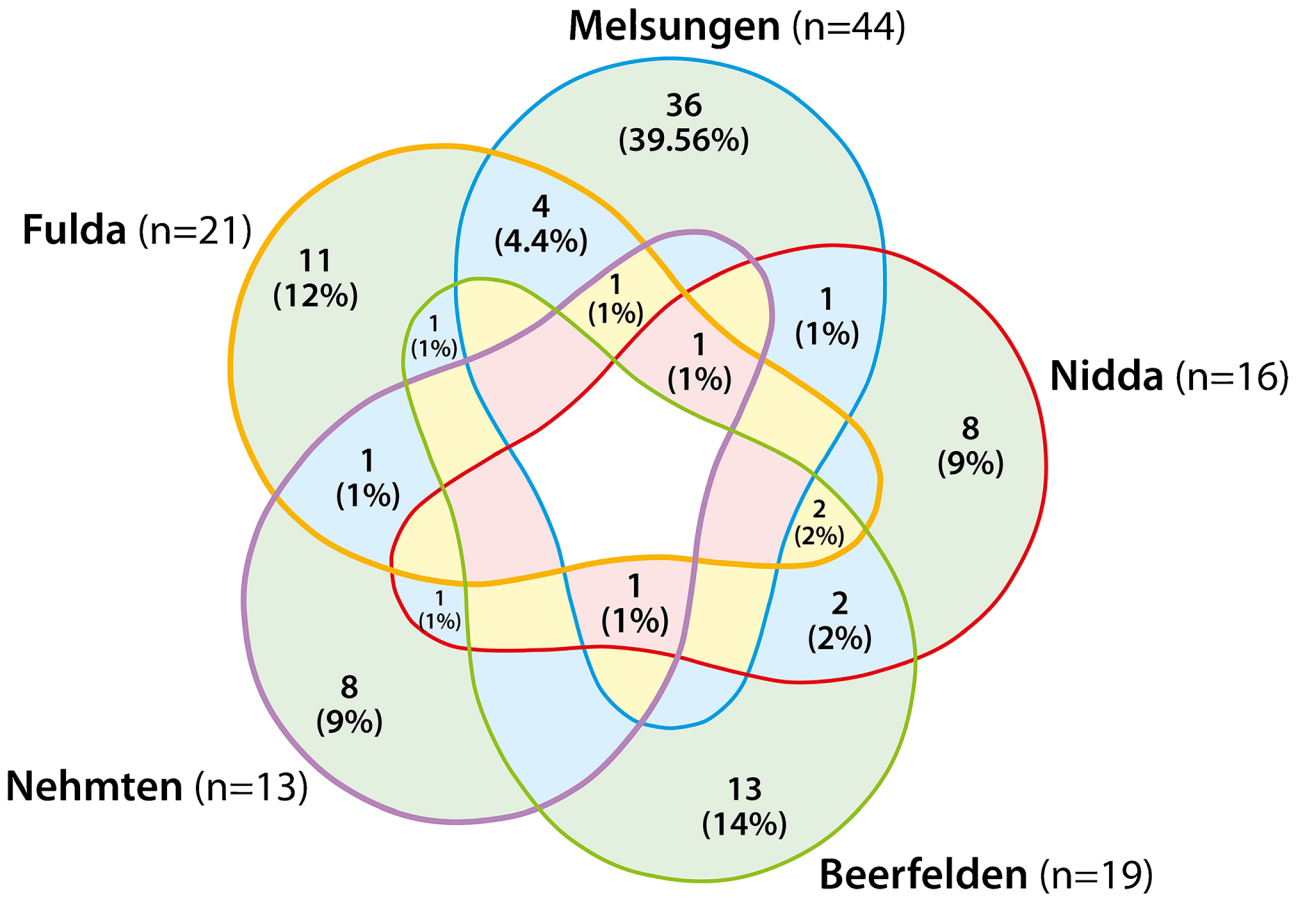

Fig. 4 Overlap between the fungi isolated from each site with the indication of how many of the isolated fungi were found at each site and between the different sites (absolute number as well as percentage); green filling, fungi isolated just at the respective site; blue, fungi isolated from two sites; yellow, fungi isolated at three sites; red, fungi isolated from four sites (RStudio 4.1.2)

five out of ten trees studied in Nidda (50%). At Nehmten, 13 morphotypes (14.29%), including *C. corticale* (10%), were isolated in total, eight of which (8.8%) were found exclusively at that site. At that location, only *Hypoxylon fragiforme* (Pers.) J. Kickx f., was isolated multiple times.

According to the statistical analyses including the distance matrix, *C. corticale* appears to have no significant influence on the fungal community observed in this study. However, eleven fungi only grew out from sampled trees where no *C. corticale* was found (*Agaricales* sp., *Angustimassarina* sp., *A. rasikravindrae*, *Cladosporium* sp. 1, *D.* cf. *rudis*, *D. pustulata*, *L. muelleri*, *Lo. turgidum*, *Neocucurbitaria acerina* Wanas., Camporesi, E.B.G. Jones & K.D. Hyde, *Neodidymelliopsis* sp., and *Neosetophoma* cf. *italica*). Only two fungi (*B. nummularia* and *Capronia* sp.) grew out from trees with *C. corticale*. The third increment taken per tree at Melsungen, increasing the sampling size in contrast to the other sampling sites, had no significant influence on the result of Melsungen being the most diverse site.

Discussion

Composition of isolated fungi

Similar to other studies on fungi isolated from tree woody tissues (Singh et al. 2017; Bußkamp 2018; Ghobad-Nejhad et al. 2018; Langer et al. 2021), in this study mainly *Ascomycota* (85.17%) and significantly less *Basidiomycota* (9.89%) were isolated. This also corresponds to studies focusing on fungi colonising different tree tissues (Petrini and Fisher 1988; Kowalski and Kehr 1992; Peršoh et al. 2010; Martínez-Álvarez et al. 2012; Sanz-Ros et al. 2015). *Basidiomycota* associated with woody tissues are found less frequently in living trees, a reason for this could be that a number of them is involved in wood decay (Langer et al. 2021).

Sieber (2007) concluded that the endophytic fungal communities in *Aceraceae* are mainly dominated by species belonging to *Diaporthales*. Furthermore, *Pleosporales* and *Xylariales* can be dominant endophytes in angiosperms. The morphotypes identified in this study mainly belong to *Pleosporales*, followed by *Xylariales* and *Hypocreales*. Isolates belonging to the *Diaporthales* constitute only the fourth most common group. In comparable studies focusing solely on woody sycamore tissue (e.g. Butin and Kowalski (1986), Kowalski and Kehr (1992), Unterseher et al. (2005), Brglez et al. (2020a)) the most commonly isolated orders include *Diaporthales*, *Helotiales*, *Hypocreales*, and *Pleosporales*. *Diaporthales* did not unequivocally dominate in any of these studies. This discrepancy in the fungal species composition could be explained by the fact that Sieber (2007) focused on different forest trees and fungi isolated from leaves and woody tissue except roots. The composition of fungal orders inhabiting leaves and woody tissue, as subsumed by Sieber (2007), might not represent the composition solely in woody tissue of *Acer* trees in specific. The fungal community of this and the aforementioned studies were not dominated by a few host-specific species as stated by Sieber (2007). In our study, only five species (*C. corticale*, *L. muelleri*, *Lo. turgidum*, *Penicillium* sp., and *X. longipes*) were found in 10% or more of the examined trees. Only one of these is host genus-specific, namely the most abundant, invasive species *C. corticale*. This might be due to the small geographical region that the analysed sites are located in, in comparison to the geographical area covered in the study of Sieber (2007), as well as the diversity of the studied plants or even the differing sampling and isolation methods used.

The total amount of isolated morphotypes from woody sycamore tissue in this study (91) is significantly higher than that in previous investigations, where 10–52 different morphotypes were detected (Butin and Kowalski 1986; Kowalski and Kehr 1992; Unterseher et al. 2005; Brglez et al. 2020a). This difference in diversity can be explained by the larger sampling size and the greater number of sites studied here in contrast to the other studies.

Many of the detected morphotypes in our study were single isolates, which may indicate a sporadic occurrence or sampling bias since only ten trees per site were studied at one specific height. However, it cannot be ruled out that these fungi occur in other stands as well as in higher abundancy and simply were not isolated from the sampled material. Due to the rather small sample size of two or three increments compared to the entire wood body of the tree, the listed fungi are likely just a small fraction of the present fungal community. Additionally, it is to be expected that the composition of fungi might differ within the tree, depending on the host tissue type (Gennaro et al. 2003) and tree age (Halley et al. 1994; Maherali and Klironomos 2007). Furthermore, a possible underestimation of fungal diversity in the studied trees may occur since not all fungi are detectable through standardised culture-based methods or in general (Guo et al. 2001; Allen et al. 2003; Unterseher 2007; Muggia et al. 2017). The composition of the forest stands combined with the nutrient and water availability could also be a factor in assessing the differences in fungal diversity per stand. The stand in Nehmten, stocked only with sycamore, had the lowest fungal diversity of all studied sites and at the same time the lowest nutrient availability.

The composition of fungi isolated in this study differed significantly between the studied forest sites, with very little overlap between the sites. It can be assumed that adding another differing site an entirely new set of fungal wood inhabitants not recorded in this study could be revealed. While some of the isolated fungi (29.1%) were already described as associated with maple (Ellis and Ellis 1985; Butin and Kowalski 1986; Chlebicki 1988; Kowalski and Kehr 1992; Unterseher

et al. 2005; Brglez et al. 2020a), most were not recorded in the aforementioned articles (70.9%). Only ten of the detected species (*C. corticale*, *D. pustulata*, *D. rudis*, *D. macrostroma*, *Eutypa maura* (Fr.) Fuckel, *N. cinnabarina*, *N. acerina*, *Petrakia irregularis* Aa, *T. vestitum*, and *X. longipes*) were listed for *A. pseudoplatanus* in the USDA fungal database (Farr and Rossman 2022). Three of them were also reported from sycamore in Germany. This further illustrates the current lack of knowledge about endophytes, specifically in woody tissue of *A. pseudoplatanus* in Germany. According to our data, no difference in the fungal composition per site was observed between trees with *C. corticale* and trees without. These results should be verified by more comprehensive studies on a larger scale.

Function of the isolated fungi

Since our goal was to isolate fungi that were expressing an endophytic life stage in *A. pseudoplatanus* wood, including the check for the presence of *C. corticale*, the far majority of the wood samples we isolated came from trees and tissue that were not exhibiting any SBD symptoms. However, a significant number of fungi have been found to switch between different lifestyles (Promputtha et al. 2010; Álvarez-Loayza et al. 2011; Eaton et al. 2011; O'Connell et al. 2012; Kuo et al. 2015). They live inside their host endophytically, inducing no visible symptoms; however, they become pathogenic, if the host plant is exposed to stress, e.g. drought (Desprez-Loustau et al. 2006; Slippers and Wingfield 2007). Therefore, inferences about the specific function of each species within the temporal-spatial succession of fungal communities linked to healthy wood of *A. pseudoplatanus* trees in Germany must be taken cautiously.

The ten most abundant fungi, including *C. corticale*, were isolated from healthy wood tissue and are discussed hereinafter. It must be assumed that all of them exhibit an endophytic life stage, even though three of them were also isolated from discoloured tissue once as well (*B. nummularia*, *C. corticale*, and *L. muelleri*). This observation for the latter three species is supported by their affiliation to the *Xylariales*, which is an order hosting many wood-decaying fungi with endophytic life stages (Hendry et al. 2002; Bußkamp et al. 2020). *Xylaria longipes* was the second most abundant species in this study and is a known endophyte in a number of different tree species. *Xylaria* species often grow and sporulate on lying deadwood and stumps (Scholtysik et al. 2013). The third most abundant morphotype, assigned to *Lo. turgidum*, belongs to the *Xylariales* as well, and has to the authors' knowledge not been reported from *Acer* before. *Cadophora prunicola*, also belonging to the ten most abundant morphotypes, is only known from its first description on *Prunus* (Bien and Damm 2020a), besides our study. This is the first report of this helotialean species from *A. pseudoplatanus*. For *C. prunicola*, an endophytic life stage is probable, since several other fungi belonging to the *Helotiales* are known endophytes (Petrini and Fisher 1988; Langer et al. 2021). Species of *Trichoderma* can occur as endophytes as described by Evans et al. (2003), while the superordinate order *Hypocreales* is a group hosting several other endophytic species as well. The isolation of *Diaporthe pustulata* in this study was of no surprise since it was originally described from *A. pseudoplatanus* (Gomes et al. 2013). Species belonging to the *Diaporthales* can occur as endophytes (Suryanarayanan 2011; Bußkamp et al. 2020; Langer et al. 2021). Therefore and because it was isolated from healthy tissue, *D. pustulata* might also have an endophytic stage in its life cycle. The pleosporalean fungus *Didymella macrostroma* is presumed to have an endophytic life stage as well, since many other fungi in the *Pleosporales* have endophytic life stages (Langer et al. 2021). *Biscogniauxia nummularia* is a known and widespread multi-host endophyte isolated from several different tree species (Petrini-Klieber 1985; Chapela and Boddy 1988; Chapela 1989; Nugent et al. 2005; Bußkamp et al. 2020) belonging to the *Xylariales* as well, and very common on beech trees (Chapela and Boddy 1988; Chapela 1989). To the authors' knowledge, this fungus has not been reported from *Acer* before.

Occurrence of SBD and spread of *C. corticale*

The observed widespread occurrence of SBD in Germany coincides with information from neighbouring central European countries (Oliveira Longa et al. 2016; Kelnarová et al. 2017; Cech 2019; Queloz et al. 2020). As shown by the SBD reports documented in this study, there are no cases of the disease in the forests of the most northern federal state of Germany, Schleswig-Holstein. This might be due to a more maritime and thus more humid climate. When comparing the site conditions of the forest stand where the fungal wood inhabitants were isolated, it can also be observed that the site in Nehmten, Schleswig-Holstein, has a different bedrock and higher water storing capacity than the other sites (Table 1). Additionally, the site in Nehmten is influenced by groundwater due to being located in a lake region. This indicates that even in drought periods, the plant water availability is more stable, resulting in less plant stress. The climate data for the summers 2018 and 2019 show that temperatures in Hesse were in general higher, as well as in relation to the average reference period with less precipitation in comparison to those in Schleswig-Holstein (DWD 2018, 2019), indicating potential higher drought stress and thus more favourable circumstances for the outbreak of SBD in Hesse, especially with reference to the respective site conditions (Table 1).

The results of Kelnarová et al. (2017) indicate an endophytic life stage of *C. corticale* as it could be detected in part from healthy woody tissue and in a quarter of all studied urban sycamore trees in Prague. However, the study only states that wood discolouration was observed in 59% of all sampled trees

and it was not clarified whether *C. corticale* was isolated from discoloured or healthy tissue, only a positive correlation between wood discolouration and the presence of *C. corticale* was mentioned. This assumption is confirmed by our observations of the latent non-symptomatic stage of *C. corticale* in forest trees in Germany. This is the first verification of an endophytic life stage for *C. corticale*. As shown in this study, 26% of all observed, apparently healthy sycamores harbour *C. corti*cale non-symptomatically, regardless of the occurrence of external symptoms in the studied forest stands or area. This leads to the assumption that latent infection with *C. corticale* is widespread in Germany. The risk of mortality due to *C. corticale* for apparently healthy sycamore trees rises with the increase of years with drought and extraordinary high temperatures, since SBD is triggered by these circumstances (Dickenson 1980; Ogris et al. 2021). Even though only vital trees with no signs of distress or injury were chosen for our sampling, 35% of the increments exhibited wood discolouration and/or wood rot. In Kelnarová et al. (2017), discolouration was observed in 59% of all sampled trees and was correlated strongly with the presence of *C. corticale*. In contrast, in this study, *C. corticale* was isolated only once from discoloured woody tissue. Besides *C. corticale*, other wood decay fungi such as *B. nummularia*, *Hymenochaetaceae* sp., and *J. cohaerens* were associated with the discoloured tissues. However, the amount of discoloured tissue observed in this study was rather low, due to our focus on vital trees.

C. corticale was isolated from two of the three stands without obvious symptoms of SBD (Beerfelden, Melsungen, and Nehmten). Especially the proof of *C. corticale* in Nehmten was surprising since symptoms of SBD have not yet been reported from forests in the entire federal state of Schleswig-Holstein. The detection of *C. corticale* in Beerfelden was unexpected, due to the high wood quality and tree vitality of the sampled stand. In contrast, the isolation of *C. corticale* in Nidda was expected, due to obvious symptoms of SBD at the site. The fungus was not detected at the stand of Fulda despite obvious symptoms. Similar to the results of Kelnarová et al. (2017), who used both a culture and a non-culture–based isolation method, the number of trees *C. corticale* was isolated from in our study was higher than expected from the occurrence of SBD with the exception of Fulda. The continuity of *C. corticale* in the presented study is 26%, similar to the results of Kelnarová et al. (2017) where the continuity, based on both isolation methods combined, was 25%.

Conclusion

The results in this paper illustrate once more that *C. corticale* can be isolated from healthy woody tissue of sycamore, thus confirming a latent life stage of this pathogenic fungus. It can be assumed that the current occurrence of the endophytic stage of *C. corticale* associated with *A. pseudoplatanus* is more widely spread than expected. This increases the risk of further outbreak of SBD in forests in the light of climate change. Therefore, the spread of *C. corticale* should be further investigated in Germany as well as in Europe in order to estimate the potential risk for *Acer* trees in this region. The presented data functions as a basis in this endeavour. An increase of negative effects due to climate change, such as rising temperature and insufficient precipitation, needs to be expected for the future, and constitutes major risks for the forest sector. The detected isolated morphotypes could be a basis for future studies concerning potential biological control agents against *C. corticale*.

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Author Contribution The study including sampling, lab work, and analysis was primarily conducted by R. Schlößer with support from G. Langer and S. Bien. The first draft of the manuscript was written by R. Schlößer and revised by S. Bien, G. Langer, and E. Langer.

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Data availability The DNA sequences generated in this study were deposited in GenBank (https://www.ncbi.nlm.nih.gov; Table 2). All sampling data is provided in the online resources (ESM 1 and ESM 2).

Declarations

Ethics approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

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