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THE ABILITY OF GROWTH, WOOD COLONIZATION AND WOOD DECOMPOSITION OF *FLAMMULINA VELUTIPES* ISOLATE *IN VITRO*

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Abstract. *Flammulina velutipes* isolate ability of growth, wood colonization and wood decomposition was studied *in vitro*. The experiments were done at three temperatures 4°C, 15°C and 22°C. The investigated isolate started to grow in one day after incubation at 15°C and 22°C. *Flammulina velutipes* mycelium colonized and decomposed wood the most intensively at 22°C. Oak wood was both the least intensively colonized and least effectively decomposed in comparison to beech and birch wood irrespective of temperature and incubation time (three and six months).

Key words: *Flammulina velutipes*, growth *in vitro*, wood colonization *in vitro*, wood decomposition *in vitro*

INTRODUCTION

Root rot pathogens are the most dangerous for both coniferous and deciduous stands in boreal hemisphere especially *Heterobasidion* spp. and *Armillaria* spp [Hood et al. 1991, Woodward et al. 1998, Sierota 2001, Mańka 2005]. In Poland, these pathogens cause economic damages on area about 400 000 ha, what makes about 4% of forest area [Sierota et al. 2002]. Control of these pathogens is very difficult, because the main source of them is the wood in the soil: roots, wood debris and also stamps. One of the proposed methods is the biological control with the use of saprotrophic fungi, which could colonize stamps and roots faster than pathogens and moreover, could exclude these pathogens from their ecological niches. The only example of commercial use of fungi as an agent of biological control against root rot pathogens in coniferous stumps is *Phlebiopsis gigantea* (Fr.: Fr.) Jülich [Sierota 1995, Pratt et al. 2002].

Scientists are looking for the alternative fungi, which are able to colonize and decay stumps and wood debris buried in the soil faster than root rot pathogens or which could colonize stumps and roots before the pathogens.

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In the past there were some attempts of using fungi against *Heterobasidion* spp. and *Armillaria* spp. – namely: *Corolius versicolor* (L.: Fr.) Quél. [=*Trametes versicolor* (L. ex Fr.) Pil.], *Hypholoma fasciculare* (Huds.: Fr.) Kummer, *Stereum hirsutum* (Willd.: Fr.) S.F. Gray [Pearce and Malajczuk 1990, Łakomy unpublished 2000, Łakomy 2004], *Phanerochaete velutina* (DC.) Parmasto, *Steccherinum fimbriatum* (Pers.: Fr.) J. Erikss. [Hagle and Show 1991], *Polyporus borealis* Fr., *Fomitopsis pinicola* (Swartz ex Fr.) P. Karsten [Orłoś 1957], *Lentinus edodes* Fr., *Pleurotus ostreatus* (Jacq.: Fr.) Kummer, *Trichoderma harzianum* Rifai [Rykowski 1990, Żółciak 2002].

Łakomy [2004] showed that *Bjerkandera adusta* (Willd.: Fr.) P. Karst*, Hypholoma fasciculare, Hypholoma sublateritium* (Fr.) Quél*, Kuehneromyces mutabilis* (Schaeff.: Fr.) Sing. et Smith*, Pleurotus ostreatus* and *Trametes versicolor* could be used in biological control against *Armillaria* species in beech, birch and oak stumps.

The aim of this study was to characterize the ability of *Flammulina velutipes* (Curtis) Singer isolate of growth, wood colonization and wood decomposition *in vitro.* This study should be considered as a first step in a wide program of biological control of *Armillaria* spp.

MATERIAL AND METHODS

Flammulina velutipes basidiomes were collected from birch stump in the Strzelce Krajeńskie Forest District (52°47'N, 15°33'E). Mycelium was isolated from basidiome's stipe and stored on 1% malt extract agar (MERCK Germany) in tubes.

The ability of growth *in vitro*. Inoculum (disc diam. 8 mm) was put in the centre of Petri dish on 1.5% malt extract agar. Petri dishes were incubated in the dark, in the temperature 4°C, 15°C and 22°C. The diameter of mycelium was measured every day starting one day after inoculation up to 15th day. The additional data were collected also 30 days after inoculation of mycelium stored in 4°C. Each combination was multiplied 10 times.

Wood colonization *in vitro*. Sterile (120°C, 1 atm., 20 min) 5 cm in length wood pieces of oak, beech and birch (cut from branches, 2 cm in diameter) were put on 1.5% malt extract agar in the centre of Petri dish. Close to the wood *F. velutipes* inoculum (disc diam. 8 mm) was put. The inoculum was tough the wood sample [Łakomy 2004]. Petri dishes were stored at 4°C, 15°C and 22°C. The wood colonization was observed after 4, 5, 6 and 7 days after inoculation. In temperature 4°C observations were done up to 54th day after inoculation.

Wood decomposition *in vitro*. Wood decomposition ability of the saprotroph was tested using the method described by Orłoś [1957], routinely applied in the Department of Forest Pathology [Łakomy 1998, Łakomy and Zarakowski 2000, Łakomy 2004, Łakomy et al. 2005] to obtain comparable results.

Wood blocks 1.5 × 2.5 × 5 cm in size were made from birch, beech and oak fresh stumps (diameter 30 cm). The wood blocks were put on well-grown mycelium of saprotroph's isolate in the centre of Kolle flask. All flasks were incubated at 4°C, 15°C and 22°C. Dry weight of wood blocks was estimated before the experiment and after three and six months of incubation on the mycelium. The percentage of wood weight loss was determined, which expressed the fungus ability of wood decomposition.

The analysis of variance and the Tukey *HSD* test was used to compare the results. Data in % were transformed before the analysis to formula of C. I. Bliss [Snedecor and Cochran 1976] of the form: $\arcsin\sqrt{percentage/100}$.

RESULTS

Growth *in vitro*. Mycelium of *F. velutipes* initiated growth one day after inoculation in 15°C and 22°C but in 7th day in 4°C. Average daily increment of mycelium was 2.2 mm in 4°C, 6.7 mm in 15°C and 6 mm in 22°C. In 9th day the mycelium stopped the growth in 22°C riches average 62 mm in diameter. Thirty days after inoculation average diameter of mycelium was 59 mm incubated in 4°C (Table 1). In 22°C the mycelium increment increased for four days and after that it was suddenly decreased and stopped in 9th day after inoculation (Fig. 1). There were statistically significant differences ($p < 0.05$, Tables 2 and 3) in the growth rate of mycelium in three incubation temperatures.

Table 1. Average *Flammulina velutipes* mycelium diameter in particular days after inoculation, mm
Tabela 1. Średnia średnica grzybni *Flammulina velutipes* w poszczególnych dniach po inokulacji, mm

Days Dni	Temperature – Temperatura		
	4°C	15°C	22°C
1		8.2	8.8
2		12.7	15.8
3		20.1	24.4
4		28.2	33.4
5		36.8	42.2
7	8.4	50.2	55.7
8	10.9	57.8	60.2
9	13.1	65.3	61.7
10	15.5	72.8	61.7
11	17.8	80.2	
12	20.1	87.8	
14	24.7		
15	27.1		
30	60.4		

Wood colonization *in vitro*. Firstly, mycelium appeared on birch and beech samples incubated at 22°C four days after inoculation. The most intensive colonization was observed in 22°C on beech wood (Fig. 2). In temperature 4°C mycelium started growth on the beech sample surfaces on 13th day, on birch in 16th and on oak on 18th day after inoculation. After fifty four days of incubation *F. velutipes* mycelium covered 55% (oak) – 84% (birch) wood samples' surface. Mycelium colonized in the whole oak samples after 15 (in 22°C) – 16 (in 15°C) days and beech samples after 14 (in 22°C) – 18 (in 15°C) days and birch wood samples after 13 (in 22°C) – 16 (in 15°C) days (Table 4).

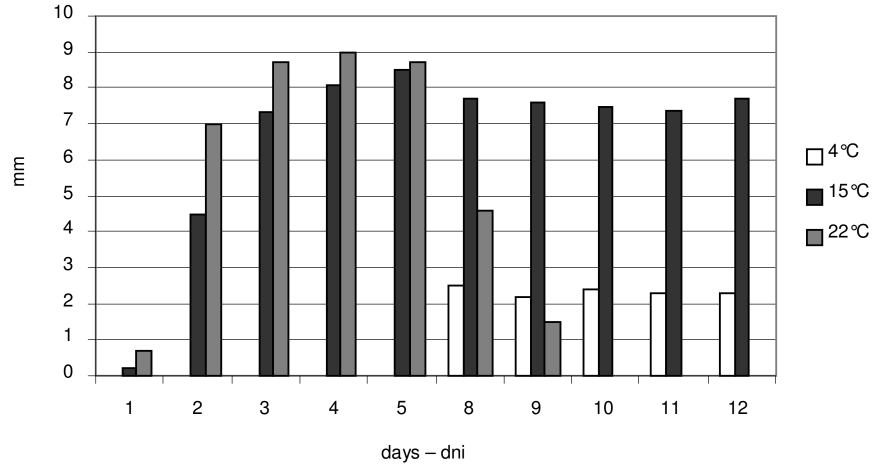

Fig. 1. Average daily increment of *F. velutipes* mycelium
Rys. 1. Średni przyrost dobowy grzybni *F. velutipes*

Table 2. Analysis of variance for diameter of mycelium growing on medium eight days after inoculation ($p < 0.05$)
Tabela 2. Analiza wariancji dla średnicy grzybni rosnącej na pożywce osiem dni po inokulacji ($p < 0,05$)

Source of variation Źródło wariancji	SS	df	MS	F	P	Test F
Among groups Pomiędzy grupami	18 426.206	2	9 213.103	36 256.2	5.08E-47	3.354131
Inside groups W obrębie grup	6.861	27	0.254111			
Total Razem	18 433.067	29				

Table 3. The results of the Tuckey HSD post-hoc comparisons of mycelium growth at different temperatures
Tabela 3. Wyniki porównania wzrostu grzybni testem HSD Tuckeya w różnych temperaturach

Temperature Temperatura	Temperature – Temperatura		
	4°C	15°C	22°C
4°C		0.000127	0.000127
15°C	0.000127		0.000127
22°C	0.000127	0.000127	

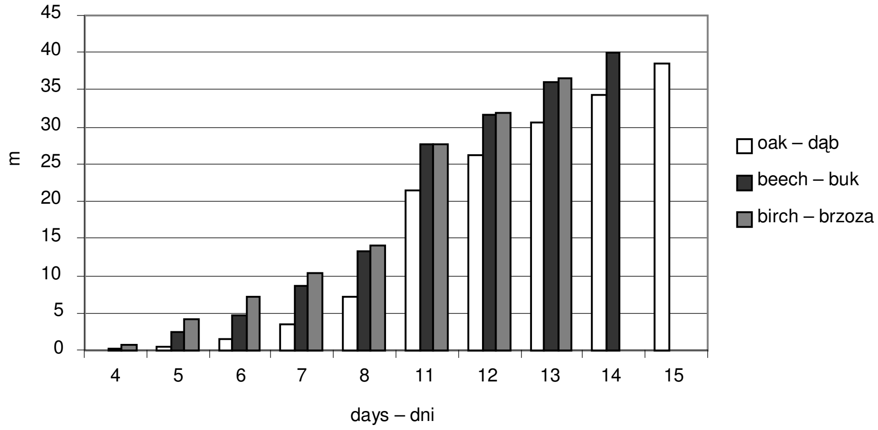

Fig. 2. Wood samples colonization by *F. velutipes* mycelium at incubation temperature 22°C
Rys. 2. Zasiedlenie próbek drewna przez grzybnię *F. velutipes* w temperaturze inkubacji 22°C

Table 4. Average wood colonization rate of *F. velutipes* mycelium, mm
Tabela 4. Średnie tempo zasiedlania drewna przez grzybnię *F. velutipes*, mm

Days after inoculation Dni po inokulacji	Oak – Dąb			Beech – Buk			Birch – Brzoza		
	Temperature, °C – Temperatura, °C								
	4	15	22	4	15	22	4	15	22
4						0.3			0.8
5		0.4	0.4		0.4	2.5		1.3	4.2
6		1.3	1.6		1	4.6		3.9	7.2
7		3.2	3.4		2.9	8.6		6.7	10.4
8		6	7.1		6.1	13.4		9.7	14.2
11		17.8	21.6		15.7	27.6		19.3	27.8
12		22.3	26.1		19.1	31.7		22.3	32
13		26.5	30.6	0.4	22.2	36		25.6	36.7
14		30.8	34.3	0.9	24.5	40		39.7	
15		35	38.5	1.1	27.3			33.6	
16		38.9		1.4	31.2		0.3	37.6	
18	0.2			1.9	39.5		1.4		
19	0.2			2			1.8		
20	0.4			2.4			2.2		
25	2			5.2			6.1		
32	4.5			9.2			11.8		
39	8.7			17.3			18		
46	15.7			23			25.9		
53	21.7			30			32.8		
54	22.1			31.2			33.7		

In some cases, there were no significant differences in wood colonization rate for different incubation temperature and for different wood species ($p > 0.05$, Tables 5 and 6). Oak samples were colonized similarly like beech and birch samples at 22°C ($p > 0.05$, Table 6). The same relation was observed for colonization of oak wood at 15°C and birch wood at 22°C or for colonization of beech and birch wood at 15°C ($p > 0.05$, Table 6).

Wood decomposition *in vitro*. *Flammulina velutipes* isolate decomposed birch and beech wood in similar extent ($p > 0.05$, Tables 7-10) at the temperature of 15°C and 22°C after three and six months of incubation. The same situation was observed for beech and birch wood decomposition at 4°C and 15°C after six months of incubation.

Table 5. Analysis of variance for *F. velutipes* wood colonization rate 14 days after inoculation ($p < 0.05$)
Tabela 5. Analiza wariancji dla zasiedlenia drewna przez *F. velutipes* 14 dni po inokulacji ($p < 0,05$)

Source of variation Źródło wariancji	SS	df	MS	F	P	Test F
Among groups Pomiędzy grupami	7 925.565	6	1 320.927	799.276	1.28E-41	2.323993
Inside groups W obrębie grup	69.41151	42	1.652655			
Total Razem	7 994.976	48				

Table 6. The results of the Tuckey HSD post-hoc comparisons of *F. velutipes* wood colonization on 14th day after inoculation at different temperatures ($p < 0.05$)
Tabela 6. Wyniki porównania zasiedlenia drewna przez *F. velutipes* testem HSD Tuckeya w 14 dniu po inokulacji w różnych temperaturach ($p < 0,05$)

Wood and incubation temperatures Drewno i temperatury inkubacji	Wood and incubation temperatures – Drewno i temperatury inkubacji					
	oak – dąb (15°C)	oak – dąb (22°C)	beech – buk (4°C)	beech – buk (15°C)	beech – buk (22°C)	birch – brzoza (15°C)
Oak – Dąb (22°C)	0.000244					
Beech – Buk (4°C)	0.000139	0.000139				
Beech – Buk (15°C)	0.000139	0.000139	0.000139			
Beech – Buk (22°C)	0.000139	0.383494	0.000139	0.000139		
Birch – Brzoza (15°C)	0.000139	0.000139	0.000139	0.64337	0.000139	
Birch – Brzoza (22°C)	0.187392	0.122822	0.000139	0.000139	0.00053	0.000139

Table 7. Analysis of variance for wood decomposition three months after inoculation ($p < 0.05$)
Tabela 7. Analiza wariancji dla rozkładu drewna trzy miesiące po inokulacji ($p < 0,05$)

Source of variation Źródło wariancji	SS	df	MS	F	P	Test F
Among groups Pomiędzy grupami	0.120944	2	0.060472	12.5496	1.44E-05	3.091188
Inside groups W obrębie grup	0.46259	96	0.004819			
Total Razem	0.583534	98				

Table 8. The results of the Tuckey HSD post-hoc variance for wood decomposition three months after inoculation ($p < 0.05$)
Tabela 8. Wyniki porównania rozkładu drewna trzy miesiące po inokulacji ($p < 0,05$)

Wood – Drewno	Wood – Drewno	
	oak – dąb	beech – buk
Beech – Buk	6.69E-06	
Birch – Brzoza	7.04E-05	0.440194

Table 9. Analysis of variance for wood decomposition six months after inoculation ($p < 0.05$)
Tabela 9. Analiza wariancji dla rozkładu drewna sześć miesięcy po inokulacji ($p < 0,05$)

Source of variation Źródło wariancji	SS	df	MS	F	P	Test F
Among groups Pomiędzy grupami	0.166481	2	0.08324	14.10218	4.27E-06	3.091188
Inside groups W obrębie grup	0.566656	96	0.005903			
Total Razem	0.733137	98				

Table 10. The results of the Tuckey HSD post-hoc variance for wood decomposition six months after inoculation ($p < 0.05$)
Tabela 10. Wyniki porównania rozkładu drewna sześć miesięcy po inokulacji ($p < 0,05$)

Wood – Drewno	Wood – Drewno	
	oak – dąb	beech – buk
Beech – Buk	4.66245E-06	
Birch – Brzoza	2.07122E-05	0.470228

The average loss of dry weight after three months differed from 1.66% of oak wood incubated at 4°C to 8.96% of birch wood incubated at 22°C (Fig. 3) and after six months respectively from 5.44% to 15.43% (Fig. 4). The oak wood was decomposed less intensive ($p < 0.05$, Tables 8 and 10) in comparison to birch and beech wood at all temperatures and both exposition time. Moreover, the oak wood was decomposed similarly at all incubation temperatures in both exposition times ($p > 0.05$, Tables 11-14). Comparing the wood decomposition of beech wood in all temperatures after three months of incubation there were no significant differences in loss of dry weight at 15°C and 22°C ($p > 0.05$, Table 12). The same situation was observed for birch wood. After six months of incubation significant differences were noted for comparison of beech wood decomposition at 4°C and 22°C and for birch wood decomposition at the same temperatures ($p < 0.05$, Table 14).

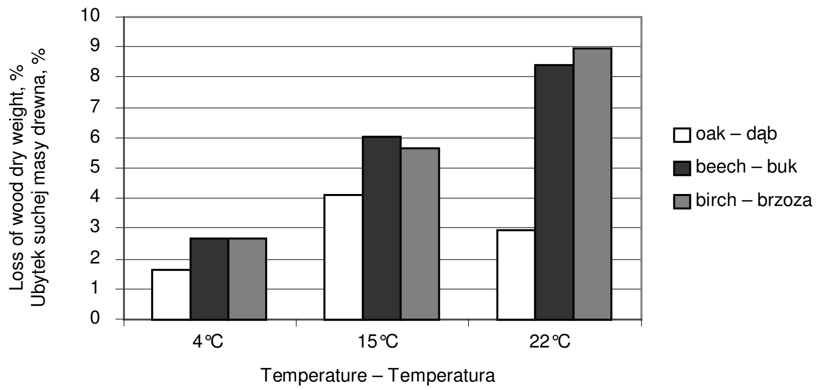

Fig. 3. Average loss of dry weight caused by *F. velutipes* after three months of incubation
Rys. 3. Średni ubytek suchej masy powodowany przez *F. velutipes* po trzech miesiącach inkubacji

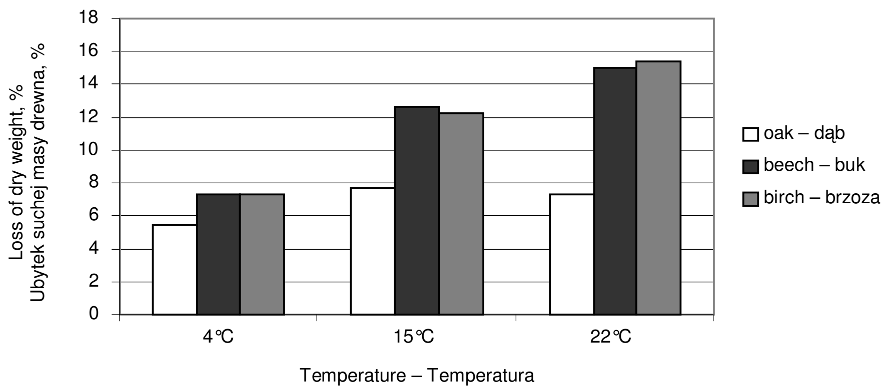

Fig. 4. Average loss of dry weight caused by *F. velutipes* after six months of incubation
Rys. 4. Średni ubytek suchej masy powodowany przez *F. velutipes* po sześciu miesiącach inkubacji

Table 11. Analysis of variance for wood decomposition three months after inoculation, separately for each wood species and for each incubation temperature ($p < 0.05$)
Tabela 11. Analiza wariancji dla rozkładu drewna trzy miesiące po inokulacji, osobno dla każdego gatunku drewna i każdej temperatury ($p < 0,05$)

Source of variation Źródło wariancji	SS	df	MS	F	P	Test F
Among groups Pomiędzy grupami	0.347034	8	0.043379	16.50798672	8.51E-15	2.042988
Inside groups W obrębie grup	0.2365	90	0.002628			
Total Razem	0.583534	98				

Table 12. The results of the Tuckey HSD post-hoc variance for wood decomposition three months after inoculation, separately for each wood species and for each incubation temperature ($p < 0.05$)
Tabela 12. Wyniki porównania rozkładu drewna trzy miesiące po inokulacji, osobno dla każdego gatunku drewna i każdej temperatury ($p < 0,05$)

Wood and incubation temperatures Drewno i temperatury inkubacji	Wood and incubation temperatures – Drewno i temperatury inkubacji							
	oak dąb (15°C)	oak dąb (22°C)	beech buk (4°C)	beech buk (15°C)	beech buk (22°C)	birch brzoza (15°C)	birch brzoza (22°C)	oak dąb (4°C)
Oak Dąb (22°C)	0.882359							
Beech Buk (4°C)	0.919477	1						
Beech Buk (15°C)	0.252506	0.005277	0.007368					
Beech Buk (22°C)	0.000445	0.000134	0.000135	0.420724				
Birch Brzoza (15°C)	0.462856	0.016712	0.022727	0.999992	0.222306			
Birch Brzoza (22°C)	0.000318	0.000134	0.000134	0.340854	1	0.169174		
Oak Dąb (4°C)	0.064355	0.773154	0.711575	0.00014	0.000134	0.000167	0.000134	
Birch Brzoza (4°C)	0.871637	1	1	0.004849	0.000134	0.015455	0.000134	0.787689

Table 13. Analysis of variance for wood decomposition six months after inoculation, separately for each wood species and for each incubation temperature ($p < 0.05$)
Tabela 13. Analiza wariancji dla rozkładu drewna sześć miesięcy po inokulacji, osobno dla każdego gatunku drewna i każdej temperatury ($p < 0,05$)

Source of variation Źródło wariancji	SS	df	MS	F	P	Test F
Among groups Pomiędzy grupami	0.355811	8	0.044476	10.60853	2.21465E-10	2.042988
Inside groups W obrębie grup	0.377326	90	0.004193			
Total Razem	0.733137	98				

Table 14. The results of the Tuckey HSD post-hoc variance for wood decomposition six months after inoculation, separately for each wood species and for each incubation temperature ($p < 0.05$)
Tabela 14. Wyniki porównania rozkładu drewna sześć miesięcy po inokulacji, osobno dla każdego gatunku drewna i każdej temperatury ($p < 0,05$)

Wood and incubation temperatures Drewno i temperatury inkubacji	Wood and incubation temperatures – Drewno i temperatury inkubacji							
	oak dąb (15°C)	oak dąb (22°C)	beech buk (4°C)	beech buk (15°C)	beech buk (22°C)	birch brzoza (15°C)	birch brzoza (22°C)	oak dąb (4°C)
Oak Dąb (22°C)	1							
Beech Buk (4°C)	1	0.999997						
Beech Buk (15°C)	0.036928	0.022168	0.05467					
Beech Buk (22°C)	0.000494	0.000311	0.000763	0.912229				
Birch Brzoza (15°C)	0.068424	0.04263	0.098063	1	0.813117			
Birch Brzoza (22°C)	0.000375	0.000251	0.00056	0.873891	1	0.756935		
Oak Dąb (4°C)	0.934596	0.972259	0.886146	0.000613	0.000135	0.001288	0.000134	
Birch Brzoza (4°C)	1	0.999998	1	0.050869	0.000701	0.091837	0.000517	0.896539

DISCUSSION

There are only a few papers about *F. velutipes* concerning the characteristic of isolates. Edgecombe [1941] found that daily increment of mycelium of *F. velutipes* was 8 mm. In this study the most intensive growth of mycelium was at 15°C, and the average daily increment of saprotroph's mycelium was similar to those describing by Edgecombe – about 8 mm from 3rd to 12th day of observation. Łakomy [2004] showed that mycelium of same fungi could grow even more than 16 mm per day (eg. *Bjerkandera adusta*), but there were some fungi, which mycelium grew less than 4 mm (eg. *Pluteus atricapillus* (Batsch.) Sing.) per day. This isolate of *F. veluitipes* might be classify to fungi of average rate of daily mycelium increment.

The most intensive colonization of wood was observed at 22°C and the less intensive at 4°C. After seven days of incubation the mycelium occupied 10 mm of birch wood samples from the inoculation point, 8.6 mm of beech wood samples and only 3.4 of oak wood. This *F. velutipes* isolate could be included to the group of fungi which colonize the wood rather slowly. Łakomy [2004] grouped isolates of nine saprotrophic fungi species in dependence on rate of wood colonization. Isolates that overgrew the wood 2-7 mm per day was classified to isolates of slow colonizing rate. The most intensive colonizing species were *B. adusta* and *T. versicolor*, which were colonizing the whole wood samples for five days.

Shanel [1966] indicated that beech wood colonizing with *F. velutipes* for 112 days lost 5.51% of its weight, while isolate of *Fomes fomentarius* (L. ex Fr.) Kickx. caused 60% loss of weight in the same time. In this study beech wood was decomposed on the average in 8% after three months but after six months in 12% at the temperature of 15°C and average 15% at 22°C. The less intensive decay was observed for oak wood but this wood species was decomposed in a similar way at three incubation temperatures. It is also interesting that beech and birch wood was decayed to the same degree at 15°C and 22°C. Łakomy [2004] also displayed that oak wood in comparison to beech and birch wood was the less intensive decayed wood irrespectively of saproteoph's species and incubation time. Moreover the beech wood was decayed by these fungi in the most intensive way. The most effective in wood decomposition isolates of *B. adusta* and *T. versicolor* caused about 41-47% loss of wood sample dry weight after three months of incubation and 77-83% after six months. At the other hand the less effective isolate of *Pholiota squarrosa* (Müll.: Fr.) Kummer decomposed oak wood in 2.67% after three months and in 16.41% after six months.

Fultz [1988] displayed that optimum for *F. velutipes* vegetative mycelium growth ranged between 22°C and 26°C, although the temperature for the initiation of basidiomes production should be below 15°C. However, in the same investigation Fultz found that about half of the tested *F. velutipes* isolates started to create basidiomes at temperatures below 22°C and the other half of isolates at the temperature typical for basidiomes production of *F. velutipes* i.e. below 15°C.

To conclude the ability of growth, wood colonization and wood decay of the investigated *F. velutipes* isolate was similar at two incubation temperatures 15°C and 22°C and totally differed at temperature 4°C. Beech and birch wood were the most effectively colonized and decomposed by *F. velutipes* isolate irrespectively of the incubation temperature. This isolate of *F. velutipes* should also be tested in forest to check its stump colonization ability and competitiveness in wood with other fungi.

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ZDOLNOŚĆ WZROSTU, ZASIEDLENIA I ROZKŁADU DREWNA IZOLATU *FLAMMULINA VELUTIPES IN VITRO*

Streszczenie. Celem badań było określenie właściwości izolatu *F. velutipes,* a w szczególności zdolności wzrostu, zasiedlania i rozkładu drewna bukowego, brzozowego i dębowego w trzech temperaturach 4°C, 15°C i 22°C *in vitro.* Grzybnia saprotrofa rozpoczynała wzrost już po dniu od inokulacji w temperaturze 15°C i 22°C. Średni dzienny przyrost grzybni wynosił 2,2 mm w temperaturze 4°C, 6,7 mm w 15°C i 6 mm w 22°C. Grzybnia rozpoczynała zasiedlanie drewna po czterech dniach od inokulacji. Najintensywniej izolat *F. velutipes* zasiedlał drewno bukowe w temperaturze 22°C. Izolat *F. velutipes* rozkładał drewno brzozowe i bukowe w takim samym stopniu w temperaturach 15°C i 22°C, zarówno po trzech, jak i sześciu miesiącach inkubacji. Średnia utrata ciężaru próbek drewna po trzech miesiącach wynosiła od 1,66% dla drewna dębowego inkubowanego w temperaturze 4°C do 8,96% dla drewna brzozowego inkubowanego w temperaturze 22°C, natomiast po sześciu miesiącach utrata ciężaru próbek drewna wynosiła odpowiednio od 5,44% do 15,45%. Najsłabiej badany izolat saprotrofa rozkładał drewno dębowe niezależnie od temperatury i czasu inkubacji. Właściwości tego izolatu co do tempa zasiedlania drewna pniakowego i jego zdolności konkurowania z innymi grzybami środowiska leśnego powinny być sprawdzone w badaniach terenowych.

Słowa kluczowe: *Flammulina velutipes*, wzrost *in vitro*, zasiedlenie drewna *in vitro*, rozkład drewna *in vitro*

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