ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol.
Silv. Colendar. Rat. Ind. Lignar. 6(1) 2007, 5-12

DEVELOPMENT OF MYCELIAL CORDS OF *HYPHOLOMA FASCICULARE* ISOLATES IN DIFFERENT FOREST SOILS

Marta Z. Dadacz, Piotr Łakomy
Agricultural University of Poznań

Abstract. The development of mycelial cords of two *Hypholoma fasciculare* isolates in two soil types and their ability to colonize the wood debris burried in these soils were studied. In this study it was shown that *H. fasciculare* could grow and colonize wood debris similarly irrespective of soil type and fungus genet.

Key words: *Hypholoma fasciculare,* mycelial cord development, forest soils

INTRODUCTION

Hypholoma fasciculare [Huds.: Fr.] Kummer is a basidimycota saprotroph which occurred in coniferous, mixed and deciduous forests. It could colonize both coniferous and deciduous stumps and wood debris burried in soil. This fungus spreads in the forest forming mycelial cords penetrating the soil and colonizing the wood as a food base [Dowson et al. 1988 a, b, Łakomy 2004]. *Hypholoma fasciculare* causes white rot of wood [Gumińska and Wojewoda 1985]. This fungal species was used in different studies to investigate its ability to suppress the growth of pathogenic fungi [Łakomy et al. 1998, 1999, Nicolotti et al. 1994], to colonize the wood in the forest [Hintikka 1993, Meredith 1959, 1960, Rayner 1978, Chapman and Xiao 2000, Łakomy 2004], and to exclude pathogens from wood [Twarowska 1972, Pearce and Malajczuk 1990, Anselmi and Nicolotti 1998, Nicolotti et al. 1999, Chapman and Xiao 2000, Łakomy 2004]. This species is considered for using as a biological control agent or in integrated pest management control against *Armillaria* spp., responsible for the most dangerous disease of forest trees all over the world. *Armillaria* spp. cause root rot and decay of trees. Stumps or wood debris in the soil are the resources for pathogen's survival and its spread in stands. Colonization and decomposition of wood debris by saprotrophic fungi could exclude *Armillaria* spp. from them and decrease the damages in future stands.

Corresponding author – Adres do korespondencji: Dr hab. Piotr Łakomy, Department of Forest Pathology of Agricultural University of Poznań, Wojska Polskiego 71 C, 60-625 Poznań, Poland, e-mail: plakomy@au.poznan.pl

The aim of this study was to investigate the growth ability of two *H. fasciculare* isolates in two different soil types and capability to colonize wood debris burried in these soils.

MATERIAL AND METHODS

Two isolates of *Hypholoma fasciculare* (no: 96022 and 96025) were used in this study. Both cultures were isolated from basidiomes collected from oak stumps in the Zielonka (96022, 52°30'N, 17°02'E) and Babki (96025, 52°23'N, 16°58'E) Forest Districts.

Hypholoma fasciculare inoculum consisted of wood segments colonized by mycelium. Wood segments in length of 5 cm and diameter of 2 cm were made from oak branches. The inoculum was put in the middle of a plastic box (26 cm × 18 cm × 6.5 cm) filled up with forest soil.

In the first variant of the experiment the inoculum was put on the soil and in the other variant the inoculum was burried in soil, at the depth of 35 mm. In both variants the soils were covered by litter taken from the same stand. In each box corners were put sterile wood segments. The segments were put at two levels of deepness in varaint I (between soil and litter and on depth of 65 mm) and at three levels in variant II (between soil and litter, on depth of 35 and 65 mm) of the experiment. At each level, which was established below and above the level of inoculum, the sterile segments of wood were put also in the middle of the box.

Two types of forest soil and litter were used in this experiment. The first was taken from fresh mixed coniferous forest (BMśw) with Scots pine as a dominant species and the other from the fresh mixed deciduous forest (LMśw) with oak as a main species. Each soil was moistened before the experiment. For control the inoculum and sterile wood segments were put in the same schedule in the sand.

Observations of mycelium development on the soil surface and in the litter were conducted after one month in variant I and after 2, 4, 6, 8 weeks in variant II. After 11 weeks wood segments were taken and analysed for mycelium colonization. Every combination of the experiment was repeated five times.

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

RESULTS

Variant I

After four weeks of growth of the mycelial cords of both *H. fasciculare* isolates were well developed and spread on average distance 13.4-16.3 cm (max. 17 cm) in soil from fresh mixed coniferous stand (BMśw) and 11.2-14.8 cm (max. 17 cm) from mixed deciduous stand (LMśw). In contrast mycelia of the both isolates grew over the sand average up to 5.7-9.7 cm (max. 13 cm) at the same time (Table 1, Fig. 1). The mycelial cords spread in the litter, on the soil and in the soil at all depth levels.

Table 1. Average mycelial cord length after four weeks of growth
Tabela 1. Średnia długość sznurów grzybniowych po czterech tygodniach wzrostu

Isolate Izolat	Soil Gleba	Length Długość cm
H. fasciculare 96022	BMśw	13.4
H. fasciculare 96025	BMśw	16.3
H. fasciculare 96022	LMśw	11.2
H. fasciculare 96025	LMśw	14.8
H. fasciculare 96022	Sand	5.7
H. fasciculare 96025	Sand	9.7

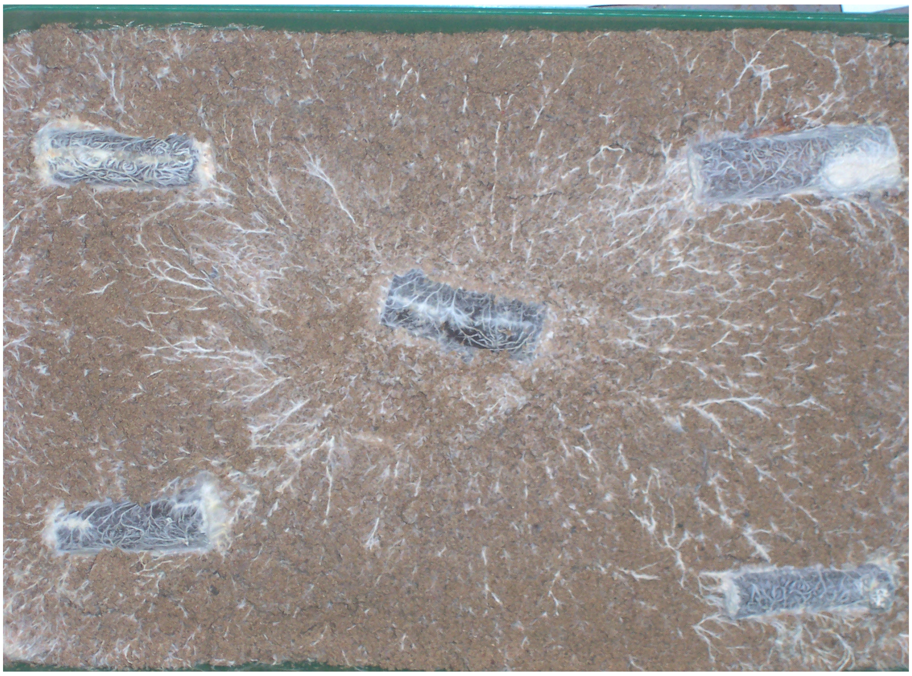

Fig. 1. Spread of mycelial cords of *H. fasciculare* 96025 in the sand 11 weeks after inoculation
Rys. 1. Rozprzestrzenianie się sznurów grzybniowych *H. fasciculare* 96025 w piasku 11 tygodni po inokulacji

Isolates of *H. fasciculare* colonized segments similarly ($p > 0.05$; Table 2) in both soils and at both levels of depth. Only isolate *H. fasciculare* 96025 colonized more wood segments in both soils than in sand ($p < 0.05$; Table 2).

The mycelium of *H. fasciculare* 96022 spread more frequently ($p < 0.05$; Table 2) on segments surfaces in the sand than in soil from mixed deciduous stand (LMśw) at level I of segments burried depth and than mycelium of *H. fasciculare* 96025 in soil from fresh mixed coniferous stand (BMśw, both levels) or at level I of soil from mixed deciduous stand (LMśw).

Table 2. Percentage of wood segments totally colonized by mycelium and with appearance of mycelium on the surface and under the bark at two levels of placing
Tabela 2. Procent całkowicie skolonizowanych segmentów drewna z obecnością grzybni na ich powierzchni i pod korą na dwóch poziomach wyłożenia

Combination Kombinacja		*H. fasciculare* 96022	*H. fasciculare* 96025
Wood colonization – Zasiedlenie drewna			
BMśw	level I – poziom I	0	55^{ab}
	level II – poziom II	0	64^{b}
LMśw	level I – poziom I	0	55^{b}
	level II – poziom II	16^{ab}	56^{b}
Sand Piasek	level I – poziom I	0	0
	level II – poziom II	0	3.33^{a}
Mycelium on the segment – Grzybnia na segmencie			
BMśw	level I – poziom I	45^{cd}	10^{d}
	level II – poziom II	56^{cd}	12^{d}
LMśw	level I – poziom I	10^{c}	10^{d}
	level II – poziom II	24^{cd}	0
Sand Piasek	level I – poziom I	41.67^{cd}	25^{cd}
	level II – poziom II	86.67^{c}	40^{cd}
Mycelium under the bark – Grzybnia pod korą			
BMśw	level I – poziom I	55^{ef}	35^{ef}
	level II – poziom II	44^{ef}	24^{f}
LMśw	level I – poziom I	80^{ef}	35^{ef}
	level II – poziom II	44^{ef}	44^{ef}
Sand Piasek	level I – poziom I	25^{ef}	75^{ef}
	level II – poziom II	13.33^{ef}	56.67^{e}

$^{a\text{-}f}$ – statistically homogeneous groups estimated by HSD Tukey's test, $p < 0.05$.
$^{a\text{-}f}$ – grupy statystycznie jednorodne wyznaczone testem HSD Tukeya, p < 0,05.

Analysing the occurrence of mycelium between bark and wood, mycelium of *H. fasciculatre* 96025 often invaded the space under the bark in sand than at level II of soil from fresh mixed coniferous stand (BMśw; $p < 0.05$; Table 2).

Variant II

Mycelal cords of *H. fasciculare* 96025 overgrew the soils faster than mycelial cords of isolate *H. fasciculare* 96022 (Table 3, Fig. 1). Both isolates spread faster in the soil from fresh deciduous mixed stand (LMśw) than in soil from fresh coniferous stand (BMsw) and in sand. Mycelial cords of both isolates also penetrated the litters.

Table 3. Average mycelial cord length after two, four, six and eight weeks of growth, cm
Tabela 3. Średnia długość sznurów grzybniowych po dwóch, czterech, sześciu i ośmiu tygodniach wzrostu, cm

Isolate Izolat	Soil Gleba	Time, weeks – Czas, tygodnie			
		2	4	6	8
H. fasciculare 96022	BMśw	0.4	1.8	4.8	7
H. fasciculare 96025	BMśw	0.8	4.8	9	14.3
H. fasciculare 96022	LMśw	0.3	3.8	8	11.5
H. fasciculare 96025	LMśw	0.5	7.3	19.3	26
H. fasciculare 96022	Sand – Piasek	0	0.5	3	8.3
H. fasciculare 96025	Sand – Piasek	0	0.8	4.5	9.8

Mycelium of both *H. fasciculare* isolates colonized wood segments and spread on its surfaces in the same way ($p > 0.05$; Table 4) both in the forest soils and in the sand irrespective of wood segments burried level.

The mycelium of *H. fasciculare* 96025 occurred only less frequenty under the bark of wood segments burried at level I in the soil from mixed deciduous stand (LMśw) than in sand ($p < 0.05$; Table 4). In other cases the mycelium penetration of both saprotroph's isolates under the bark was similar both in the forest soils and in the sand ($p > 0.05$; Table 4).

Table 4. Percentage of wood segments totally colonized by mycelium and with appearance of mycelium on the surface and under the bark at three levels of placing
Tabela 4. Procent całkowicie skolonizowanych segmentów drewna z obecnością grzybni na ich powierzchni i pod korą na trzech poziomach wyłożenia

Combination – Kombinacja		*H. fasciculare* 96022	*H. fasciculare* 96025
1		2	3
Wood colonization – Zasiedlenie drewna			
BMśw	level I – poziom I	0	15
	level II – poziom II	0	6.25
	level III – poziom II	0	5
LMśw	level I – poziom I	0	25
	level II – poziom II	25	56.25
	level III – poziom II	30	55
Sand Piasek	level I – poziom I	0	0
	level II – poziom II	0	0
	level III – poziom II	0	0
Mycelium on the segment – Grzybnia na segmencie			
BMśw	level I – poziom I	10^{ab}	30^{ab}
	level II – poziom II	0	25^{ab}
	level III – poziom II	0	20^{ab}

Table 4 – cont.

	1	2	3
LMśw	level I – poziom I	40[ab]	5[b]
	level II – poziom II	31.25[ab]	0
	level III – poziom II	10[ab]	0
Sand Piasek	level I – poziom I	30[ab]	60[ab]
	level II – poziom II	56.25[ab]	56.25[ab]
	level III – poziom III	55[ab]	70[a]
Mycelium under the bark – Grzybnia pod korą			
BMśw	level I – poziom I	20	20
	level II – poziom II	12.5	6.25
	level III – poziom II	10	5
LMśw	level I – poziom I	25	70
	level II – poziom II	25	43.75
	level III – poziom II	20	45
Sand Piasek	level I – poziom I	20	15
	level II – poziom II	6.25	18.75
	level III – poziom II	20	20

[a-b] – statistically homogeneous groups estimated by HSD Tukey's test, $p < 0.05$.
[a-b] – grupy statystycznie jednorodne wyznaczone testem HSD Tukeya, $p < 0,05$.

DISCUSSION

Fungi which spread in forest soil by cords are interested in view of biological control of tree pathogens or even insects. They could penetrate the ecological niche in the soil relatively fast, colonize all wood debris and make it then inaccesible for root rot pathogens such as *Armillaria* spp.

Dowson et al. [1988 a] tested cord-forming fungi *H. fasciculare, Phallus impudicus* (L.) Pers., *Phanerochaete laevis* (Fr.) Erikss. & Ryv., *P. velutina* (DC ex Pers.) Parmasto, *Steccherinum fimbriatum* (Pers. ex Fr.) Erikss. and *Tricholomopsis platyphylla* (Pers. ex Fr.) Sing. The pieces of wood overgrown by mycelium burried in the soil and development of cords were investigated. After three months all species except *T. platyphylla* spread radially around the burried wood, between soil and litter. After three or six months *H. fasciculare* and *P. velutina* grew fastest than other species. These findings indicated that these fungi could survive in forest soil and spread in a similar way to *Armillaria* species.

In this study both isolates of *H. fasciculare* spread not only between soil and litter but also through the litter and soil. The radial growth of *H. fasciclare* was also noted.

Dowson et al. [1988 b] showed that three factors determine spread and survival of mycelial cords in the environment. They found that micrococmos, food base and occurrence of other competitive microorganisms were the most important factors in fungi spreading. *Hypholoma fasiculare, S. fimbriatum* and *P. velutina* grew very fast and colonized wood debris before other fungi species. Moreover *P. velutina* colonized wood earlier occupied by other fungi and in addition this species was able to exclude other species from wood debris. Dowson et al. [1988 b] showed that burried wood could be colonized by other soil fungi (*Trichoderma* spp., *Penicillium thomii* Maire, *Ascocoryne sarcoides* (Jacq. ex S.F. Gray] Groves et Wilson, *Hypoxylon serpens* (Pers.: Fr.) Fr., *Xylaria hypoxylon* (L.) Grev., *Coprinus domesticus* (Bolt: Fr.) S.F. Grey, *Sistotrema brinkmannii* (Bres.) Erikss. or *Botrytis cinerea* Pers.) before tested species.

In this study it was shown that *H. fasciculare* could grow and colonize wood debris similarly irrespective of soil type and fungus genet.

Łakomy et al. [1999], Łakomy [2004] showed that fungi wood decay ability, wood colonization, penetration speed, competitive capability to other orgasnisms depended on individual features of fungus genet.

Cord-forming *Hypholoma fasciculare* was also tested in natural environment. Pearce and Malajczuk [1990], Łakomy [2004], Chapman and Xiao [2000] found that *Hypholoma* may colonize stumps, spread by cords in forest soil and even successfully exclude *Armillaria* from wood. Rayner [1978] found that only *P. velutina* could exclude *H. fasciculare* from the wood. *Hypholoma fasciculare* is a species which is considered in biological control or integrated pest management against *Armillaria* spp. [Łakomy 2004].

REFERENCES

Anselmi N., Nicolotti G., 1998. Biological control of *Heterobasidion annosum* in the forest by non-pathogenic wood-destroying fungi. In: Root and Butt Rots of Forest Trees (9th International Conference on Root and Butt Rots). Eds C. Delatour, J.J. Guillaumin, B. Lungescarmant, B. Marcais. INRA Editions (France), Les Colloques no 89, 421-428.

Chapman B., Xiao G., 2000. Inoculation of stumps with *Hypholoma fasciculare* as a possible means of control *Armillaria* root disease. Can. J. Bot. 78, 129-134.

Dowson C.G., Rayner A.D.M., Boddy L., 1988 a. Inoculation of mycelial cord-forming basidiomycetes into woodland soil and litter. I. Initial establishment. New Phytol. 109, 335-341.

Dowson C.G., Rayner A.D.M., Boddy L., 1988 b. Inoculation of mycelial cord-forming basidiomycetes into woodland soil and litter. II. Resource capture and persistance. New Phytol. 109, 343-349.

Gumińska B., Wojewoda W., 1985. Grzyby i ich oznaczanie [Fungi and its identification]. PWRiL Warszawa [in Polish].

Hintikka V., 1993. Occurrence of edible fungi and other macromycetes on tree stump over a sixteen year period. Acta Bot. Fen. 149, 11-17.

Łakomy P., 2004. Środowiskowe uwarunkowania zasiedlenia pniaków drzew liściastych przez wybrane gatunki grzybów saprotroficznych oraz grzyby rodzaju *Armillaria* [Environmental conditions of deciduous tree stumps colonization by selected saprotrophic fungi species and *Armillaria* spp. Eng. summary]. Rocz. AR Pozn. Rozpr. Nauk. 355 [in Polish].

Łakomy P., Zieniewicz J., Świdkiewicz T., 1998. The influence of *Hypholoma fasciculare* and *Phlebiopsis gigantea* on the growth of *Heterobasidion annosum* in vitro. Acta Mycologica 33 (1), 147-154.

Łakomy P., Świdkiewicz T., Zieniewicz J., 1999. Different influence of *Hypholoma fasciculare* (Huds.: Fr.) Kummer on the growth of *Armillaria ostoyae* (Romagnesi) Herink *in vitro*. Phytopathol. Pol. 17, 91-98.

Meredith D.S., 1959. The infection of pine stumps by *Fomes annosus* and other fungi. Ann. Bot. 23, 445-476.

Meredith D.S., 1960. Further observation on fungi inhabiting pine stumps. Ann. Bot. 24, 63-78.

Nicolotti G., Gangemi D., Lanata F., Anselmi N., 1994. Antagonistic activity of wood decay *Basidiomycetes* against European *Armillaria* species. In: Proceedings of 8th International Conference on Root and Butt Rots. Wik, Sweden and Haikko, Finland, August 9-16 1993. Eds M. Johansson, J. Stenlid. IUFRO Working Party S2.06.01. Swed. Univ. Agric. Sci. S-750 Uppsala, Sweden, 725-735.
Nicolotti G., Gonthier P., Varese G.C., 1999. Effectiveness of some biocontrol and chemical treatments against *Heterobasidion annosum* on Norway spruce stumps. Eur. J. For. Path. 29, 339-346.
Pearce M.H., Malajczuk N., 1990. Inoculation of karri (*Eucalyptus diversicolor* F. Muell.) thinning stumps with wood decay fungi for control of *Armillaria luteobubalina.* Mycological Res. 94, 32-37.
Rayner A.D.M., 1978. Interactions between fungi colonizing hardwood stumps and their possible role in determining patterns of colonization and succession. Ann. Appl. Biol. 89, 131-134.
Snedecor W., Cochran W.G., 1976. Statistical methods. The Iowa State Univ. Press Ames, Iowa, USA, 327-329.
Twarowska I., 1972. Badania nad zwalczaniem huby korzeniowej metodą biologiczną [Investigation of *Heterobasidoon annosum* biological control]. Pr. Inst. Bad. Leśn. 405, 1-56 [in Polish].

ROZWÓJ SZNURÓW GRZYBNIOWYCH IZOLATÓW *HYPHOLOMA FASCICULARE* W RÓŻNYCH GLEBACH

Streszczenie. Celem pracy było zbadanie rozwoju sznurów grzybniowych dwóch izolatów *Hypholoma fasciculare* w glebach leśnych pochodzących drzewostanów z siedlisk BMśw i LMśw. W pierwszym wariancie doświadczenia zasiedlone przez grzybnie segmenty, uzyskane z pędów dębowych o średnicy 2 cm i długości 5 cm, wykładano centralnie na powierzchni gleby umieszczonej w pudełku (26 cm × 18 cm × 6,5 cm). W narożnikach pudełka umieszczano także sterylne segmenty drewna na powierzchni gleby i na głębokości 65 mm. Glebę przykrywano sciołą pobraną z tych samych drzewostanów. W drugim wariancie doświadczenia inokulum zostało umieszczone na głębokości 35 mm, a sterylne segmenty drewna na powierzchni gleby, na głębokości 35 i 65 mm. W kontrolach glebę zastąpiono piaskiem. Każda kombinacja doświadczenia została wykonana w pięciu powtórzeniach. Analizę rozwoju sznurów grzybniowych oraz zasiedlenia drewna przeprowadzono po miesiącu w wariancie I doświadczenia oraz po dwóch, czterech, sześciu i ośmiu tygodniach w wariacie II. Analizowano rozwój sznurów grzybniowych i zasiedlenie drewna przez grzybnię izolatów *H. fasciculare.* Stwierdzono, że sznury grzybniowe penetrują intensywniej glebę pobraną z LMśw niż z BMśw. Izolaty różniły się także pod względem długości sznurów grzybniowych wytwarzanych w tych samych warunkach glebowych. Natomiast nie stwierdzano różnic w zasiedleniu drewna pomiędzy izolatami w różnych glebach. Pojedyncze różnice wystąpiły po porównaniu kontroli z odpowiednią kombinacją doświadczenia.

Słowa kluczowe: *Hypholoma fasciculare,* rozwój sznurów grzybniowych, gleby leśne

Accepted for print – Zaakceptowano do druku: 9.02.2007

For citation – Do cytowania: Dadacz M.Z., Łakomy P., 2007. Development of mycelial cords of Hypholoma fasciculare isolates in different forest soils. Acta Sci. Pol., Silv. Colendar. Rat. Ind. Lignar. 6(1), 5-12.