

Fungi associated with *Armillaria* spp. rhizomorphs on *Populus tremula* L.

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Abstract. The economic significance of aspen *Populus tremula* L. is only marginal, which is the main reason for its low share in the structure of stands. However, aspen can play a decisive ecological role as a pioneer tree in the succession of forest communities and may decrease the occurrence of pathogenic fungi.

Aspen is also dying out and in order to determine possible biotic causes, we examined taxa of fungi using classical and molecular methods in the area of the Czerwony Dwór Forest District, Subdistrict Rogale, compartments 74b and 75j. The health of aspen trees and the quantitative and qualitative structure of microorganisms associated with *Armillaria* spp. was evaluated.

Based on our health assessment of the aspen, we conclude that the main reason for its dying out is the intensification of root rot, which is a result of *Armillaria* spp weakening the trees. In the general pool of *Armillaria*-associated microorganisms, saprotrophs were dominating, and the fungus *Fomitopsis pinicola* commonly inhabited the poplar stems, whereas pathogen antagonists comprised the lowest proportion. In the quantitative and qualitative structure of fungi found in rhizomorphs, the composition of ecological groups inhabiting stumps was found to be favourable nevertheless. The increasing frequency of pathogens on the logs is a major premise for removing trees affected by *Armillaria*, because aspen stumps are a good food base for *A. cepistipes* and *A. ostoyae* which ultimately spread within the stand. Treatment of stumps with preparations stimulating the decomposition of wood is not recommended for logs that form the food base for pathogenic fungi.

Keywords: Common Aspen, *Armillaria cepistipes*, *Armillaria ostoyae*

1. Introduction

Common aspen *Populus tremula* L. occurs over a wide range, covering the area from Europe, except for southern Spain, to Siberia, northern Africa and Asia Minor (Caudullo, De Rigo 2016). In Poland, common aspen is a widespread tree, and its mountain ecotype occurs as far as the upper montane zone (Gorzela 1999). Aspen covers about 1% of Poland's area, with 0.4% in the State Forests National Forest Holding (PGL LP) and 3% in private forests (Milewski 2017).

Species of marginal economic importance, such as common aspen, are valued in tree stands for their quick ability to afforest areas, e.g. wasteland, re-cultivated gravel pits or slag heaps. They can be a pioneer species in the succession of forest communities, acting as a natural cover for accompanying species, improving their growth rate and speeding up the pro-

cess of tree trunks dropping their branches. Common aspen roots, similarly to birch, loosen deep soil levels, which in future natural regeneration may be settled by pine (Jaworski 2011). It is worth noting that common aspen is an attractive food for deer, which seriously damage forest crops; therefore, it is recommended that it be left at sites under pressure from these game animals (Białobok et al. 1973; Gach 2013; Murat 2014). Aspen is also a tree eaten by the European beaver *Castor fiber* L., and its leaves are food for the western capercaillie *Tetrao urogallus* L. (Czyżowski et al. 2009).

The elimination of *P. tremula* from commercial tree crops or stands is dictated by their susceptibility to infection and their role as the secondary host of pathogenic pine fungi, e.g. *Melampsora populnea* (Pers.) P. Karst. Additionally, common aspen trees may be colonised by polyphagous pathogens: *Heterobasidion annosum* (Fr.) Bref. and *Cytospora chrysosper-*

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ma (Pers.) Fr. (Domański 1983; Sierota, Szczepkowski 2014). Other fungi noted on poplars are *Ceratocystis fimbriata* Ellis and Halst., *Armillaria* spp. and economically less dangerous *Fomesfomentarius* (L.) Fr. and *Phellinus igniarius* (L.) Quél. Aspen can also be colonised by viruses, causing poplar mosaic (viral poplar mosaic), and *Xanthomonas populi* Ride, a bacteria causing poplar canker (Manny 2005, 2011).

The aim of this study was to determine the health status of *P. tremula* trees and to identify the assemblage of fungi colonising the numerous rhizomorphs of the confirmed *Armillaria* fungi.

2. Study site, material and methods

The research was conducted in the Warmińsko-Mazurskie Province within the boundaries of the Regional Directorate of State Forests in Białystok. The health of 50 randomly selected *P. tremula* trees was assessed in the summer and autumn of 2017 in the Rogale Subdistrict of the Czerwony Dwór Forest District. The natural-forest regionalization system places the Forest District in the II Mazurian-Podlasie region, District 1 of the Mazurian Lake District, the meso-regions of the Elk Lakeland, Węgorapa Land and the Land of the Great Masurian Lakes (Bańkowski et al. 2003). The Rogale Subdistrict is located in the northern part of the Forest District and belongs to the Skalisko unit. The tree stands under observation, from which the material for the research was collected, were located in Sections 74j (GPS 54.2943; 22.0863) and 75b (GPS 54.2915; 22.0831). These sections consist of commercial stands occupying fresh deciduous forest habitat, 1.47 ha and 20.79 ha respectively. The share of common aspen in the first section was below 5%, and in the second one – 10%.

The health assessment of the aspens was verified in the field, where the share of trees inhabited by fungi was determined, and subsequently, wood samples with etiological signs of fungi presence were collected and transported to the laboratory. This material consisted mainly of *Armillaria* rhizomorphs taken from two sites located in each of the selected forest sections.

In Section 74j, rhizomorphs were taken in 2017 from a stump and log remaining after felling in 2016, while in Section 75b, material was taken from two objects – a tree that had fallen in 2014 in the second stage of decomposition according to the five-step Maser scale (Maser et al. 1979) and a stump (from a felling in 2016). In order to verify the species of the genus *Armillaria*, representative samples from the surface were marked with the symbol AR and the code for the location and origin of the biological material, where the number 1 designated the fallen tree (75b), 2 – stump (75b), 3, 5 – stump (74j), 4 – log (74j).

The research material in the form of 0.5 cm of cut rhizomorph fragments was placed on an agar-maltose medium

(seven sections each) in Petri dishes. Each fragment was sterilised superficially in a burner flame. The material prepared in this way (10 repetitions for one point of the collected material) was left in a thermostat on the medium for 10 days, after which clean cultures were grafted onto glucose-potato slants. Then, using an Axio Imager A2 microscope manufactured by Carl Zeiss, fungi species or genera were determined (Ellis 1971; Domsch, Gams 1993; Kubicek, Harman 1998; Watanabe 2002; Leslie, Summerell 2006).

The next step was the molecular identification of *Armillaria* species from fragments of the rhizomorphs taken from the four objects mentioned. The work protocol included isolating the DNA with the GeneMATRIX Soil DNA Purification Kit (2008) and identifying the *Armillaria* species from the sample's isolated DNA with nested PCR. The PCR products obtained using AR1 and AR2 primers (separated from the nested PCR) were sequenced at Genomed SA. The resulting nucleotide sequences of the ITS1-5.8S-ITS2 region were compared with the GenBank database (www.ncbi.nlm.nih.gov/genbank/) using the Basic Local Alignment Search Tool (BLAST) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>; Lochman et al. 2004), developed by Altschul et al. (1990). Deposited sequences of other *Armillaria* fungi were obtained from the National Centre for Biotechnology Information (NCBI-GenBank) database and a phylogenetic analysis was performed in MEGA 6 (Tamura et al. 2013). Sequence matching was performed using the built-in Clustal W function (Thompson et al. 1994). In the next stage, the evolutionary model calculated on the basis of the matched sequences was established, which enabled the method to be chosen for constructing the phylogenetic tree. The ITS sequence of *Fomes fomentarius* with accession number AY849306 was used to root the tree. *A. cepistipes* isolates were deposited in GenBank under accession numbers MT764333, MT764334, MT764335, MT764336 and *A. ostoya* – under number MT765062 (NCBI Taxonomy Browser, Index Fungorum).

3. Results

The assessment of common aspen health in the field made it possible to identify potential threats to the species in the stand. Among the biotic factors, typical and specific symptoms in the form of the fruiting body of large-fruited fungi deserved attention. Among the 50 standing trees assessed, 37 (74%) were inhabited by fungi. The species identified on the basis of the morphological features of the fruiting body was the red-belted conk *Fomitopsis pinicola* (Sw.) P. Karst., causing a strong brown decomposition of the heartwood and sapwood. In Section 75b, the fruiting bodies of this taxon were numerous recorded on the entire trunk length of 70-year-old specimens. The second type, found on the

basis of macroscopic etiological signs, was *Armillaria* spp., whose fruiting bodies and rhizomorphs were numerous on 13 living trees (26% of observed trees) and *P. tremula* dead wood. Rhizomorphs of different ages occurred both on the stem under the bark and on the roots. Young rhizomorphs were thin, with a white cross-section, while older ones were characterised by a much larger diameter and a darker cross-section. Mycelial cords and mycelium populated the logs, stumps and roots remaining in the stand after felling.

In the genetic tests, the patterns of AR1 to AR4 strains showed the presence of *A. cepistipes*, while the AR5 strain had a different RFLP pattern of the ITS subunit. Testing the matched sequences in terms of the evolutionary model revealed that the best way to determine the variability of the analysed sequences was with the two-parameter Kimura model with +0.28 Gamma distribution (Kimura 1980). This allowed us to determine the effective reconstruction of phylogenetic dependencies for the used sequences using the neighbour-joining method. The similarity of the AR1–AR4 isolates was 100% identical with the *A. cepistipes* sequences, while the best result obtained for the AR5 isolate was attributed to the dark root rot *A. ostoyae* (99%).

The phylogenetic reconstruction showed that the variation within the ITS *Armillaria* spp. sequences used was small (the largest variation observed was about 0.1 nucleotide changes per site). The genetic material of *A. cepistipes* was homogenous in terms of the ITS sequence and the ITS-RFLP method. This indicates a high probability of the occurrence of one individual in a given habitat or the low genetic differentiation between strains of this species. The tested isolate of *A. ostoyae* differed only slightly from the NC1091-B test strain of *A. ostoyae*.

The analysis of the colonisation by microorganisms of the collected rhizomorphs allowed us to determine the genera or species of the fungi, which were assigned to appropriate ecological groups. In Section 75b, taking into account both the mycobiota of the rhizomorphs inhabiting the fallen tree and the stump, a total of 91 isolates of microorganisms were indicated, including 17 taxa of fungi from the fallen tree and 11 from the stump, as well as one fungal-like organism (Table 1). *Mortierella isabelina* (16%), *Trichoderma* spp. (12%) and *Artrihinium phaeosphaerum* (8%) were the dominant species in this location. The share of other species and genera did not exceed 7%.

In the samples taken from Section 74j, a total of 136 isolates of microorganisms were determined, including 19 fungi and 4 fungal-like taxa from the log and 10 fungi and one fungal-like organism from the stump. Representatives of the genus *Aspergillus* spp. were most often identified. (21%), next the species *Pythium echinulatum* (15%) and then the genus *Penicillium* spp. (10%). The share of other

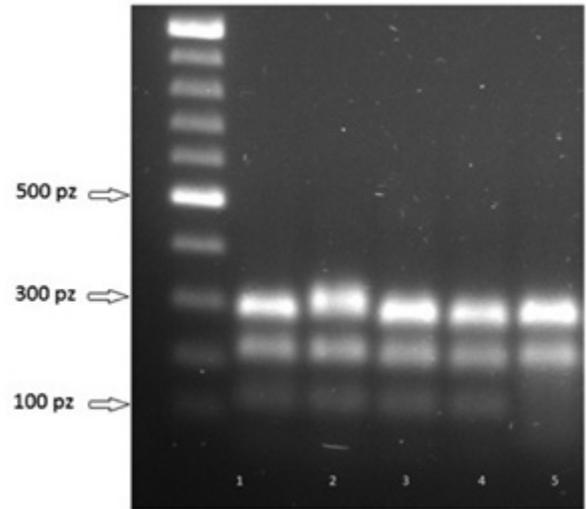


Figure 1. Distribution of base pairs (pz) of a DNA sequence by restriction cleavage with the HinfI enzyme (1 – rhizomorph from tree fall (75b), 2 – from stump (75b), 3, 5 – from stump (74j), 4 – from log (74j))

microorganisms in the assemblage from Section 74j did not exceed 8%.

On the basis of the analysis of the frequency of occurrence of all identified species and genera, the dominant ecological group was found to be saprotrophs, representing a total of 54%. Pathogens were the next group (27%), and antagonistic microorganisms constituted a less numerous group (19%). Antagonistic microorganisms occurred in a similar proportion in each section: 15.3% on the stump and 17.7% on the log in section 74j, 20.3% on the fallen tree and 28.1% on the stump in section 75b. The share of pathogens differed significantly in the case of the log as the main food base in Section 74j, as it contained as much as 45.8% of all microorganisms. The dominant taxon of the assemblage in the obtained isolates was the genus *Pythium*, found mainly in rhizomorphs from Section 74j. The remaining objects exhibited a similar occurrence of pathogens, from 16.1 to 21.9% (Table 1, Fig. 2).

In the culture on artificial medium, isolates representing the genus *Trichoderma* were also obtained, which may exhibit antagonistic features towards pathogens belonging to Oomycetes. Saprotrophic fungi predominated in Section 75b on the fallen tree and in Section 74j on the stump, with shares of 28% and 35% respectively. The 75b stump had a 50% share of saprotrophic fungi, a rich source of carbon compounds, while the share on the 74j log was 38.9%. A similar frequency of this ecological group was obtained for the share of pathogens on the stump and fallen tree in Section 75b (Fig. 3).



Figure 2. Phylogenetic tree and rooting of obtained isolates AR1–AR5

4. Discussion and summary

The properties of *P. tremula* determine the marginal role of this species in commercial stands. Although common aspen is a typical forest species, it only plays an admixture role as a pioneer tree (Gil 1999). Its dominant share in open areas and the edges of stands result from its characteristic requirements of light and resistance to high thermal amplitudes, successive heat and frost, as well as low soil requirements (Murat 2014). In the studied fresh deciduous forest site, common aspen gave way to birch, pine and spruce, reaching, depending on the location, up to 5–10% of species composition. This share means that in a given stand, common aspen could have a significant impact on neighbouring tree species, both due to the phenomenon of competition and their share of roots in the rhizosphere and rhizoplane biota of the soil profile.

Armillaria fungi threatened the condition of the stands in both sections. As perceived as a substrate for both *A. cepistipes* and *A. ostoyae*, which was confirmed by identifying the taxa with molecular methods, these pathogens are a serious

problem in commercial crops and stands, mainly in coniferous stands, but also in deciduous ones in different stages of development (Żółciak 2002), which indicates the difficulty of successfully renewing a given area. Mycelial cords are long lasting and characterised by the ability to remain dormant in the soil for many years until they are stimulated by the chemotrophic signal of a fresh substrate or physical damage, e.g. by wild boars digging in the leaf litter or by mechanical soil preparation (Heather, Sierota 2012). The presence of rhizomorphs on aspen stumps, the fallen tree and log was found in all analysed sites. The growth ability of the rhizomorph apex and the enzymatic decomposition of tissues in the zone next to the cambium confirms that rhizomorphs, as an active inoculum of both *Armillaria* species, can penetrate the soil and threaten the health of other species of trees.

The trends of climate change with numerous weather extremes and pest foraging or the inappropriate selection of habitat are conducive to the intensification of root rot (Basham 1988; Kubiak et al. 2017). According to Żółciak (2003), *A. cepistipes* occurs massively in places where there is no in-

Table 1. Isolatet microorganisms from *Armillaria* spp. rhizomorphs in the studied locations

Taxon	Subcomp. 75b		[%]	Subcomp. 74j		[%]
	fallen tree	stump		log	stump	
<i>Alternaria alternata</i> (Fr.) Keissler	2.00	3.00	5.50	0.00	0.00	0.00
<i>Arthrinium phaeosphaerum</i> (Corda) M.B. Ellis	4.00	3.00	7.70	1.00	7.00	5.80
<i>Arthrotrrys</i> spp.	3.00	0.00	3.30	0.00	0.00	0.00
<i>Aspergillus</i> spp.	0.00	0.00	0.00	6.00	22.00	20.60
<i>Aureobasidium pullulans</i> (De Bary) Arn.	3.00	0.00	3.30	8.00	3.00	8.10
<i>Botrytis cinerea</i> Pers.	0.00	0.00	0.00	1.00	0.00	0.72
<i>Cheatomium</i> spp.	6.00	0.00	6.60	2.00	2.00	3.00
<i>Cladosporium cladosporioides</i> (Fresen.) de Vries	0.00	0.00	0.00	1.00	0.00	0.72
<i>Epicoccum nigrum</i> Link	3.00	0.00	3.30	0.00	0.00	0.00
<i>Fusarium</i> spp.	5.00	1.00	6.60	0.00	0.00	0.00
<i>Gliomastix cerealis</i> (P. Karst.) C.H. Dickinson	0.00	0.00	0.00	0.00	1.00	0.72
<i>Gliomastix</i> sp.	1.00	0.00	1.10	0.00	0.00	0.00
<i>Microsphaeropsis</i> sp.	0.00	0.00	0.00	1.00	0.00	0.72
<i>Mortierella isabellina</i> Oudem.	9.00	6.00	16.50	0.00	1.00	0.72
<i>Mucor racemosus</i> Bull.	0.00	0.00	0.00	3.00	0.00	2.20
<i>Mucor</i> spp.	1.00	0.00	1.10	1.00	0.00	0.72
<i>Oidiodendron</i> spp.	0.00	0.00	0.00	1.00	1.00	1.50
<i>Paecilomyces</i> sp.	0.00	0.00	0.00	1.00	0.00	0.72
<i>Penicillium</i> spp.	3.00	0.00	3.30	5.00	9.00	10.30
<i>Periconia</i> spp.	0.00	0.00	0.00	3.00	0.00	2.20
<i>Phoma</i> spp.	2.00	0.00	2.20	3.00	0.00	2.20
<i>Pythium catenulatum</i> V.D. Matthews	0.00	0.00	0.00	2.00	0.00	1.50
<i>Pythium debaryanum</i> R. Hesse	0.00	0.00	0.00	1.00	0.00	0.72
<i>Pythium echinulatum</i> V.D. Matthews	0.00	0.00	0.00	10.00	10.00	14.70
<i>Pythium</i> spp.	0.00	2.00	2.20	6.00	0.00	4.40
<i>Rhizoctonia solani</i> J.G. Kühn	0.00	1.00	1.10	0.00	0.00	0.00
<i>Rhizopus</i> spp.	0.00	3.00	3.30	0.00	4.00	3.00
<i>Scopulariopsis</i> spp.	1.00	1.00	2.20	0.00	0.00	0.00
<i>Trichoderma harzianum</i> Rifai	0.00	0.00	0.00	3.00	0.00	2.20
<i>Trichoderma</i> spp.	4.00	7.00	12.00	0.00	0.00	0.00
<i>Trichoderma viride</i> Pers.	0.00	0.00	0.00	2.00	1.00	2.20
<i>Trichoderma aureoviride</i> Rifai	1.00	0.00	1.10	0.00	1.00	0.72
<i>Trichoderma hamatum</i> (Bonord.) Bainer	3.00	2.00	5.50	1.00	0.00	0.72
<i>Verticillium</i> spp.	3.00	3.00	6.60	10.00	0.00	7.40
Non sporulating mycelium	2.00	3.00	5,0	2.00	0.00	1.50
Total numer of isolates	56.00	35.00	100.00	74.00	62.00	100.00

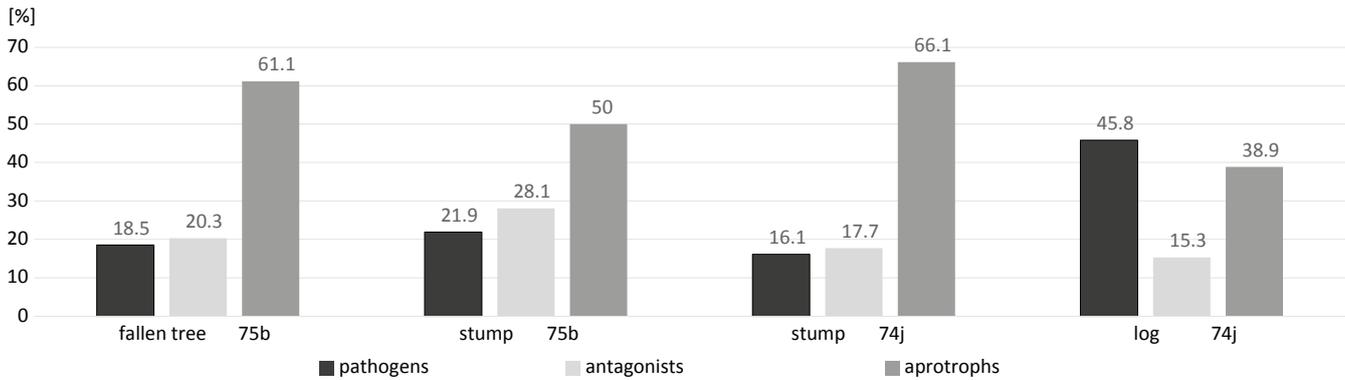


Figure 3. Percentage [%] of ecological groups of microorganisms isolated from rhizomorphs

tensive commercial forest management, in areas that are most often under passive or active protection, whereas the study sites are intended for economic purposes. It should also be emphasised that the presence of *A. cepistipes* is favoured by a large accumulation of organic material in the form of dead wood, stumps and fallen trees and high ground moisture. This species occurs more frequently than *A. ostoyae* in sites with higher nitrogen content (Oliva et al. 2009). In addition, *A. cepistipes* is most commonly found in old, highly decomposed stumps of both deciduous and coniferous trees. This fact is reflected in our own research, where rhizomorphs were collected from a fallen tree and stumps. Oliva et al. (2009) report that beech, silver birch or common aspen in the Spanish Pyrenees together comprise only 7% of the admixture in fir stands; hence, there is little research on the colonisation of these species by *Armillaria* spp. (Oliva, Colinas 2007; Oliva et al. 2009). In commercial forest management practice, sites with common aspen should be monitored, because if infected by the culprit of root rot, it could be a potential reservoir of pathogenic species inoculum in renewals.

Armillaria cepistipes is often a secondary pathogen, worsening the health of already weakened trees and co-occurring with *A. ostoyae*. In Żółciak's study (2007) conducted in the Czerwony Dwór Forest District, *A. cepistipes*, *A. borealis* and *A. ostoyae* were found on spruce and fir, while only *A. ostoyae* was found on pine. Therefore, when preparing the soil for renewals in areas where *Armillaria* spp. rhizomorphs are found on the surface, plowing furrows should be avoided so as not to tear the rhizomorph, which could be a factor stimulating the pathogen to grow and develop in the soil (Rykowski 1985; Baumgartner et al. 2011; Kubiak et al. 2017).

The mycological analysis of the accompanying microorganisms *A. cepistipes* and *A. ostoyae* showed that together, saprotrophs constituted the highest percentage of taxa in the assemblage, both on the stumps and log; antagonistic microorganisms were found in a lower percentage, and the lowest

percentage was attributed to pathogens. The dominant taxon determining the frequency of pathogenic microorganisms was the genus *Pythium*. Representatives of the genus *Pythium* and *Phytophthora* together with *Phytophthora* (belonging to the Oomycetes) may be factors instigating the health deterioration of deciduous trees, e.g. oaks (Jankowiak et al. 2015). Heavy precipitation during the season may stimulate the share of these microorganisms in the assemblage accompanying *Armillaria* spp. This is both the result of zoospore production by Oomycetes and the specific structure of the rhizomorph, in which its hollow core not only plays a role in gas exchange, but is also used by entomofauna – vectors of other microorganisms (Tomalak et al. 2013; Tomalak 2017).

The demonstrated share of *Trichoderma* could balance the potential of the pathogens, which was confirmed by Lefortai et al. (2013), where isolates of the genus *Pythium* and *Phytophthora* from deciduous oak and beech trees were limited by saprotrophs with antagonistic attributes, such as *Trichoderma* and *Gliocladium*. The mycological analysis and assessment of the frequency of occurrence of individual ecological groups on the stumps showed that the share of fungi with antagonistic potential against the pathogens is more favourable in comparison to the studied log assemblage, where a balance in the number of pathogens and their potential antagonists was not found. The share of pathogens associated with *Armillaria* spp. on the log was more than three times higher than on the stumps and fallen tree. This may mean that leaving dead wood inhabited by *Armillaria* spp. in a commercial tree stand may further increase the risk of disease to trees by Oomycetes organisms present in the rhizomorphs of root rot and indicates the need for the timely collection and removal of raw wood material from the felling area.

The red-belted conk found in the monitored plots on common aspen, known for its co-occurrence with other fungi deteriorating tree health, characterised weakened and dying specimens. The presence of *Fomitopsis pinicola* as a

parasite of weakened specimens resulted in the strong brown decomposition of aspen wood. This parasite, using mainly the cellulose and hemicellulose of the cell walls of both deciduous and coniferous tree species, can infect co-existing individuals through wounds. After taking into account the species composition in the verified sections, the red-belted conk may unfavourably impact the condition of the dominant trees, exposed to numerous spore releases from the fruiting bodies (BULiGL 2013; Łakomy, Kwaśna 2015).

On the basis of the health assessment of common aspen in both sections (74j and 75b), we found that the most probable cause of decline among common aspens were the fungi *A. cepistipes* and *A. ostoyae*. In the total pool of microorganisms accompanying the rhizomorphs of the studied *Armillaria* species, the dominant ones were fungi belonging to the saprotroph group, and the lowest share was recorded for the antagonistic fungi of pathogens. In the quantitative and qualitative structure of the fungi occurring in the rhizomorphs, the proportion of ecological groups was found to be favourable for the balance of matter and energy circulation: saprotrophs, including antagonistic fungi colonising stumps, in relation to the unfavourable trend of the increased occurrence of pathogens on the log. Although the interaction *Armillaria* and *Phlebiopsis* genera fungi is not clear, and it has been shown in vitro that these genera inhibit each other's growth and lose their biological potential after their mycelia interact, the mycelium and metabolites of *P. gigantea* act as an inhibitor on the rhizomorphs (Keča 2009). Rykowski and Sierota (1997) showed that both species of fungi: *P. gigantea* and *A. mellea* sensu lato cause equally intensive wood decomposition, and a similar rate of wood decomposition by these fungi has been proven on beech wood. This may be a practical premise for removing trees infected by *Armillaria* spp. from areas and the need to protect the stumps with bio-preparations to stimulate wood decomposition in order to exclude the food base of pathogenic fungi.

Conflict of interest

The authors declare a lack of conflicts of interest.

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References

- Altschul S.F., Gish W., Miller W., Myers E.W., Lipman D.J. 1990. Basic local alignment search tool. *Journal Molecular Biology* 215: 403–410.
- Bańkowski J., Cieśla A., Czerepko J., Czępińska-Kamińska D., Kliczkowska A., Kowalkowski A., Krzyżanowski A., Mąkosa K., Sikora E., Zielony R. 2003. Siedliskowe podstawy hodowli lasu. Dyrekcja Generalna Lasów Państwowych, Warszawa, 282 s.
- Białobok S., Bugała W., Hejnowicz A., Jakuszewski T., Jankiewicz L.J., Obmiński Z., Siwecki R., Środoń A., Surmiński J., Suszka B., Wojterski T., Zabielski S., Żelawski W. 1973. *Topole Populus L.* Tom 12. Państwowe Wydawnictwo Naukowe, Warszawa – Poznań, 515 s.
- Basham J.T. 1988. Decay and stain 10 years later in aspen suckers subjected to scarification at age 3. *Canadian Journal of Forest Research* 18: 1507–1521. DOI 10.1139/x88-232.
- Baumgartner K., Coetzee M.P.A., Hoffmeister D. 2011. Secrets of the subterranean pathosystem of *Armillaria*. *Molecular Plant Pathology* 12: 515–534. DOI 10.1111/j.1364-3703.2010.00693.x.
- BULiGL. 2013. Plan Urządzenia Lasu Nadleśnictwa Czerwonny Dwór, Obrębu Skalisko, Leśnictwo Rogale na okres 01.01.2014-31.12.2023. Opisy taksacyjne i wykazy. Tom 2. Biuro Urządzenia Lasu i Geodezji Leśnej, Białystok.
- Caudullo G., de Rigo D., 2016. *Populus tremula* in Europe: distribution, habitat, usage and threats, in: San-Miguel-Ayaz J., de Rigo D., Caudullo G., Houston Durrant T., Mauri A. (eds.) European Atlas of Forest Tree Species. Publ. Off. EU, Luxembourg, 138–139. ISBN 978-92-79-36740-3.
- Czyżowski P., Karpiński M., Drozd L. 2009. Preferencje pokarmowe bobra europejskiego (*Castor fiber*) na terenach zurbanizowanych oraz chronionych. *Sylvan* 153(6): 425–432
- Domański S. 1983. Fungi that destroyed a *Populus tremula* stand in Lagow Lubuski. *Forest Pathology* 13(3): 166–173. DOI 10.1111/j.1439-0329.1983.tb01414.x.
- Domsch K.H., Gams W., Anderson T.H. 1993. Compendium of soil fungi. IHW-Verlag, London, 1264 s. ISBN 9783980308380.
- Ellis M.B. 1971. Dematiaceous hyphomycetes. X. *Mycological Papers* 125: 1–30.
- Gach P. 2013. http://drzewa.nk4.netmark.pl/atlas/topola/topola_osika/topola_osika.php [15.11.2018].
- GeneMATRIX Soil DNA Purification Kit. 2008. Zestaw do izolacji DNA z ziemi. Protokół prac molekularnych. Eurx. Molecular Biology Products. Kat. Nr E3570.
- Gorzelać A. (red.) 1999. Zalesianie terenów porolnych. Instytut Badawczy Leśnictwa, Warszawa, 174 s. ISBN 83-87647-08-X.
- Jankowiak R., Stępniewska H., Bilański P. 2015. Notes on some *Phytophthium* and *Pythium* species occurring in oak forests in southern Poland. *Acta Mycologica* 50(1): 1052. DOI 10.5586/am.1052.
- Jaworski A. 2011. Charakterystyka hodowlana drzew i krzewów leśnych. Tom 3. Powszechne Wydawnictwo Rolnicze i Leśne. Warszawa, 556 s. ISBN 978-83-09-01076-0.
- Keča N. 2009. In vitro interactions between *Armillaria* species and potential biocontrol fungi. *Bulletin of the Faculty of Forestry* 100: 129–142. DOI 10.2298/GSF0900129K.
- Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111–120.
- Kubiak K., Żółciak A., Damszel M., Lech P., Sierota Z. 2017. *Armillaria* pathogenesis under climate changes. *Forests* 8:100. DOI 10.3390/f8040100.

- Kubicek Ch.P., Harman G.E. 1998. *Trichoderma* and *Gliocladium*. Basic biology, taxonomy, and genetics. Volume 1. Taylor and Francis, London, 293 s. DOI 10.1201/9781482295320.
- Łakomy P., Kwaśna H. 2015. Atlas hub. Multico Oficyna Wydawnicza, Warszawa, 181 s. ISBN 978-83-7763-336-6.
- Lefort F., Pralon T., Nowakowska J., Oszako T. 2013. Screening of bacteria and fungi antagonist to *Phytophthora* and *Pythium* species pathogenic of forest trees. *Biological Control of Fungal and Bacterial Plant Pathogens. IOBC-WPRS Bulletin* 86: 185–186.
- Leslie J.F., Summerell B.A. 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing, Oxford, UK, 402 s. ISBN 1-281-31794-2.
- Lochman J., Sery O., Mikes V. 2004. The rapid identification of European *Armillaria* species from soil samples by nested PCR. *FEMS Microbiology* 1(237): 105–110.
- Mańka K. 2005. Fitopatologia leśna. Państwowe Wydawnictwo Rolnicze i Leśne, Warszawa, 350 s. ISBN 83-09-01793-6.
- Mańka M. 2011. Choroby drzew leśnych. Powszechnie Wydawnictwo Rolnicze i Leśne. Warszawa, 113 s. ISBN 978-83-09-01071-5.
- Maser C., Anderson R.G., Cromack K. Jr., Williams J.T., Martin R.E. 1979. Dead and down woody material, w: Wildlife habitats in managed forests. The Blue Mountains of Oregon and Washington, Thomas J.W. (red. techn.). USDA Forest Service, Agriculture Handbook No. 553, Portland – Washington D.C., 78–95.
- Milewski W. 2017. Lasy w Polsce 2017. Centrum Informacyjne Lasów Państwowych, Warszawa. ISBN 978-83-65659-22-4.
- Murat E. 2014. Brzoza, olsza, topola – hodowla. Eko-Las, Pruszków, 303 s. ISBN 9788393592944.
- Oliva J., Suz L. M., Colinas C. 2009. Ecology of *Armillaria* species on silver fir (*Abies alba*) in the Spanish Pyrenees. *Annals of Forest Science* 66: 603. DOI 10.1051/forest/2009046.
- Oliva J., Colinas C. 2007. Decline of silver fir (*Abies alba* Mill.) stands in the Spanish Pyrenees: Role of management, historic dynamics and pathogens. *Forest Ecology and Management* 252: 84–97. DOI 10.1016/j.foreco.2007.06.017.
- Product information Hinfl. Thermo Scientific. Lot: 00558656. Expiry date: 08.2020.
- Rykowski K. 1985. Niektóre troficzne uwarunkowania patogeniczności *Armillaria mellea* (Vahl) Quèl. w uprawach sosnowych. *Prace Instytutu Badawczego Leśnictwa* 640: 1–140
- Rykowski K., Sierota Z. 1997. Badania nad przygotowaniem do produkcji biopreparatu z grzybem *Phlebia gigantea* (Fr.) Donk. *Prace Instytutu Badawczego Leśnictwa* 534: 73–90.
- Sierota Z., Szczepkowski A. 2014. Rozpoznawanie chorób infekcyjnych drzew leśnych. Centrum Informacyjne Lasów Państwowych, Warszawa, 120 s. ISBN 978-83-63895-37-2.
- Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30(12): 2725–2750. DOI 10.1093/molbev/mst197.
- Thompson J.D., Higgins D.G., Gibson T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22(22): 4673–80. DOI 10.1093/nar/22.22.4673.
- Tomalak M. 2017. Parasitic association of the mycetophagous wood nematode, *Bursaphelenchus fraudulentus* with the honey fungus *Armillaria ostoyae*. *Forest Pathology* 47(3): 1–10. DOI 10.1111/efp.12325.
- Tomalak M., Worrall J., Filipiak A. 2013. *Bursaphelenchus masseyi* sp. n. (Nematoda: Parasitaphelenchinae) – a nematode associate of the bark beetle, *Trypophloeus populi* Hopkins (Coleoptera: Curculionidae: Scolytinae), in aspen, *Populus tremuloides* Michx. affected by sudden aspen decline in Colorado. *Nematology* 15(8): 907–924. DOI 10.1163/15685411-00002729.
- Watanabe T. 2002. Pictorial Atlas of Soil and Seed Fungi. Morphologies of Cultured Fungi and Key to Species. CRC Press.
- Wrzosek M., Sierota Z. 2012. Grzyby jakich nie znamy. Centrum Informacyjne Lasów Państwowych. Warszawa, 160 s. ISBN 978-83-61633-87-7.
- Żółciak A. 2002. Rozkład drewna różnych gatunków drzew leśnych przez *Armillaria* sp. *Prace Instytutu Badawczego Leśnictwa. Seria A* 936: 111–123.
- Żółciak A. 2003. Rozmieszczenie grzybów z rodzaju *Armillaria* w Polsce oraz ich rośliny żywicielskie. *Prace Instytutu Badawczego Leśnictwa. Seria A* 956: 7–22.
- Żółciak A. 2007. *Armillaria* species in coniferous stands. *Acta Mycologica* 42(2): 211–217. DOI 10.5586/am.2007.024.
- Żółciak A. 2015. Zagrożenia upraw i drzewostanów przez grzyby z rodzaju opieńka (*Armillaria*). *Biblioteczka Leśniczego* 375, 18 s.

Authors' contributions

M.D. – concept, field and laboratory work, literature review, manuscript writing; S.P. – laboratory work, graphics preparation; K.D. – field and laboratory work, literature review; J.M. – literature review.