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DECAY OF OAK HEARTWOOD BY SELECTED FUNGI SPECIES

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Abstract. One of the methods limiting the damages done by *Armillaria* species is to decrease the number of dead wood stumps attacked by it, particularly it refers to deciduous tree stumps.The objective of the presented paper was an attempt to estimate the possibility to select some saprotrophic fungi species whose ability of decaying oak wood is higher and faster than that of *Armillaria ostoyae* (Romagn.) Herink and *Armillaria gallica* Marxm & Romagn, and which could be utilized in a biological protection method preventing *Armillaria* root-wood attack. All analyzed wood samples were colonized by tested mycelia. Among the fungi which could be utilized in a biological method of protection against root mould caused by *Armillaria*, the fungus *Tramestes versicolor* has shown to be the best suited for this purpose. Four different isolates were tested, which have confirmed their efficiency in decaying oak heartwood. Therefore, this fungus could be used for the elaboration of a biopreparation protecting effectively against *Armillaria* root rot.

Key words: *Armillaria* root rot, *Trametes versicolor*, oak heartwood, stumps

INTRODUCTION

Dead wood represents an important element of forest ecosystem. Stumps and logs contribute to the formation of forest floor microrelief and they participate in the circulation of nitrogen and carbon compounds. They create the habitats where many organisms live and form their associations. Next to the positive aspects of leaving dead woods in the forest, there also are some negative sides of such procedure, because it supplies the nutritional base for pathogenic fungi. One of the genera causing enormous losses in tree stands all over the world is *Armillaria* (Fr.) Staude genus which lives in dead wood and subsequently, it is displaced by rhyzomorphs which migrate in the soil and attack living trees. One of the methods limiting the damages done by *Armillaria* species is to decrease the number of dead wood stumps attacked by it, particularly it refers to deciduous tree stumps.

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Another method of reducing *Armillaria* root rot is to utilize pathogenic fungi which compete for food with *Armillaria*, but their processes of wood decay are shorter than those of Armillaria, so once they attack an object first, they leave no chance for *Armillaria* species invasion.

So far, there were some attempts to investigate for such purposes the following fungi: *Hypholoma fasciculare* (Huds.) P. Kumm., *Tricholomopsis rutilans* (Schaeff.) Singer [Sierota 2001], *Polyporus borealis* Fr., *Fomitopsis pinicola* (Sw.) P. Karst. [Orłoś 1957], *Lentinus edokes* Fr., *Phlebiopsis gigantea* (Fr. Fr.), Julich, *Pleurotus ostreatus* (Jacq. Fr.) Kummer, *Trichoderma harzianum* Rifai [Rykowski 1990, Sierota and Sternak 1993, Łakomy and Zarakowski 2000].

The objective of the presented paper was an attempt to estimate the possibility to select some saprotrophic fungi species whose ability of decaying oak wood is higher and faster than that of *Armillaria ostoyae* (Romagn.) Herink and *Armillaria gallica* Marxm & Romagn, and which could be utilized in a biological protection method preventing *Armillaria* root-wood attack.

MATERIAL AND METHODS

Isolates of different fungi species causing wood decay (mainly in the deciduous trees) were selected for the studies (Table 1). Mycelium of the tested species was introduced into sterile Kolle flasks with 2% maltose nutritive substance. Then, the flasks were transferred, for a period of four weeks, to an incubator with a constant temperature of 23°C.

Table 1. Isolates of fungi used in the studies
Tabela 1. Izolaty grzybów wykorzystane w badaniach

Isolat Izolat	Species of fungi Gatunek grzyba	Substrate Substrat	Localization Lokalizacja
1	2	3	4
6095	*Armillaria ostoyae*	dead pine	Experimental Forest District Siemianice
7012	*Armillaria ostoyae*	dead pine	Experimental Forest District Siemianice
7038	*Armillaria ostoyae*	dead pine	Experimental Forest District Siemianice
8003	*Armillaria ostoyae*	dead pine	Experimental Forest District Siemianice
8025	*Armillaria ostoyae*	dead pine	Experimental Forest District Siemianice
8036	*Armillaria ostoyae*	dead pine	Experimental Forest District Zielonka
8042	*Armillaria ostoyae*	dead pine	Experimental Forest District Zielonka
90Z021	*Trametes hirsuta* (Wulfen) Pilát	stump of oak	Experimental Forest District Zielonka
90Z09	*Trametes versicolor*	stump of beech	Experimental Forest District Zielonka
90Z04	*Trametes versicolor*	log of willow	Experimental Forest District Zielonka
90Z16	*Pleurotus ostreatus*	log of oak	Experimental Forest District Zielonka
90T08	*Trametes versicolor*	stump of birch	Forest District Tuchola

Table 1 – cont. / Tabela 1 – cd.

1	2	3	4
90Z14	*Pleurotus ostreatus*	indefinite	Forest District Tuchola
90W02	*Armillaria gallica*	ryzomorfs from soil	Forest District Wołów
90W10	*Armillaria gallica*	ryzomorfs from soil	Forest District Wołów
90W02	*Armillaria gallica*	ryzomorf from surface of oak roots	Forest District Wołów
6037	*Armillaria ostoyae*	dead pine	Forest District Złotów
6045	*Armillaria ostoyae*	dead pine	Forest District Złotów
6067	*Armillaria ostoyae*	dead pine	Forest District Złotów
6069	*Armillaria ostoyae*	dead pine	Forest District Złotów
6072	*Armillaria ostoyae*	dead pine	Forest District Złotów
7035	*Armillaria ostoyae*	dead pine	Forest District Złotów
90P03	*Panellus serotinus* (Schrad.) Kühner	log of oak	National Park of Wielkopolska
90P14	*Panellus serotinus*	log of oak	National Park of Wielkopolska

Samples of oak wood (5.0 × 2.5 × 1.5 cm) were prepared of oak heartwood. Subsequently, the wood was dried and its dry matter weight was determined. Afterwards, the wood samples were sterilized twice in autoclave (at 120°C, 1 atm.). After wetting of the wood samples in sterile water, they were placed on the developed mycelium in the Kolle's flasks and they were incubated for six months at 24°C. After incubation, the wood was dried and its dry matter weight was determined again. Each combination was done in six replications. The obtained results were statistically analysed using the Kruskal-Wallis test.

RESULTS

All the analysed wood samples were colonized by tested mycelia. All isolates of *Trametes versicolor* showed the most abutand mycelia. Wood samples tested with *Armillaria* spp. Were covered with a sclerotic mycelium. The tested fungi isolates decayed wood samples in 1.13 to 64.52% (Fig. 1). Wood isolates of *Trametes versicolor* originating from the area of the Zielonka Experimental Forest District (90Z09) decayed wood samples in the shortest time. The remaining *T. versicolor* isolates also decayed oak wood samples very fast and the wood loss ranged from 36.36% (isolate 90T07) to 63.63% (isolate 90Z04). Statistical analysis showed significant differences in wood loss by *T. versicolor* isolates, in comparison with isolates of *Armillria* spp. Isolates of *Armillaria ostoyae* decayed wood samples in 2.71 to 8.52%. Isolates of *A. gallica* decayed wood in a similar way (3.19-6.06%). Isolates of *Panellus serotinus*, *Pleurotus ostreatus* and *Trametes hirsute* fungi did not exceed a 10% threshold of wood decay. Results of multiple (two sided) comparisons referring to the percentage of wood dry matter losses are shown in Table 2.

Table 2. Results of Kruskal-Wallis test for the significance level P = 0.0000 of dry matter loss in oak wood samples decayed by the particular isolates
Tabela 2. Wynik testu Kruskala-Wallisa, dla poziomu istotności p = 0,0000 dla ubytku suchej masy próbek drewna dębowego rozkładanego przez poszczególne izolaty grzybów

	90W01	90P03	90Z04	90P14	90Z14	90Z21	90Z16	90T07	90T08	90W02	90Z09	90W10
90W01		1.0000	**0.0010**	0.8277	1.0000	0.0627	1.0000	**0.0015**	**0.0092**	1.0000	**0.0008**	1.0000
90P03	1.0000		1.0000	1.0000	1.0000	1.0000	0.8739	1.0000	1.0000	1.0000	1.0000	1.0000
90Z04	**0.0010**	1.0000		1.0000	1.0000	1.0000	**0.0000**	1.0000	1.0000	1.0000	1.0000	1.0000
90P19	0.8277	1.0000	1.0000		1.0000	1.0000	0.1551	1.0000	1.0000	1.0000	1.0000	1.0000
90Z14	1.0000	1.0000	1.0000	1.0000		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
90Z21	0.0627	1.0000	1.0000	1.0000	1.0000		**0.0085**	1.0000	1.0000	1.0000	1.0000	1.0000
90Z16	1.0000	0.8739	**0.0000**	0.1551	1.0000	**0.0085**		**0.0001**	**0.0010**	1.0000	**0.0000**	1.0000
90T07	**0.0015**	1.0000	1.0000	1.0000	1.0000	1.0000	**0.0001**		1.0000	1.0000	1.0000	1.0000
90T08	**0.0092**	1.0000	1.0000	1.0000	1.0000	1.0000	**0.0010**	1.0000		1.0000	1.0000	1.0000
90W02	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000		1.0000	1.0000
90Z09	**0.0008**	1.0000	1.0000	1.0000	1.0000	1.0000	**0.0000**	1.0000	1.0000	1.0000		1.0000
90W10	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	
6037	1.0000	1.0000	0.1254	1.0000	1.0000	1.0000	1.0000	0.1609	0.6570	1.0000	0.1025	1.0000
6045	1.0000	1.0000	0.3313	1.0000	1.0000	1.0000	1.0000	0.4179	1.0000	1.0000	0.2743	1.0000
6067	1.0000	1.0000	1.0000	1.000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
6069	1.0000	1.0000	**0.0003**	0.4037	1.0000	**0.0265**	1.0000	**0.0005**	**0.0035**	1.0000	**0.0002**	1.0000
6072	1.0000	1.0000	0.1820	1.0000	1.0000	1.0000	1.0000	0.2320	0.9125	1.0000	0.1494	1.0000
6095	0.3313	1.0000	1.0000	1.0000	1.0000	1.0000	0.0550	1.0000	1.0000	1.0000	1.0000	1.0000
7012	1.0000	1.0000	**0.0002**	0.3198	1.0000	**0.0200**	1.0000	**0.0003**	**0.0026**	1.0000	**0.0002**	1.0000
7035	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
7038	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
8003	1.0000	1.0000	0.8367	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.7022	1.0000
8025	1.0000	1.0000	**0.0348**	1.0000	1.0000	1.0000	1.0000	**0.0456**	0.2106	1.0000	**0.0280**	1.0000
8036	1.0000	1.0000	0.3512	1.0000	1.0000	1.0000	1.0000	0.4427	1.0000	1.0000	0.2910	1.0000
8042	1.0000	1.0000	**0.0028**	1.0000	1.0000	0.1387	1.0000	**0.0039**	**0.0224**	1.0000	**0.0022**	1.0000

Table 2 – cont. / Tabela 2 – cd.

6037	6045	6067	6069	6072	6095	7012	7035	7038	8003	8025	8036	8042
1.0000	1.0000	1.0000	1.0000	1.0000	0.3313	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.1254	0.3313	1.0000	**0.0004**	0.1820	1.0000	**0.0003**	1.0000	1.0000	0.8368	**0.0348**	0.3512	**0.0028**
1.0000	1.0000	1.0000	0.4038	1.0000	1.0000	0.3199	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1.0000	1.0000	1.0000	**0.0265**	1.0000	1.0000	**0.0201**	1.0000	1.0000	1.0000	1.0000	1.0000	0.1387
1.0000	1.0000	1.0000	1.0000	1.0000	0.0550	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.1609	0.4179	1.0000	**0.0005**	0.2320	1.0000	**0.0004**	1.0000	1.0000	1.0000	**0.0456**	0.4427	**0.0039**
0.6570	1.0000	1.0000	**0.0036**	0.9126	1.0000	**0.0026**	1.0000	1.0000	1.0000	0.2106	1.0000	**0.0224**
1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.1025	0.2743	1.0000	**0.0003**	0.1494	1.0000	**0.0002**	1.0000	1.0000	0.7022	**0.0280**	0.2910	**0.0022**
1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1.0000		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1.0000	1.0000		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1.0000	1.0000	1.0000		1.0000	0.1532	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1.0000	1.0000	1.0000	1.0000		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1.0000	1.0000	1.000	0.1532	1.0000		0.1193	1.0000	1.0000	1.0000	1.0000	1.0000	0.6718
1.0000	1.0000	1.0000	1.0000	1.0000	0.1193		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000		1.0000	1.0000	1.0000	1.0000	1.0000
1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000		1.0000	1.0000	1.0000	1.0000
1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000		1.0000	1.0000	1.0000
1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000		1.0000	1.0000
1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000		1.0000
1.0000	1.0000	1.0000	1.0000	1.0000	0.6718	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	

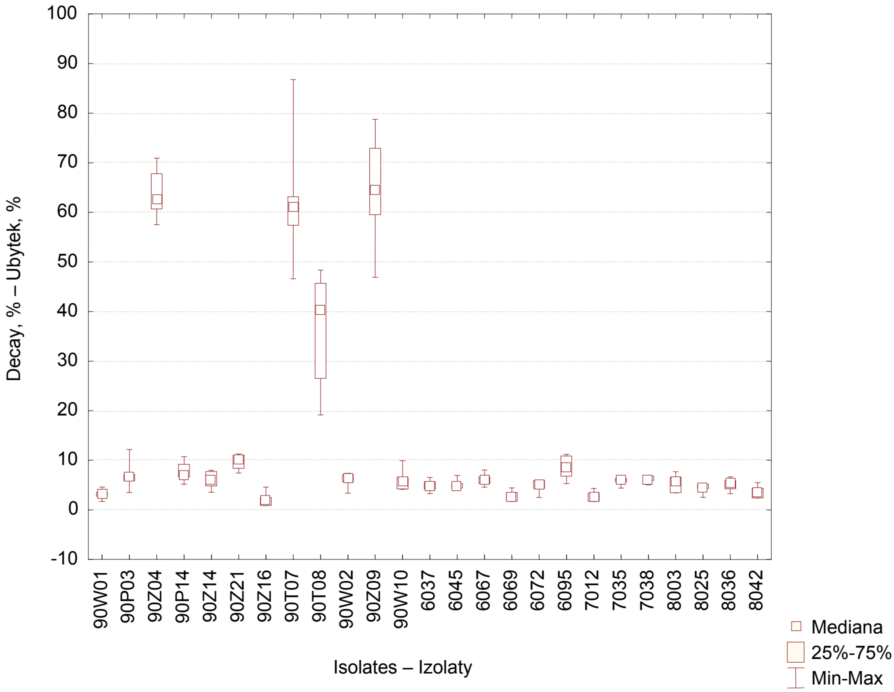

Fig. 1. Mean values of wood dry matter losses
Rys. 1. Średnie wartości ubytku suchej masy drewna

DISCUSSION

Oak wood was selected for the presented experiments because its decay by fungi is rather difficult, in comparison with other forest-creating tree species like e.g. beech [Kordik 2001, Żółciak 2002 b]. Oak wood, because of its content of tannin substances, is less easily available for the majority of fungi since in oak heart wood, there occur both the hydrolyzing and the condensed tannins. Therefore, they are characterised by high natural hardness [Prosiński 1984]. The studied *Armillaria* isolates did not decay wood very quickly. In spite of the fact that the samples were covered by mycelium, there quickly developed a sclerotic mycelium. It is known that the mycelium of *Armillaria* can exist very long in a colonized wood. Situations have been recorded where the mycelia existed for 30-40 year and even as long as 70 years [Redfern and Filip 1991, Rishbet 1972]. Studies carried out by Zółciak [2002 a] indicated that the isolates of *Armillaria gallica* decayed oak wood in 10.04 to 13.96% while the isolates of *A. ostoyae* decayed the same wood samples in 10.54 to 21.50 %. Łakomy [1998], who tested the mycelium of *A. ostoyae*, obtained after months a wood loss from 4.1 to 4.78%.

Among the fungi which could be utilized in a biological method of protection against root mould caused by *Armillaria*, the fungus *Trametes versicolor* has shown to be the best suited for this purpose. Four different isolates were tested, which have confirmed their efficiency in decaying oak heartwood. This fact had been known already earlier, since in the work of Łakomy [2004], this fungus contributed to the decay of oak wood in 54.44 to 83.35%. In similar studies with other isolates [Łakomy et al. 2005], *Trametes versicolor* decayed oak wood in 22.57 to 61.03%. Another well known species, *Pleurotus ostreatus* was analysed in several papers referring to the biological protection against *Armillaria* root rot. In Poland, Rykowski and Sierota [quoted after Żółciak 2002 b] attempted to use *P. ostreatus* for the wood decay of deciduous tree stumps. In their experiment, they used two different isolates of *P. ostreatus*, which had not been selected earlier for tests referring to the speed of wood decay. Their effects showing 2.13 to 6.00% of decayed wood are not convincing. In the work by Łakomy [2004], isolates of this species (*P. ostreatus*) decayed oak wood in 35.8 to 44.4%. In other studies on the possibility of utilizing *P. ostreatus* in the biological protection method against *Armillaria* root rot [Żółciak 2002 a, Szczepkowski and Piętka 2007], oak stumps were less eagerly settled by *Armillaria*, in comparison with beech stumps. There is no information referring to any possibility of utilizing *Trametes hirsuta* and *Panellus serotinus* in the biological protection against *Armillaria* root rot. However, it is known that *Trametes hirsute* eargerly settles in oak stumps [Szczepkowski and Piętka 2007]. On the basis of the studies carried out so far, one may conclude that *Trametes versicolor* decayed oak wood in the shortest time. Therefore, this fungus could be used for the elaboration of a biopreparation protecting effectively against *Armillria* root rot.

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ROZKŁAD DREWNA TWARDZIELI DĘBA PRZEZ WYBRANE GATUNKI GRZYBÓW

Streszczenie. *Armillaria* (Fr.) Staude jest jednym z rodzajów powodujących ogromne straty w drzewostanach na całym świecie. Bytuje w martwym drewnie, by następnie atakować żywe drzewa za pomocą ryzomorf, które przemieszczają się w glebie. Jednym ze sposobów ograniczania szkód ze strony opieniek jest ograniczenie ilości drewna pniakowego, szczególnie gatunków liściastych. Celem pracy była próba oceny możliwości rozkładu drewna dębowego przez wybrane gatunki grzybów saprotroficznych w stosunku do *Armillaria ostoyae* (Romagn.) Herink i *Armillaria gallica* Marxm. & Romagn. Do badań wybrano izolaty różnych gatunków grzybów rozkładających drewno, głównie liściaste. Wszystkie analizowane próbki drewna były skolonizowane przez testowane grzybnie. Z grzybów możliwych do wykorzystannia w biologicznej metodzie ochrony przed opieńkową zgnilizną korzeni najlepiej rozkładał drewno *T. versicolor.* Testowano cztery różne izolaty, które potwierdziły sprawność rozkładu części twardzielowej drewna dębowego.

Słowa kluczowe: opieńka, *Trametes versicolor*, drewno twardzielowe dębu, pniaki

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