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Trophic Abilities of *Trametes Gibbosa* (Polyporales, Basidiomycota) With Respect to The Wood of *Fagus Sylvatica*

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Trametes gibbosa is a widespread polypore fungus occurring throughout Europe and Asia. It is considered a saprotroph occasionally exhibiting parasitic activity. The aims of the present experimental work were to: (1) determine the ability of *T. gibbosa* pure culture to decompose beech wood under laboratory conditions, (2) test the trophic abilities of *T. gibbosa* by comparative analysis of the development of its mycelium inoculated into the stems of living beech trees and into beech rollers (stem segments – dead substrate). The laboratory wood decay experiment revealed considerable weight loss of the beech wood samples exposed to *T. gibbosa* (32.7% after 120 days). Identification of the mycelium used for inoculation in natural forest conditions was done by morphological analysis of the cultures and molecular barcoding. Attempts to recover *T. gibbosa* mycelium were made after two and eight years for the living trees, and after two years for the rollers. While *T. gibbosa* could not be reisolated from the inoculated beech trees, basidiomata were observed on the beech rollers, and genetically verified *T. gibbosa* mycelium was extracted from them. We conclude that *T. gibbosa* exhibits strong saprotrophic inclinations.

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Introduction

Trametes gibbosa (Pers.) Fr., which belongs to the family Polyporaceae in the order Polyporales [www.mycobank.org], is a widespread fungus throughout the forests of Europe and Asia [Kotlaba 1984; Breitenbach and Kränzlin 1986; Ryvarden and Gilbertson 1994; Dai 2012]. Recently, this species was also reported in North America [Kout and Vlasák 2007. In Europe, *Trametes gibbosa* was recorded on the wood of numerous deciduous tree species, including *Acer* (*A. campestre*), *Aesculus* (*A. hippocastanum*), *Ailanthus*, *Alnus* (*A. glutinosa*), *Betula* (*B. pendula*), *Carpinus* (*C. betulus*), *Castanea*, *Corylus* (*C. avellana*), *Crataegus*, *Eucalyptus*, *Fagus* (*F. sylvatica*), *Fraxinus*, *Juglans* (*J. regia*), *Laburnum* (*L. anagyroides*), *Malus*

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(*M. domestica*), *Morus*, *Platanus*, *Populus* (*P. alba*), *Prunus*, *Pyrus*, *Rhamnus*, *Quercus*, *Salix* (*S. alba*, *S. fragilis*), *Sorbus* (*S. aucuparia*), *Tilia* (*T. cordata*), and *Ulmus* (*U. glabra*), and rarely on the wood of conifers, such as *Abies* and *Picea* [Domański et al. 1973; Kotlaba 1984; Kreisel 1987; Ryvarden and Gilbertson 1994; Wojewoda 2003, Bernicchia 2005]. However, *T. gibbosa* most often colonizes beech wood [Kotlaba 1984; Breitenbach and Kränzlin 1986; Kreisel 1987; Ryvarden and Gilbertson 1994; Bernicchia 2005]. According to Kotlaba [1984] its distribution in Europe is coextensive with that of the European beech (*Fagus sylvatica*). The preference of *T. gibbosa* for *F. sylvatica* is much stronger in the northern part of its European range than in the south of the continent [Ryvarden and Melo 2014]. The basidiomata and mycelium of *Trametes gibbosa* are readily colonized by mycetobiontic beetles [Hammond and Lawrence 1989; Piętka and Borowski 2011; Andrési and Tuba 2018], which may indicate their participation in the spread of this species of fungus.

T. gibbosa causes simultaneous white rot decay, as well as other species of the genus *Trametes* (e.g. *Trametes versicolor*) [Bari et al. 2016; Karim et al. 2017; Chi and Zhang 2022] and the following enzymes are involved in the decomposition process: laccase, manganese peroxidase (MnP), glutathione S-transferase (GST) and cellobiose dehydrogenase (CDH) [Nyanhongo et al. 2007; Zhang et al. 2021; Chi and Zhang 2022] and is considered a predominantly saprotrophic species [Domański et al. 1973; Kotlaba 1984; Kreisel 1987; Ryvarden and Gilbertson 1994; Wojewoda 2003]. Nevertheless, according to some authors, it may occasionally be a weak parasite [Breitenbach and Kränzlin 1986] developing on damaged trees, or even invading living trees, turning into a saprotroph only after the host dies or is felled [Ripaček 1967]. *T. gibbosa* has also been reported as a mycoparasite specifically on members of the basidiomycete genus *Bjerkandera* [Rayner et al. 1987], which may indicate substantial trophic plasticity. It also grows well on wheat straw [Knežević et al. 2017]. This species has the potential to be used in medicine and biotechnology [Czarnecki and Grzybek 1995; Nyanhongo et al. 2007; Ga and Kaviyarasana 2011; Ma et al. 2013; Knežević et al. 2018; Zhang et al. 2021; Jouybari et al. 2022].

T. gibbosa is a secondary colonizer of angiosperm wood, which is involved in lignocellulose decomposition after colonization by primary and other secondary colonizers. The typical lifespan of the active mycelium of this fungus ranges from 2 to 5 years [Boddy and Heilmann-Clausen 2008]. In Poland, *T. gibbosa* basidiomata have been observed on the stems of living trees (e.g. *Fagus sylvatica*, *Fraxinus excelsior*, *Populus nigra*) at wound sites, e.g. resulting from branch breakage [A. Szczepkowski, unpublished observation].

The objectives of the present work were to: (1) determine the ability of *T. gibbosa* pure culture to decompose beech wood under laboratory conditions, (2) test the trophic abilities of *T. gibbosa* by comparative analysis of the development of its mycelium inoculated into the stems of living beech trees and into beech rollers (stem segments – dead substrate). These observations may be of particular value to assess the trophic abilities of *T. gibbosa* and its impact on colonized beech wood in Europe.

Material and methods

Pure culture of *Trametes gibbose*

Both the field and laboratory experiments were conducted using a pure culture of *T. gibbosa* from the pure culture collection of the Department of Forest Protection, Warsaw University of Life Sciences. The pure culture was isolated from a *T. gibbosa* basidioma collected in August 2007 from a *Fagus sylvatica* stump in the Młynary Forest District (northern Poland). The identity of the species was verified by molecular barcoding (see below).

Determination of degree and rate of beech sample decay by *T. gibbosa* mycelium

The beech wood used in the experiments was from a 41-year-old stand growing on a fresh forest site in the Mińsk Forest District (central Poland). The wood decay study was based on the PN-EN 350-1 [2000] and EN 113 [2004] standards. The beech wood samples measured 50 × 25 × 15 mm. The beech wood samples were taken from the middle part of cross-sectional radii, from the basal part of the trunk (0.5-1.5 m) of beech trees. The longest edge of the sample was parallel to the direction of the fibers. Prior to experiments, the samples were numbered, weighed with an accuracy of 0.001 g to determine their air-dry weight, and measured with calipers with an accuracy of 0.01 mm to calculate their exact volume. The density of the wood samples was determined by the oven-dry method [Kokociński 2004]. The moisture content of the samples of beech wood was 6.03%. Five randomly selected samples were dried in a Memmert oven at 105 °C until completely dry and then weighed. The obtained data were used to calculate the absolute dry weight of each sample prior to the decay tests.

Before the experiments, 40 beech wood samples were autoclaved twice on consecutive days at 121 °C (for 20 min on the first day and 10 min on the second

day). Following sterilization, the samples were transferred to a laminar flow chamber and left for several hours to cool down. They were then soaked for 1 h in cool sterile distilled water to increase their initial moisture content (on average up to 29.15% absolute humidity).

Two beech wood samples were placed in each of the previously prepared 20 Kolle flasks containing 3-week-old *T. gibbosa* mycelium cultured on 30 mL of an agar-maltose medium (Figure 1). The composition of the medium was as follows: 1 L of distilled water, 20 g of Bacto agar and 20 g of Difco maltose extract (both from Becton, Dickinson and Company, Sparks, USA). The flasks were placed in a Q-Cell 700 incubator (Poll Lab, Wilkowice, Poland) at the constant temperature of 22 °C and relative humidity of 80 ± 5% for 120 days. After 60 days, 10 flasks (with 20 samples) were selected at random. The samples were removed from the flask, cleaned of the mycelium growing on them, and then dried at 105 °C until absolutely dry. The percentage of wood weight loss was calculated by comparing the initial and final absolute dry weight of the samples.

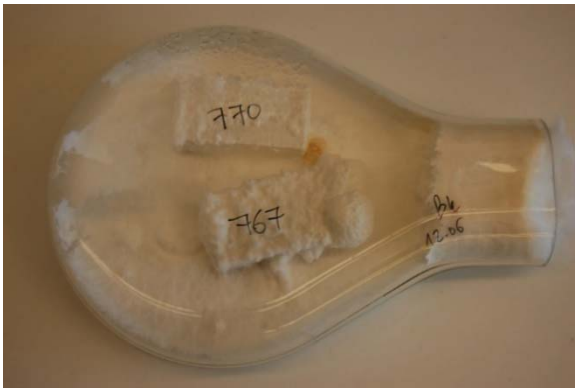

Fig. 1. Beech wood samples colonized by *Trametes gibbosa* mycelium in a Kolle flask (photographed by Jacek Piętka)

Inoculation of living beech stems and beech rollers

In the spring of 2008, 10 stems of healthy 85-year-old beech trees were inoculated at the Forest Experimental Station of the Warsaw University of Life Sciences in Rogów (central Poland; N 51°54.731′, E 19°54.279′). The mean diameter at breast height (DBH) of those trees was 32.9 cm, and the inocula were installed on a sunny day at heights of 1 and 2 m. The inoculates for the field tests were prepared from pieces of beech wood measuring 30 × 5 × 5 mm. The pieces of wood were put into a 200 ml flask, then 5 ml of beer wort and 10 ml of distilled water were added to cover the bottom of the flask. The flasks were autoclaved for 0.5 hour at 121 °C. After sterilization, the pieces of wood were inoculated with *T. gibbosa* mycelium. Subsequently, the flasks were placed in an incubator at 22 °C for 6 weeks.

The inoculation holes were made with a drill (8 mm wide and approx. 10 cm deep). An inoculum was introduced into each hole using sterile tweezers, and then the holes were plugged with sterile beech dowels. Two inoculated beech trees were felled two years after inoculation (in 2010), and another two trees were felled eight years after the inoculations (in 2016). Two one-meter long segments were cut from each tree stem (from heights of 0.5 m to 1.5 m and from 1.5 m to 2.5 m) and then split to produce wood samples. The samples were placed in envelopes, labelled, and transferred to the laboratory. In a laminar flow chamber, the original inoculum was removed and samples of wood with an area of approx. 0.5 cm^2 were taken using a sterile chisel at 2 cm intervals, beginning 1 cm from the inoculation site (that is, at 1 cm, 3 cm, 5 cm, etc.). The obtained material was placed in Petri dishes containing an agar-wort medium (1 L of the medium contained 750 mL of distilled water, 250 mL of unhopped wort from a brewery, and 20 g of Bacto agar from Becton, Dickinson and Company, Sparks, USA) to test for the presence of *T. gibbosa* mycelium. The obtained cultures were morphologically compared with the reference pure culture of *T. gibbosa*, and then the identity of the mycelium was verified by molecular barcoding (the internal transcribed spacer (ITS) of nuclear ribosomal DNA was selected as a region for taxonomic identification).

In the second part of the inoculation experiment, rollers of wood (60 cm long and approx. 20 cm wide stem segments) were cut from one living beech felled in the Mińsk Forest District and left to dry. After two weeks, they were inoculated halfway along their

length, on opposite sides, with wood fragments colonized by *T. gibbosa* mycelium, similarly as in the case of the living trees. The five inoculated rollers were placed under the canopy of a spruce stand, spaced at approx. 1 m intervals, with the bottom part of the rollers being slightly buried in the ground.

After two years, two rollers were subjected to detailed laboratory analysis. Following external visual evaluation, the rollers were split open along the line defined by the drilled holes. The wood fragments were placed in envelopes, labelled, and transferred to the laboratory for analysis using the procedure described above.

Molecular identification of isolates recovered from Inoculations

The identity of the pure cultures of fungi isolated during the experiments was established using DNA barcoding, by sequencing a selected DNA marker [Seifert 2009].

The total genomic DNA was extracted from mycelial fragments taken from pure cultures and placed for storage in Eppendorf tubes in 600 µL of CTAB buffer. The DNA was isolated using the Doyle and Doyle CTAB procedure [1987], with modifications as described by Ronikier et al. [2002]. In the final stage, purified DNA pellets were dissolved in 50 µL of TE buffer (pH 8).

The internal transcribed spacer (ITS) of nuclear ribosomal DNA was selected as a region for taxonomic identification of the studied mycelial samples as it is widely used in studies on the genetic variability of fungi and universally adopted as a molecular barcoding standard for fungal taxa [Seifert 2009; Xu 2016]. The first step involved the analysis of a pure culture of *T. gibbosa* mycelium, directly obtained from a carpophore and further used for inoculations, to obtain a reference sequence for the experiment (GenBank accession number: MN096596). All the ITS sequences of the fungal isolates recovered from the beech trees or wood in the course of the experiment were compared to the reference sequence.

All the amplifications were conducted in GeneAmp 9700 (Applied Biosystems, Foster City, CA, USA) or Mastercycler Nexus GSX1 and Nexus SX1e (Eppendorf, Hamburg, Germany) thermal cyclers. Amplification of the ITS region was carried out using ITS1F and ITS4 primers [Gardes et al. 1991; Gardes and Bruns 1993]. The PCR mixture contained 2.5 µL of a dedicated reaction buffer (10 × RedTaq PCR reaction buffer; Sigma-Aldrich, St. Louis, Missouri, USA), 0.5 µL of dNTP (10 mM of each nucleotide; Sigma-Aldrich), 0.5 µL of each primer (10 mM), 0.2 µL of BSA (10 mg/mL), 1.25 µL of RedTaq DNA polymerase (1 U; Sigma-Aldrich), and 1 µL of 50 × diluted genomic DNA isolate, adjusted with sterile deionized water to 25 µL). A touchdown PCR profile was used in PCR to minimize non-specific amplification as follows: preliminary denaturation for 3 min at 94 °C followed by 35 cycles consisting of 30 s at 94 °C, 30 s at 60 °C (with the annealing temperature decreased by 1 °C/cycle for 10 cycles and then kept at 50 °C), and 1 min at 72 °C, and with a final extension for 7 min at 72 °C. The PCR products were purified enzymatically to remove the excess reaction components by incubation with a mixture of 45 µL of exonuclease I 10 U/µL (EUR-X, Gdansk, Poland) and 85 µL of shrimp alkaline phosphatase 1 U/µL (USB Affymetrix, Santa Clara, CA, USA) at the ratio of 4:1 (4 µL of PCR product and 1 µL of enzyme mix) for 15 min at 37 °C, and for another 15 min at 80 °C. The purified product was subsequently used for sequencing.

Sequencing was carried out in two directions with the ITS1F and ITS4 primers, using BigDye Terminator 3.1 and a dedicated 5 × sequencing buffer (Applied Biosystems), according to the manufacturer's instructions. The sequencing products were purified by precipitation using the ethanol/EDTA procedure. The purified and dried products were suspended in 12 µL of highly deionized formamide and separated in either ABI 3130 or ABI 3500 automated sequencers using POP7 polymer and capillaries with a length of 36 cm and 50 cm, respectively (all from Applied Biosystems).

The obtained sequences were analyzed using BioEdit v.7.2.5 [Hall 1999] and Geneious v.10.1.3 (Biomatters, Ltd.) [Kearse et al. 2012] programs to generate consensus sequences, verify sequence records, and manually introduce corrections, if necessary. The reference sequences obtained from the starter cultures were examined by comparing with DNA sequence libraries in the databases of the National Center for Biotechnology Information [NCBI, http://www.ncbi.nlm.nih.gov/genbank].

In the analyses of the mycelia recovered from the inoculated wood, the barcoding ITS sequences were aligned and ordered based on similarity. In the first step, sequences identical to the reference one were identified and classified as a positive result. In the next step, the other sequences (different from the reference sequence – belonging to other fungal species) were analyzed by comparison with the sequences stored in the GenBank (NCBI) database using the BLASTn algorithm. The genetic identification of those sequences was assessed at the level of species, genus, or higher taxa, depending on the accuracy and reliability of the comparison results.

Statistical analysis

The statistical significance of the differences and the mean weight of the wood blocks before and after decay by *T. gibbosa* was analyzed using Statistica 12 software [StatSoft, Inc. 2014]. The normality of distribution was evaluated using the Shapiro-Wilk test, while the homogeneity of variances was evaluated with the Levene test. The parametric Tuckey test was employed to analyze differences between the mean percentage of weight loss of the beech wood samples prior to the experiment and on days 60 and 120.

Results

Wood block test: weight loss of beech wood

The density of the beech wood samples used for this experiment ranged from 0.62 to 0.79 g/cm^3, with a mean of 0.66 g/cm^3. The mean percentage of weight loss of beech wood decayed by *T. gibbosa* mycelium was 21.07 ± 3.26% after 60 days of the experiment and 32.73 ± 4.38% after 120 days, which means that it increased over time (Figure 2). The difference between the weight loss [%] of the wood blocks after *T. gibbosa* colonization for 60 and 120 days was statistically significant at $p<0.0005$.

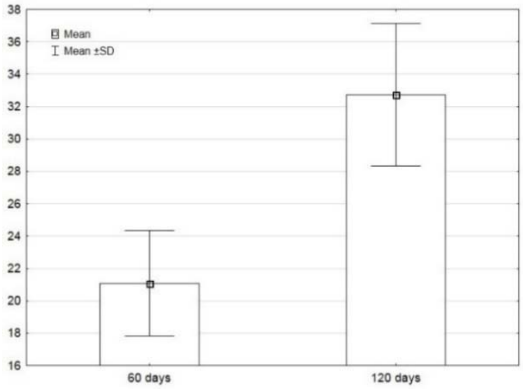

Fig. 2. Mean weight loss [%] of beech wood caused by *Trametes gibbosa* decay

Colonization of wood of living *Fagus sylvatica*

The split stem segments cut from the inoculated beech trees after felling revealed dark stains in the vicinity of the inoculation holes. The discolorations extended up to 8.5 cm upward and 8 cm downward from the holes two years after inoculation, and up to 20 cm upward and 19 cm downward eight years after inoculation (Figure 3). Dark-stained zones ranging in width from several millimeters to several centimeters were observed at the margins of the lesions. However, the inner zone of the lesion had a color similar to that of healthy wood. The radial depth of discolorations was the same as that of the inoculation holes. Multiple wood fragments (approx. 0.5 cm^2 each) were taken from the stained areas, which were characterized by a very high moisture content (water emerged when a chisel was applied to the wood). The samples were cultured in the agar-wort medium and gave rise only to microscopic fungi and bacteria. *T. gibbosa* was not isolated either two or eight years after inoculation. On the other hand, several other fungal taxa were identified using molecular analysis (e.g. *Arthrinium arundinis*, *Clonostachys rosea*, *Geomyces auratus*, *Isaria farinosa*, *Elaphocordyceps* sp., *Penicillium* spp., and *Pleosporales* sp. – see Table 1).

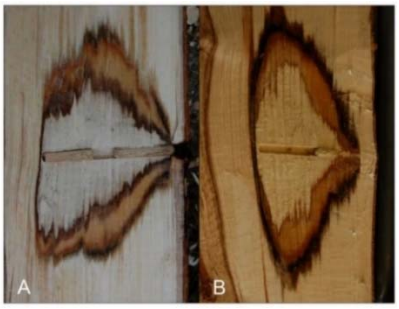

Fig. 3. Changes in wood of living beech trees two years (A) and eight years (B) after inoculation (photographed by Jacek Piętka)

Table 1. Molecular analysis (ITS sequencing) of selected cultures of fungi grown from wood samples collected eight years after inoculation of healthy European beech trees with *Trametes gibbosa*. M1-1-1 – first number – sample height (1 or 2 m), second number – hole no., third number – distance from the inoculum (in cm), U – upward penetration; B – downward penetration. Accession – GenBank accession number of ITS sequences from cultures grown from wood isolates. Organism – taxonomic identification of sequences with highest similarity retrieved by BLAST analysis (taxonomic description given as originally annotated in NCBI database) with sequence accession numbers

Tree number	Isolation site	Accession no.	Related organism (BLAST)
1	Wood (M2-1-1U)	MN096584	Fungal sp. (JF340245)
	Wood (M2-1-3U)	MN096585	Fungal sp. (JF340245)
	Wood (M2-1-7U)	MN096595	Pleosporales (KR909176)
	Wood (M2-1-1B)	MN096586	Fungal sp. (JF340245)
	Wood (M2-1-3B)	MN096587	Fungal sp. (JF340245)
	Wood (M1-1-5U)	MN096582	*Elaphocordyceps* sp. (KF753860)
	Wood (M1-1-3B)	MN096592	*Geomyces auratus* (MF106206)
2	Inoculum (M1-2)	MN096588	*Isaria farinosa* (HQ115724)
			Paecilomyces hepiali (EF555097)
			Paecilomyces farinosus (AB083033)
			Paecilomyces hepiali (KX237743)
	Inoculum (M1-2)	MN096594	*Penicillium* sp. (KX100384)
			Penicillium murcianum (NR138358)
			Penicillium janczewskii (KP016839)
	Wood (M1-2-7U)	MN096591	*Arthrinium arundinis* (GU566268)
	Wood (M1-2-3B)	MN096583	*Epicoccum nigrum* (MF509753)
	Wood (M1-2-7B)	MN096593	*Geomyces auratus* (MF106206)
			Geomyces pannorum (KJ755500)
	Inoculum (M2-1)	MN096589	Fungal sp. (FJ857928)
			Clonostachys rosea (MF509749)
			Clonostachys rosea (KT215192)
			Bionectria sp. (HG937118)
			Bionectria ochroleuca (AF106532)
	Wood (M2-2-1B)	MN096590	*Isaria farinosa* (HQ115724)
			Paecilomyces hepiali (EF555097)
			Paecilomyces farinosus (AB083033)
			Paecilomyces hepiali (KX237743)

Colonization of beech rollers by mycelium of *Trametes gibbosa*

In the field experiment, the beech rollers inoculated with *T. gibbosa* mycelium revealed the presence of sporocarps of five fungal species, namely *Exidia plana*, *Hypoxylon fragiforme*, *Nectria* sp., *Stereum hirsutum*, and *T. gibbosa* (Table 2).

T. gibbosa basidiomata were found on three out of five rollers of wood. After two years of the experiment, two beech rollers with *T. gibbosa* basidiomata were split and subjected to detailed laboratory analysis. In roller no. 1, *T. gibbosa* penetrated into the wood from one drill hole (the mycelium was also present in the original inoculum). White rot caused by *T. gibbosa* was observed up to 19 cm downward and 11 cm upward from the inoculation hole (Figure 4). The other pure cultures isolated from the roller were *Trametes hirsuta* and *Peniophora* sp. (identified using DNA sequencing). In roller no. 2, *T. gibbosa* penetrated the wood also from one drill hole. Here, the mycelium mostly spread upward, up to 21 cm. A young *T. gibbosa* basidioma was found on the upper surface of the roller, which means that the mycelium penetrated the entire distance of 30 cm from the drill hole to the top of the roller (*T. gibbosa* basidiomata were also found on the top on three beech rollers). However, pure cultures of *T. gibbosa* were not isolated from a distance greater than 21 cm from the drill hole. Furthermore, *Hypoxylon fragiforme* was isolated from the upper fragment of the roller; its sporocarps were observed on the upper surface and sides of the roller. Below the inoculation hole, *T. gibbosa* mycelium was recovered only from a distance of 3 cm. *Stereum hirsutum* was found between that point and approx. 15 cm below the drill hole. Finally, *Hypholoma fasciculare* was identified in the roller segment between 19 cm and 29 cm below the inoculation hole (with identity confirmed by molecular barcoding). All the mycelium cultures of *T. gibbosa* were confirmed genetically through comparison with the reference sequence obtained from the initial material.

Table 2. Ascomycota and Basidiomycota identified in two analyzed European beech rollers inoculated with *Trametes gibbosa* based on presence of sporocarps and molecular analysis of pure cultures

Fungal species	Roller no. 1		Roller no. 2	
	sporocarps	ITS	sporocarps	ITS
Exidia plana Donk	+		+	
Hypholoma fasciculare (Huds.) P. Kumm.		+		+
Hypoxylon fragiforme (Pers.) J. Kickx f.	+		+	+
Nectria sp.	+		+	
Peniophora sp.		+		
Stereum hirsutum (Willd.) Pers.			+	+
Trametes gibbosa (Pers.) Fr.	+	+	+	+
Trametes hirsuta (Wulfen) Lloyd		+		

Fig. 4. Inoculation site in beech roller no. 1 two years after inoculation (photographed by Jacek Piętka)

Discussion

The density of the studied beech wood samples (0.62-0.79 g/cm^3) is similar to the literature data [Wagenführ and Scheiber 1974; Bouriaud et al. 2004; Hering et al. 2012]. The weight loss of the beech wood samples exposed to *T. gibbosa* for 4 months was 32.7%, which confirms that this species is capable of rapid and significant wood colonization. Most of the other decay fungi applied to the samples taken from beech trees of varied age and health status from different provenances decayed the wood at a slower rate. Contrary to the above data, Cartwright and Findlay [1958] found a slightly higher (36.6-39.5%) loss of beech wood mass caused by brown-rot fungi (*Coniophora puteana*, *Gloeophyllum trabeum* (Pers.) Murrill, *Serpula lacrymans* (Wulfen) J. Schröt.) and white-rot fungi (*Pycnoporus sanguineus* (L.) Murrill, *Trametes versicolor*) than by *T. gibbosa* in our study (Table 3). The laboratory tests of the resistance of the beech wood using the basidiomycete fungus *Trametes gibbosa* allow the wood to be classified as DC5 (Not durable) [EN 350: 2016].

The wood of *Fagus sylvatica* is a good substrate for many wood-decay fungi because it does not contain extractive substances and contains easily soluble nutrients [Fengel and Wegener 1984; Eriksson et al. 1990; Kubiak and Laurow 1994]. Both our laboratory and field experiments (the inoculation of beech rollers) demonstrated that physiologically inactive wood with a suitable moisture content is readily colonized and decayed by *T. gibbosa*. One year after inoculation, *T. gibbosa* basidiomata were found on three beech rollers. Extensive wood colonization was confirmed by the reisolation of pure cultures and molecular studies of the obtained mycelium.

In the course of our field experiment, the beech rollers were colonized by a number of other saprotrophic fungi. Heilmann-Clausen and Boddy [2005] suggest that primary decay agents influence the subsequent development of fungal communities on decaying wood, which is associated with the inhibiting or stimulating effects of the early colonizers. The considerable success rate of *T. gibbosa* reisolation from the central segments of the test rollers is probably attributable to the absence of competition in some inoculation holes and high mycelial activity. Other fungal species competing for the beech wood nutrients spontaneously colonized the rollers mostly at their ends additionally at bark wound sites, and thus were predominantly found at the top and bottom of the rollers as well as around the circumference. In the case of the two drill holes which did not reveal *T. gibbosa* growth, the inoculation failure may have been caused by an airborne infection. *Peniophora* sp. mycelium was found around one drill hole in roller no. 1 and *Stereum hirsutum* was found around another hole in roller no. 2. In their laboratory study, Heilmann-Clausen and Boddy [2005] noted that wood invaded by *S. hirsutum* delayed or entirely inhibited the growth of most other decay fungi, which indicates the use of secondary metabolites for defense purposes.

The inoculation of the beech rollers with *T. gibbosa* should be deemed a success as after two years viable mycelium of this fungal species was recovered from both rollers and identified by comparative DNA analysis (vs. the original mycelial inoculum) using rDNA ITS sequencing. Examination of the beech roller with the most advanced rot showed that *T. gibbosa* grew extremely rapidly in the axial direction. The wood decay extended from the inoculation hole to the end of the roller, with a young basidioma that had formed on the face of the cut. Also in the study of Deflorio et al. [2008], the extent of fungal decay in trees was the greatest in the axial direction (vs. the radial and tangential directions).

The felled living beech trees inoculated with *T. gibbosa* mycelium revealed a distinct, dark-stained zone coinciding with the inoculation holes and extending along their entire depth. The inner region of the lesion was of a color similar to that of healthy wood and revealed an increased moisture content, which is an inhibiting factor for many fungal species (water emerged while taking test samples with a chisel). The agar-wort cultures of those wood fragments gave rise only to colonies of microscopic fungi and bacteria. In a similar study on *Fagus grandifolia* conducted by Shigo and Sharon [1968], who used different fungal species, discoloration was found at all the wounds. Deflorio et al. [2008] counted beech wood (*F. sylvatica*) among species highly susceptible to discoloration and decay by the studied fungi. Analyzing the concentration of phenolics in discolored wood (discoloration was also caused by the control treatment without fungal inoculation), those authors found that the host response was nonspecific with respect to wounding and fungal inoculation. In a study by Loyd et al. [2018] the internal xylem discoloration observed across all tree species (*Pinus elliottii*, *P. taeda*, *Quercus shumardii*, *Q. virginiana* and *Butia odorata*) and the inocula appeared to be the result of wounding, and not *Ganoderma* infection. Trees respond to wounding of the sapwood by producing antimicrobial compounds that often stain the wood and function by deterring decay organisms from establishing themselves [Deflorio et al. 2008; Shigo 1984]. Antimicrobial

chemicals are also naturally produced in sapwood when it converts to heartwood [Schwarze et al. 2000]. Also, Johansson and Stenlid [1985] reported that in response to wounding and penetration by decay fungi, high amounts of phenolics were accumulated in the living tissues around the wounds. According to Schwarze et al. [2000], the optimum wood moisture content for decay fungi is 40–70%. Nonetheless, recently Meyer and Brischke [2015] found that beech wood can be decayed by some fungi (*Coniophora puteana*, *Gloeophyllum trabeum*, *Trametes versicolor* and *Donkioporia expansa*) at much lower moisture values (the minimum MC growth border) respectively: 13.5, 14.6, 12.3 and 13.7%. Albert et al. [2003] found the moisture content of fresh *F. sylvatica* tissues to range from 35% to 47%. In turn, Barański et al. [2017] reported the moisture content of beech sapwood and false red heartwood to be 64-71% and 53-56%, respectively. According to Boddy and Rayner [1983], a high moisture content of wood and the resulting restricted gas exchange are known to limit the activity of wood decay fungi. Thus, the substantial increase in moisture content around the inoculation holes probably constituted a barrier to the development of *T. gibbosa* mycelium.

The development of fungal hyphae is also hindered by inhibiting substances (mostly polyphenolics) exuded by parenchyma cells. Trees in the genus *Fagus* form distinct boundaries to confine the decay zone, which is associated with the production of such substances [Schwarze et al. 2000]. According to Vek et al. [2013], wound-associated tissues possess a higher content of phenolic extractives. High phenolic concentrations are particularly characteristic of extracts from wound wood and reaction zone tissues.

The lesions brought about by the inoculation of living beech stems appear to be consistent with the compartmentalization of decay in trees (CODIT) model, according to which trees respond to fungal invasions by forming so-called "walls" to prevent the spread of colonization. Those walls may be associated either with the anatomical structures already present in the wood at the time of wounding or with structures and substances produced afterwards [Shigo 1984]. In the presented experiment involving the inoculation of living beech trees, new growth rings are clearly isolated from the discolored zone. This is probably attributable to the presence of suberin in the barrier zone (wall 4 of the CODIT model), which has been found in several broadleaved species, including *Fagus* [Pearce and Holloway 1984; Schmitt and Liese 1993]. In a study by Pearce [1990], suberization induced by the fungal colonization of sapwood was observed in 30 out of the 37 examined tree species. This reaction increases the resistance to decay and may protect the hydraulic integrity of the neighboring tissues, contributing to the antimicrobial defense of functional sapwood. On the other hand, Schwarze and Baum [2000] found that the reaction zones in beech wood may be effectively overcome by white rot fungi.

The present study showed the growth of numerous fungal taxa (Table 1), as well as bacteria, in the discolored regions of beech stems. Similar results were reported by Manion and French [1968], who inoculated living aspen trees (*Populus tremuloides*) with *Phellinus igniarius* basidiospores. Several weeks later, they isolated many species of other competing fungi and bacteria from the vicinity of the inoculation wounds. According to Johnston et al. [2016], bacteria interact with wood decay fungi, but little is known about the mechanism of those interactions, with both antagonistic and synergistic effects observed. Many authors have reported the presence of bacteria in *F. sylvatica* wood decayed by fungi [Folman et al. 2008; Hoppe et al. 2014, 2015]. The failure to reisolate the inoculated fungal species is often attributable to the presence of other microorganisms, mostly *Penicillium* and *Trichoderma* [Deflorio et al. 2008].

Table 3. Weight loss of *Fagus sylvatica* wood caused by various white-rot and brown-rot fungi under laboratory conditions

Fungus species	Weight loss (%)	Time of incubation (months)	Comments	References
Armillaria borealis	19.68 - 9.74	3		Żółciak 2002a
Armillaria cepistipes	20.88 - 22.59	3		Żółciak 2002a
Armillaria gallica	11.87 - 20.26	3		Żółciak 2002a
Armillaria mellea	17.32 - 20.03	3		Żółciak 2002a
A. mellea	8.67 - 8.91	4	stumps wood	Łakomy 1998
Armillaria ostoyae	0.5	3		Schwarze et al. 2000
A. ostoyae	4.52 - 6.84	4	stumps wood	Łakomy 1998
Coniophora puteana	25.9 - 30.7[a] 25.3 - 32.5[b]	4	a - healthy trees, b - damaged trees	Szczepkowski 2010a

Fungus species	Weight loss (%)	Time of incubation (months)	Comments	References
C. puteana	30.9 - 36.5	4		Cartwright and Findlay 1958
C. puteana	12.7	4		Bari et al. 2021
C. puteana	20.1[a] 26.8[b]	6	a - age of trees 20-30 years, b - age of trees 70-80 years	Rosnev and Stiptzov 1986
Fibroporia vaillantii	13.2	4		Cartwright and Findlay 1958
Flammulina velutipes	ca. 8.3 ca. 15.0	3 6		Miłkowski and Łakomy 2007
Fomes fomentarius	9.76 - 15.91[a] 13.93 - 18.72[b] 8.24 - 16.88[c]	3	a - healthy trees, b - moderately damaged trees, c - declining trees	Szczepkowski 2001
F. fomentarius	9.0 - 25.9[a] 14.0 - 28.4[b]	4	a - healthy trees, b - damaged trees	Szczepkowski 2010a
F. fomentarius	21.0	3		Schwarze et al. 2000
F. fomentarius	4.4[a] 1.8[b]	6	a - age of trees 20-30 years, b - age of trees 70-80 years	Rosnev and Stiptzov 1986
F. fomentarius	17.4	4		Bari et al. 2021
Fomitopsis pinicola	21.0	3		Schwarze et al. 2000
Ganoderma applanatum	23.0	3		Schwarze et al. 2000
Ganoderma resinaceum	13.0	3		Schwarze et al. 2000
Ganoderma pfeifferi	11.2 - 17.9	4		Szczepkowski and Konsencjusz 2006
Gloeophyllum trabeum	37.6 - 38.6	4		Cartwright and Findlay 1958
Heterobasidion annosum	5.0 - 6.0	4		Schmidt et al. 1986
Hypholoma fasciculare	14.79	4	stumps wood	Łakomy 1998
Inonotus dryadeus	2.0	3		Schwarze et al. 2000
Inonotus obliquus	10.4 - 23.6	4		Szczepkowski et al. 2021
Laetiporus sulphureus	22.96 - 38.24[a] 31.23 - 35.94[b] 30.5 - 33.5[c]	3	a - healthy trees, b - moderately damaged trees, c - declining trees	Szczepkowski 2001
Lentinula edodes	23.72 - 24.07	3		Zółciak 2005
Lentinus sajor-caju	27.0	4		Bari et al. 2021
Meripilus giganteus	11.0	3		Schwarze et al. 2000
Pholiota squarrosa	6.0	3		Schwarze et al. 2000
Pleurotus ostreatus	27.45 - 33.10	3		Zółciak 2002b, 2005
P. ostreatus	20.6	4		Szczepkowski and Piętka 2007-2008
P. ostreatus	26.3	4		Bari et al. 2021
Pleurotus pulmonarius	25.6	4		Bari et al. 2021
Pycnoporus sanguineus	37.7	4		Cartwright and Findlay 1958
Sarcodontia crocea	19.7 - 30.8	4		Szczepkowski 2010b
Serpula lacrymans	26.2 - 39.5	4		Cartwright and Findlay 1958

Fungus species	Weight loss (%)	Time of incubation (months)	Comments	References
Trametes versicolor	19.6 - 31.0[a] 21.9 - 29.5[b]	4	a - healthy trees, b - damaged trees	Szczepkowski 2010a
T. versicolor	23.0 - 26.0	4		Schmidt et al. 1986
T. versicolor	10.2[a] 5.0[b]	6	a - age of trees 20-30 years, b - age of trees 70-80 years	Rosnev and Stiptzov 1986
T. versicolor	36.6 - 37.7	4		Cartwright and Findlay 1958
T. versicolor	31.6	4		Bari et al. 2021
Ustulina deusta	5.0	3		Schwarze et al. 2000
Xylaria longipes	26.3	4		Bari et al. 2021

Conclusions

The present study showed that *T. gibbosa* exhibits strong saprotrophic inclinations and seems incapable of attacking and colonizing fully vital, unwounded trees. The inoculation of living beech trees failed as no viable mycelium of that fungal species was recovered from them after two and eight years of the experiment. On the other hand, *T. gibbosa* actively invaded beech rollers, where it competed for a substrate with other saprotrophic species and was successfully reisolated two years after inoculation. The growth of *T. gibbosa* on dead wood was confirmed by laboratory tests; under controlled conditions, its mycelium caused intensive decay of the beech wood samples.

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