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SHORT COMMUNICATION

Fibroporia gossypium in northeastern Poland – a preliminary study

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Abstract
A *Fibroporia gossypium* (Speg.) Parmasto fruit-body was found on a Norway spruce [*Picea abies* (L.) Karst.] stump in the northeast of Poland (Waliły forest district). The mycelium from wood samples was sequenced (KF725876), identified and used to examine: (***i***) linear growth of the mycelium on malt-extract medium and (***ii***) the decay of spruce wood samples. We observed slow mycelium growth (84 mm colony diameter after 28 days). After the first 3 months of wood decay, the average loss of dry mass was 6.7%. After next 3 months, a further loss of 15.4% (the average loss) was recorded until finally 35.98% loss (the maximum loss of wood for a single sample) occurred. *Fibroporia gossypium* can be considered as a protective biological agent against root rot in threatened Norway spruce stands.

Keywords
Fibroporia gossypium; Norway spruce; wood decay; biological control

Introduction

Fibroporia gossypium [syn. *Antrodia gossypium* (Speg.) 1968, Ryvarden 1973; Basidiomycota (Agaricomycetes) › Hymenochaetales (Polyporales) › Schizoporaceae (Fomitopsidaceae)] is a wood decay fungus occurring in coniferous stands in Europe [1,2].

Thirty-two species of *Antrodia* are known in Europe [3–8]. Among them, 15 species of *Antrodia* were described in Poland [9,10]. As *F. gossypia*, the fungus was described by Domański [11] and as *F. gossypium*, specimens were provided from the Białowieża National Park by Karasiński and Wołkowycki [10]. Actually, *F. gossypium* has been reported in 38 localities in Estonia (23 records), Norway (five records), Sweden (five records), Finland (three records) and Spain (two records) [2].

Parmasto [12] proposed the genus *Fibroporia* as the generic type species including the following species: *Fibroporia vaillantii* (DC.) Parmasto, *F. gossypium* (Speg.) Parmasto, *F. destructor* (Fr.) Parmasto, *F. radiculosa* (Peck) Parmasto, and *F. oveholtsii* (Pilát) Parmasto. According to Ryvarden [13], there was not enough evidence to justify a generic separation so he synonymized *Fibroporia* to *Antrodia*. However, molecular studies support the monophyletic group of *F. vaillantii* and *F. gossypium* [14]. Rajchenberg [15] has distinguished *Fibroporia* from *Antrodia* taking into consideration irregularly thickened walls, thick-walled basidiospores and tetrapolar sexuality.

In the present study, *F. gossypium* was found in the Waliły forest district (53°05′47.28″ N, 23°50′08.11″ E) in 2010 as a fruiting body covering a Norway spruce stump occurring in a 33-year-old spruce stand (Fig. 1). The observed record of *F. gossypium* seems to be the most northeasterly documented occurrence of this species in Poland. The fungus causes brown wood decay (Fig. 2), indicating that it is

Fig. 1 Young (left) and older (right) fruit-body of *F. gossypium* on a Norway spruce stump (photo: Z. Sierota).

Fig. 2 Brown decay of the wood of the stump visible after cutting the disc from the stump (photo: Z. Sierota).

able to secrete hydrolytic enzymes, especially endoglucanase, cellobiohydrolase, and β-glucosidase as well as H_2O_2. The cellulose and hemicelluloses are broken down in the wood substrate, while lignin remains preserved in a slightly modified form [16,17].

The *F. gossypium* strain was isolated from a Norway spruce stump that remained as a result of routine tree thinning conducted three years earlier. The fruit-body occupied the entire stump surface, part of the side and a small fragment of litter around the root collar. The spruce stand in which the *F. gossypium* fruit-body was found also bore symptoms of disease caused by *Heterobasidion parviporum*. The natural presence of *F. gossypium* seems to be a good indicator of natural competition with the pathogen in the spruce stumps. At present, a saprotrophic fungus *Phlebiopsis gigantea*, a protective agent against *Heterobasidion* spp., is commercially used [18–20]. However, studies performed by Żółciak et al. [21] and Małecka et al. [22,23] showed that in the case of

spruce stumps, the prophylactic effect of *P. gigantea* is not always satisfactory over a short period of time. Hence, it is important to search for other saprotrophic species that could be more effective in the decay of spruce stumps and roots under natural conditions and could therefore find practical application.

For this purpose, we conducted an investigation of mycelium growth of a strain of *F. gossypium* on an artificial medium and a preliminary study of wood spruce decomposition by this fungus under controlled conditions.

Material and methods

PCR amplification and sequencing

The isolate of *F. gossypium* was grown on 2% malt extract agar (MEA) medium for 7 days in the dark at 22°C. The mycelium was ground in liquid nitrogen to disrupt the cells prior to the DNA extraction. Total DNA was extracted using DNeasy® Plant Mini Kit (Qiagen, USA), following the manufacturer's protocol. The amplification conditions were performed in a Peltier Thermal Cycler PTC-200 (MJ Research, USA) in a 25-µl-volume mixture containing 50 ng of genomic DNA and 200 nM of each primer. The amplification reaction with the pair of primers ITS1/ITS4 [24] was carried out under the following protocol: initial denaturation step at 95°C for 3 min followed by 40 cycles of denaturation, annealing, and elongation, respectively 25 s at 95°C, 25 s at 56°C, and 50 s at 72°C, with a final extension step at 72°C for 10 min. Directly after the PCR reaction, 1 µl of PCR product was checked on the 1% agarose gel with the 1 kb DNA Ladder Plus (Invitrogen, USA) as a molecular weight marker. The PCR product was purified using the CleanUp kit (A&A Biotechnology, Poland), following the manufacturer's protocol. Sequencing was conducted on a 3730XL DNA Analyzer (Applied Biosystems®, USA). The chromatogram was analyzed in FinchTV v.1.4.0 (Geospiza®, Inc., USA) and a consensus sequence was created using Bioedit v.7.1.3. The sequence obtained was compared with references deposited in the GenBank database.

Culture condition

Stock cultures of pure *F. gossypium* mycelium were maintained in Petri dishes containing MEA medium. From this culture, inoculum discs 5 mm in diameter were cut and put in the middle of MEA agar in Petri dishes 85 mm in diameter. The mycelium was cultivated at 24°C in darkness. Radial growth of the mycelium was measured daily using a stereomicroscope Zeiss Stemi 2000-c with an accuracy of 1 mm. Each colony was measured in two directions starting from the second day after the inoculation until the time when the mycelium covered the entire surface of the dish. Results from three replications (six measurements) were averaged. We also determined the percentage of daily mycelium increment. For colony diameter (d) the coefficient of variation (*V*%), standard error (*SE*), and standard deviation (*SD*) as well as the trend algorithm were calculated. Changes in colony diameter over the duration of growth and *p* values for counts and expected values were evaluated using the Monte Carlo permutation (MC) (Statistica ver. 10, StatSoft, Inc.).

Wood decay assessment

Norway spruce wood decay activity of the *F. gossypium* strain was tested using in total 20 wood blocks (measuring 0.5 × 1.0 × 2.0 cm with volumes 1 cm^3), following the methodology described by Żółciak et al. [21]. The dry weight loss of 10 samples after 3 and 6 months was recorded as $U_{0–3}$ and $U_{0–6}$, respectively, and the difference between the two terms was calculated as $U_{3–6}$. We also calculated the decay intensity index (Ir) as the quotient of the loss of dry wood in both periods: $Ir = U_{3–6}/U_{0–3}$.

For decay data, the *SE* and *SD* were determined (Statistica ver. 10, StatSoft, Inc.).

Results

The amplified ITS region was 662 bp and contained partial sequences of 18S and 28S ribosomal RNA genes (flanking regions) and complete sequences of internal transcribed spacer 1 (ITS1), 5.8S ribosomal RNA gene, and internal transcribed spacer 2 (ITS2). Bioinformatic analysis of the sequence showed a 100% identity with the corresponding DNA fragment of *Fibroporia gossypium* isolate HUBO 7725 (GU991576). After successful identification, the nucleotide sequence of *Fibroporia gossypium* voucher FG2011 was deposited in GenBank with accession number KF725876.

The aerial mycelium of *F. gossypium* was white and fluffy when grown on the MEA medium. The diameter of 85 mm was obtained after 29 days of cultivation. Within 24 hours, mycelium growth was in the range of 3–4 mm. Mycelial growth was rather slow, but steady, and a curve showing the mycelium growth was most suited to the 6° polynomial trend ($y = 1E{-}06x^6 - 0.0001x^5 + 0.0047x4 + 0.0732x^3 + 0.6061x^2 - 0.4384x + 5.4732$). The values of colony diameter (d) and basic statistics (*V*%, *SE*, *SD*) are presented in Fig. 3. The *p* values were significant ($p < 0.05$) only near the initial and final periods of growth – at 5, 6, 7, 25, 26, and 28 days (marked * in Fig. 3).

After the first 3 months of *F. gossypium* cultivation, loss of spruce wood dry mass was 6.66% and 15.38% for the subsequent 3 months on average (Tab. 1). Values of the decay intensity index Ir, defining activity of the *F. gossypium* strain in various stages of wood destruction (shown here after 3 and 6 months), ranged from 0.98–5.89 (where the value of Ir = 1 is a linear increase).

While in the first 3 months, decomposition rates of the replicates were similar (with Replication 5 showing the highest rates), we noticed a wide variation in decomposition rates between the replicates for months 3 to 6 (Fig. 4). The fungus showed the highest decomposition rates on sample numbers 7, 2, and 9, which could be confirmed by the values of their respective indexes (Ir > 3; Tab. 1). The *SD* values for 3- and 6-months' decay were 1.84 and 8.49, respectively (Fig. 5).

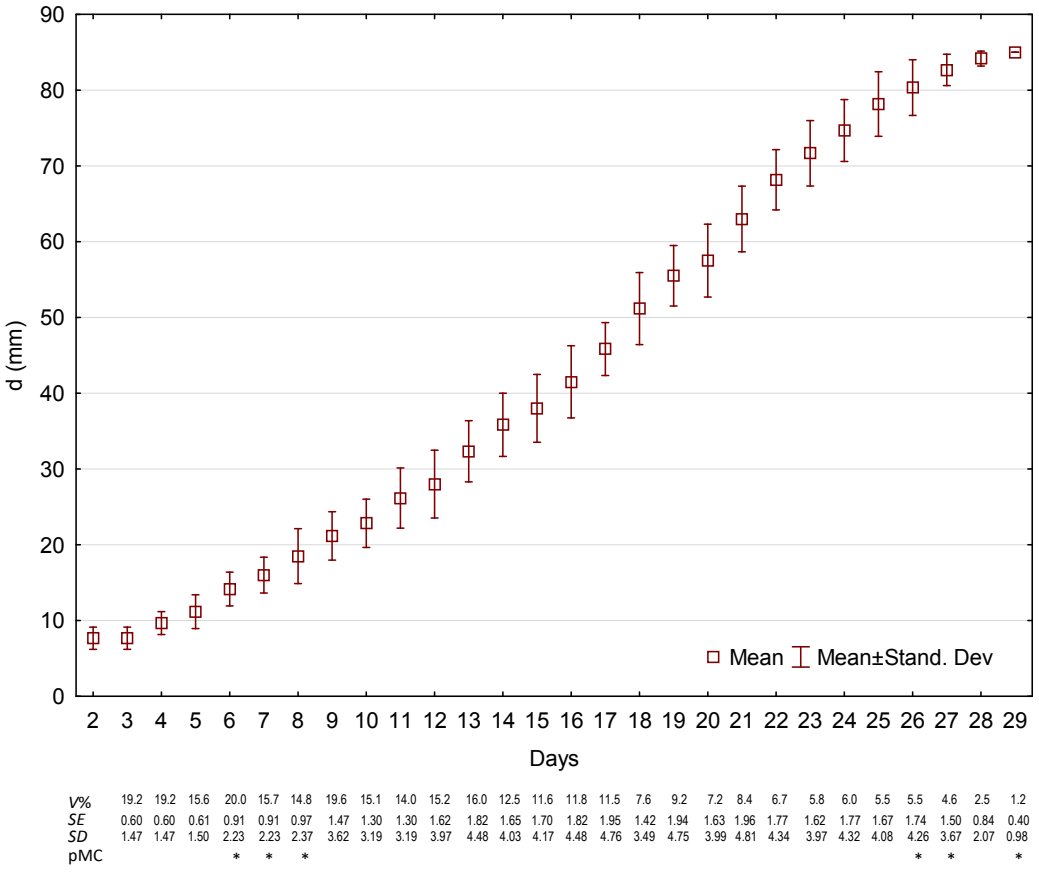

Fig. 3 Colony diameter during 29 days of *F. gossypium* mycelium growth and statistics.

Tab. 1 Loss of wood dry mass (%) and the wood decay intensity ratio (Ir) of *F. gossypium* strain.

Replication	Loss of wood dry mass (%)		Coefficient Ir
	after 3 months	between 3rd and 6th month	
1	5.27	12.50	2.37
2	5.50	23.67	4.30
3	8.57	8.54	0.99
4	5.05	9.50	1.88
5	9.96	9.81	0.98
6	4.51	10.43	2.31
7	5.22	30.76	5.89
8	8.36	10.47	1.25
9	7.38	27.65	3.74
10	6.79	10.47	1.54
Mean	6.33	14.53	2.53
SD	1.84	8.49	1.63
V%	29.10	58.46	64.37

SD – standard deviation; *V*% - coefficient of variation.

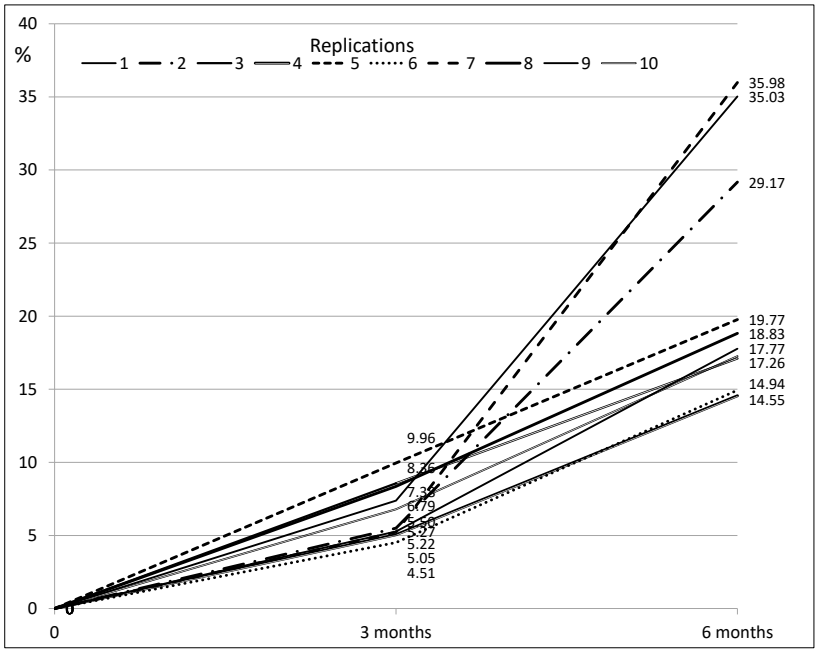

Fig. 4 Loss of dry mass (%) of spruce wood inoculated by *Fibroporia gossypium* after 3 and 6 month of fungal growth.

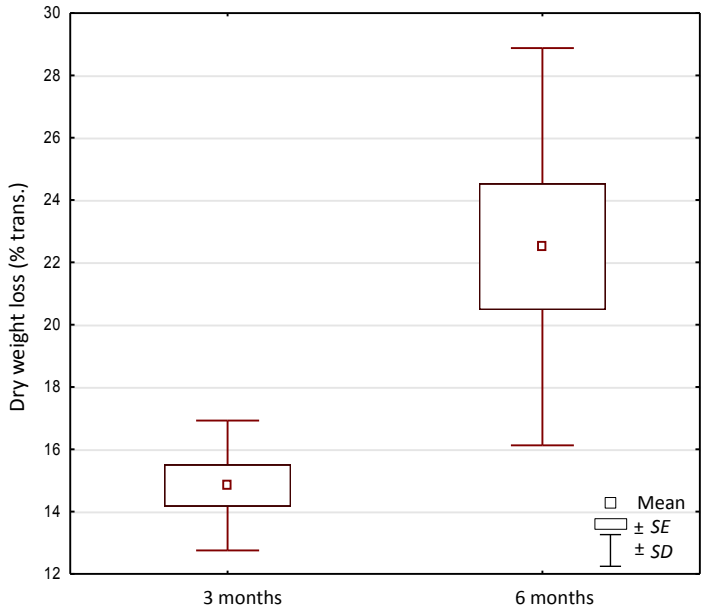

Fig. 5 Mean, standard error (*SE*), and standard deviation (*SD*) for dry mass loss after 3 and 6 months.

Discussion

A high acceleration of wood decomposition (Ir > 2.5) was found in some samples, a slight acceleration in others, and a steady, almost linear degree of decomposition (Ir ~ 1) in Replications 3 and 5. The large differences in wood decay by a homogenous *F. gossypium* mycelium after a period of 6 months (differentiation in Ir values and *V*% coefficient) suggest a changing wood quality of the samples, actually a different wood density [25,26]. After 3 months, the variability of decomposition of particular samples (*SD*) was much lower than after 6 months, which is due to the variability of sample wood density and different levels of enzymatic action of the fungus [21,26]. This seems to be an important methodological remark in the study of the decomposition of wood.

The further study of *F. gossypium* in this area under controlled conditions and in comparison with the other wood-destroying fungi is required (see also [25]). In addition, field trials are necessary to examine the potential use of *F. gossypium* as a biological method of stump protection against root rot in spruce stands. A quite intense, brown wood decay of spruce is a good sign for the expansion of a variety of biological agents against root pathogens; *P. gigantea* causes white rot [18].

The ex situ promotion of *F. gossypium* in natural habitats seems to be not only a preventive and therapeutic method against root rot but also a way to increase the inoculum of this rare fungus in forest sites.

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