DESCRIPTION OF *HETEROBASIDION ANNOSUM* POPULATION OCCURRING IN SCOTS PINE STANDS IN CZŁOPA FOREST DISTRICTS II. MYCELIUM DEVELOPMENT IN A DEAD WOOD

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Abstract. The aim of this study was to test the ability of dead wood colonization of *Heterobasidion annosum* isolates with known aggressiveness to the Scots pine seedlings. The experiment was established in Scots pine stand localized in the Człopa Forest District. Roots of six-month-old stumps were inoculated with mycelium of 19 genotypes of *H. annosum* Genotypes colonized wood of Scots pine roots on distance from 8.10 cm to 43.27 cm. The genotypes that colonized wood on a shortest distance and the most extensively differed significantly from others. The analysis of the ability of wood colonization by mycelium of genotypes in relations to its origin showed that genotypes collected from stumps overgrew the roots slightly stronger (about 14.5%) than those collected from trees, but differences were not significant. There were no differences between genotypes isolated from pines and beaches, either. Population of *H. annosum* differed in genotypes features conditioning the rate of its development in the environment and disease dynamics. Differentiation of these features of pathogen populations caused an unequal infection and disease development rate in stands. It seems that the most aggressive isolates colonize the stand fastest through root system.

Key words: *Heterobasidion annosum*, genets, Scots pine, saprotrophic growth

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INTRODUCTION

Heterobasision spp. are the most important pathogens in coniferous stands in boreal climate zone. They play a particular role in stands growing both in the first and second generation on the post-arable soil. Stands are infested by basidiospores, which infect the stumps surfaces or get into the soil with rain and colonize via the root wounds. In addition basidiospores infected wounds in root collar of Norway spruce. The secondary spread of pathogen is caused by mycelium transfer through root system connections from stumps to neighbour stumps or trees (Korhonen et al., 1998; Redfern and Stenlid, 1998; Stenlid and Redfern, 1998; Mańka, 2005).

Stumps and roots are the most important source of the pathogens in infested stand. After primary infection by basidiospores in stands only small genotypes are present that occupied one stump, but after several years of disease development the mycelium is transferred via root contacts. In the second generation of stand on the post arable soil the mycelium of single genotypes spread for longer distance and cover even more than 200 m^2 of forest area (Dalke and Łakomy, 2009; Cieślak et al., 2011).

The share of root and butt rot diseases of the whole area of all diseases appearing in Polish forests reached 76.7% in 2013. Moreover, the pathogens cause severe damages in stands on the area of 220 thousands hectares, i.e. 2.89% of the whole area of State Forests in Poland.

A lot of geneotypes occurred in the stands influenced by a high diversity in pathogen's population, consisting of many organisms, which differ between each other of virulence, speed of wood colonization, and the ability of competitions with other microorganisms coexisting in the same ecological niche (Łakomy et al., 2007; Dalke and Łakomy, 2009).

The aim of this study was to test the ability of dead wood colonization of *Heterobasidion annosum* isolates with known aggressiveness (Cieślak et al., 2011) to the Scots pine seedlings.

MATERIAL AND METHODS

Pathogen

Nineteen isolates represented different genotypes of *H. annosum* have been used in these experiments. Pathogens were isolated from Scots pine stumps and dead trees localized in three stands – two 45-year-old (A, C) and from 12-year-old (B) pines and beeches planted in gaps of 33-year-old stand. All stands were localized in the Człopa Forest District and growing on the post-agricultural soil (pedzol). Identification of the pathogen's species was made with the aid of mating tests (Korhonen, 1978), and genets diversity on the base of somatic compatibility (Stenlid, 1985). Genetic relationships of population were described using DNA extraction in previous work (Cieślak et al., 2011). The inoculum consisted of 5 cm long and 1 cm in diameter wooden sticks overgrown by *H. annosum* mycelium.

The rate of growth in Scots pine stumps

The experiment was established in division 279d localized in the Człopa Forest District, Jaglice Range. The six-months-old stumps leaving after last thinning were chosen and the main roots of them were cleaned from the soil. In each root a hole of 1 cm in diameter was made by a drill 50 cm far from the stump. Inoculum of pathogen was put in each hole and next the place was covered by artificial cork (Lac Balsam®, Germany) and a root by soil. The control roots were inoculated with sterile wooden sticks. Three main roots of single stumps were inoculated with one pathogen genotype. Twelve months after inoculation the roots were collected and transferred to the laboratory. Root were cut 50 cm away from the inoculation point the both sides (towards and from stumps). In the laboratory all rots were tap water washed and dried with sterile filter paper. After that roots were split along fibers. The lengths of discoloration zone were measured in two directions. In addition the isolation of mycelium was made from each root. Inoculums were put on 1% malt extract agar (Merck, Germany) in Petri dishes and after 4-5 days were investigated on the presence of *Siiniger meinekellus* (A.J. Olson) Stalpers conidial stage of *Heterobasidion annosum* (Stenlid and Swedjemark, 1988; Werner and Łakomy, 2002).

The multifactorial analysis of variance was used (ANOVA/MANOVA) and HSD Tuckey's test. The Statistica v. 6 (2003) (Bobrowski, 1980; Kala, 2002) was used to verify the basic hypothesis.

RESULTS

The length of discoloration zone caused in stumps roots by *H. annosum* genotypes was shown in Table 1. The most extensive genotype BCZ3 in colonization spread in dead wood of stump roots on average distance 43.27 cm and the less intensively growth of mycelium displayed genotypes BCZ1 (8.10 cm). Other genotypes colonized wood of Scots pine roots on distance from 18.40 cm (BCZ4) to 40.47 cm (ACZ1). The mycelium of genotypes collected from plot A overgrew the wood (average 30.17 cm) 17% more expansively than mycelium of genotypes from plot B (average 25.32 cm) and 3% from plot C (average 29.44 cm). The mycelium of genotypes originated from plot A spread in roots' wood on distance from 19.60 cm (ACZ2) to 40.47 cm (ACZ1), from plot B on distance 8.20 cm (BCZ1) to 43.27 cm (BCZ3) and from plot C on distance from 20.03 cm (CCZ10) to 35.17 cm (CCZ11) (Table 1). The only genotype BCZ1 colonized wood on a distance shorter than 10 cm. Six genotypes (31%) overgrew the wood on distance from 20 cm to 30 cm, seven genotypes (37%) on distance from 30 cm to 40 cm and two genets colonized wood on distance longer than 40 cm.

The differences among genotypes in ability of wood colonization were significant ($p < 0.05$; Table 2). The genotypes that colonized wood on the shortest distance differed significantly ($p < 0.05$) from others. And in the opposite site those genotypes which overgrew roots wood on distance more than 30 cm differed significantly ($p < 0.05$) from

Table 1. Length of wood discoloration in stumps roots inoculated by *H. annosum* genotypes, twelve months after inoculation

Tabela 1. Długość przebarwienia drewna korzeni pniakowych inokulowanych przez genotypy *H. annosum* dwanaście miesięcy po zakażeniu

Genotype code Kod genotypu	Discoloration length, cm – Długość przebarwienia, cm		
	max	min	average średnia
ACZ1	41.20	40.00	40.47
ACZ2	22.70	16.80	19.60
ACZ3	31.50	29.30	30.47
BCZ1	9.60	5.20	8.10
BCZ2	20.6	19.00	20.00
BCZ3	43.50	43.00	43.27
BCZ4	19.50	16.90	18.40
BCZ5	26.40	24.50	25.50
BCZ6	38.10	36.20	36.63
CCZ1	35.80	31.10	33.63
CCZ2	32.20	30.90	31.33
CCZ4	30.70	18.70	25.60
CCZ5	28.00	25.00	26.63
CCZ6	31.00	28.70	29.50
CCZ7	31.20	28.00	29.17
CCZ8	35.00	27.80	32.17
CCZ9	36.10	22.80	31.20
CCZ10	25.80	13.00	20.03
CCZ11	38.30	31.80	35.17
Control Kontrola	4.45	0.05	2.17

others. The analyze of the ability of wood colonization by mycelium of genotypes in relations to its origin showed that genotypes collected from stumps (A, C) overgrew the roots slightly stronger (about 14.5%) that those collected from trees (B), but differences were not significant ($p > 0.05$). There were no differences between genotypes isolated from pines and beaches ($p > 0.05$), either (Table 3).

Table 2. Analysis of variance of wood discoloration length of Scots pine roots infected by different genotypes of *H. annosum*, α = 0.05
Tabela 2. Analiza wariancji długości przebarwienia drewna korzeni sosny zwyczajnej inokulowanych różnymi genotypami *H. annosum,* α = 0,05

	SS	Degree Stopień	MS	*F*	*p*
Genotype – Genotyp	5819.95	19	306.31	30.715	0.00
Error – Błąd	398.92	40	9.97		

Table 3. Homogenous groups on the base of HSD Tuckey test in terms of mycelium ability to overgrow the roots' wood
Tabela 3. Grupy homogeniczne pod względem zdolności przerastania drewna korzeni na podstawie testu HSD Tuckeya

Genets Genotypy	Mycelium range Rozmiar grzybni cm	Homogenous groups – Grupy homogeniczne							
		1	2	3	4	5	6	7	8
Control Kontrola	2.16	****							
BCZ1	8.1	****							
BCZ4	18.4		****						
ACZ2	19.6		****	****					
BCZ2	20		****	****	****				
CCZ10	20.03		****	****	****				
BCZ5	25.5		****	****	****	****			
CCZ4	25.6		****	****	****	****			
CCZ5	26.63		****	****	****	****			
CCZ7	29.17			****	****	****	****		
CCZ6	29.5				****	****	****		
ACZ3	30.47					****	****		
CCZ9	31.2					****	****	****	
CCZ2	31.33					****	****	****	
CCZ8	32.17					****	****	****	
CCZ1	33.63					****	****	****	****
CCZ11	35.17					****	****	****	****
BCZ6	36.63						****	****	****
ACZ1	40.47							****	****
BCZ3	43.27								****

DISCUSSION

Individual pathogen's genotype, that was tested in this study grew in the wood of stumps' root at different rate. After one year of inoculation the mycelium was isolated from 8.1 cm (BCZ1) to 43.27 (BCZ3) from inoculation point (average 28.26 cm). *Heterobasidion annosum* genotypes belonged to 8 homogenous group in the rate of wood colonization. The only isolate BCZ1, that grew in the wood at the lowest rate, differed significantly from other isolates.

Results of this work are comparable to other infection experiments. Roll-Hansen and Roll Hansen (1981) showed that the *Heterobasidion* mycelium overgrew wood in roots on average from 20 cm to 38 cm after inoculation in Norway spruce roots and from 11 cm to 12 cm in Sitka spruce roots per year. In addition Pagony (1981) found that a year after stumps inoculation the mycelium colonized wood up to 40 cm from inoculation point in the environment favourable to pathogen.

The growth rate in stumps wood could be also higher than we obtained in this work. In infection experiments the growth rate depended on the climate or host species. Meredith (1960) found that mycelium grew 7.2 cm per month in Scots pine stumps, whereas Cobb and Barber (1968) in warmer climate of California showed the mycelium growth of 15–21 cm per month.

The ability of dead wood colonization plays a crucial role in a pathogen's secondary spread in stands through stumps its roots to roots and stem of live trees. Mańka et al. (1974) proved that infection and colonization intensity depended on width of wood ring increment. Stumps with wider wood rings were faster colonized than stumps with narrow wood rings. The ratio of summer wood to late wood is an important factor in rate of wood colonization by *Heterobasidion* mycelium.

Oliva et al. (2011) found that probability of pathogen's transfer from inoculated stumps to neighbour trees via roots contacts depended on stump diameter and suggested that this fact is related to size of late wood in annual rings. After fifteen years Norway spruce stumps inoculations by different genotypes of *H. patrviporum* mycelium spread on distance from 1.96 m to 4.23 m. During this time *H. parviporum* genets infested appropriately 1.26 to 3.01 trees, while genotypes of *H. annosum* colonized distance 1.51 to 2.92 trees. In this experiment the pathogen was inserted on stumps surface by conidial spores.

Isolates, which mycelium overgrew the wood on distance more than 30 cm significantly differed ($p < 0.05$) of those that mycelium colonize the wood on distance shorter than 20 cm. Genotypes, which mycelium had the highest growth rate in dead wood were the most aggressive to Scots pine seedlings (according to previous study Cieślak et al., 2011). On the other hand isolates that overgrew the smallest portion of wood displayed the average level of aggressiveness. Genets isolated from dead trees had a lower growth rate than those isolated from stumps but differences were not statistically significant. Similar results were obtained by Cieślak et al. (2011) in infection experiment with the same isolates on Scots pine seedlings. Isolates collected from stems of Scots pine had lower aggressiveness than those isolated from stumps. It seems that the ability of wood

colonization by *H. annosum* mycelium, both in live and dead wood is rather an ontogenetic feature than it is connected with a colonized source in the forest environment.

Stumps after thinning and clear cuttings are the most important pathogen source in current and even next generation of forest. Greig and Pratt (1976) showed that *Heterobasidion* is able to survive in stumps for decades. They found active pathogen's mycelium in 33-62-year-old Scots pine stumps, 30-46-year old larch and 30-year-old Douglas fir stumps, Norway and Sitka spruce.

Not only colonized stumps could increase a disease risk in stands. Similarly to stumps and roots, lying logs or wood debris could be infected by *Heterobasidion* and become a disease source. Schütt and Schock (1979) emphasized that released dead wood (logs with *Heterobasidon* decay) in Norway spruce stand could increase infection risk. On wood debris *Heterobasidion* sporocarps producing a lot of basidiospores could appeared. Sporocarps appeared on 50% of decayed stems. Nitisa et al. (2013) present similar results. They found that wood debris left in stand could influence a faster pathogen spread. They showed that sproocarps appeared on windthrown Norway spruces were more frequent and bigger in comparison with those created on roots. In turn sporocarps produced on excavated stumps were bigger from those on standing stumps. The most frequent sporocarps occurred in stands on wet sites growing on drained peat soil.

In addition Pratt and Greig (2013) found that *Heterobasidion* could survive in saprotrophic stage colonizing small wood debris of Scots pine roots about 2 cm in diameter even for 13 years. The longevity of active mycelium in stumps varied from 25 years in Scots pine stumps (Rishbeth, 1951) to even 46 years for *Picea abies* (Geig and Pratt, 1976; Laine, 1976; Piri, 1996). So even the less extensive in growth rate genotypes through years of stumps existence after thinning could create a dangerous source in the wood of stumps and its roots for the neighbour trees infection.

That is why the stumps excavation seems to be a very important treatment to decrease the risk of *Heterobasidion* infection and amount of pathogen source in stumps. After this treatment the possibility of pathogen spread is smaller both in generative and vegetative way (Greig, 1984; Piri, 2013; Arhipova et al., 2013).

In conclusion population of *H. annosum* differed in genotypes features conditioning the rate of its development in the environment and disease dynamics. Differentiation of these features of pathogen populations caused an unequal infection and disease development rate in stands. Genets with high pathogenic potential have also a high level of saprotrophic features. It seems that the most aggressive isolates colonize the stand fastest through root system.

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CHARAKTERYSTYKA POPULACJI *HETEROBASIDION ANNOSUM* WYSTĘPUJĄCYCH W DRZEWOSTANACH SOSNOWYCH NADLEŚNICTWA CZŁOPA
II. ROZWÓJ GRZYBNI W MARTWYM DREWNIE

Streszczenie. Do badań wybrano populacje patogena o znanej agresywności w stosunku do sadzonek sosnowych (Cieślak i in., 2011). W doświadczeniu wykorzystano 19 izolatów reprezentujących różne genotypy *H. annosum* pochodzące z dwóch 45-letnich drzewostanów sosnowych oraz z 12-letnich sosen i buków rosnących w gniazdach zlokalizowanych w drzewostanie 33-letnim. Wszystkie drzewostany w Nadleśnictwie Człopa rosły na gruntach porolnych. Identyfikację grzybów przeprowadzono dzięki testom genetycznej zgodności grzybni, a rozróżnienie genotypów – testami somatycznej kompatybilności.

Podobieństwo genetyczne populacji patogena określono we wcześniejszych badaniach za pomocą reakcji PCR (Cieślak i in., 2011). Inokulum były kołki drewniane o długości 5 cm i średnicy 1 cm przerośnięte przez grzybnię patogena. Inokulum wprowadzano w dziury wywiercone wiertłem w korzeniach pniaków w odległości 50 cm od karpy. Miejsce wprowadzenia zaklejano sztuczną korą, a korzenie przykrywano glebą. Kontrolę tworzyły korzenie inokulowane sterylnymi kołkami drewna. Po 12 miesiącach korzenie odkopywano i pozyskiwano, odcinając w odległości 50 cm w każdą stronę od miejsca inokulacji. Po rozłupaniu korzeni analizowano długość strefy przebarwienia spowodowaną przez rozwijającą się grzybnię *H. annosum*. Dodatkowo wykonano izolację z miejsc przebarwionych dla potwierdzenia obecności grzybni patogena. Grzybnia zasiedliła drewno i spowodowała jego przebarwienie od 8,10 cm (genotyp BCZ1) do 43,27 cm (izolat BCZ3). Drewno korzeni zostało przerośnięte najsilniej przez izolaty pochodzące z powierzchni A: o 17% silniej niż z powierzchni B i 3% z powierzchni C. Analiza zdolności przerastania drewna korzeni pniakowych przez genotypy patogena w zależności od pochodzenia wykazała, że genotypy pochodzące z pniaków przerastały drewno korzeni nieco silniej niż pochodzące z drzew, ale różnica nie była statystycznie istotna ($p > 0,05$). Nie było także różnic pomiędzy genotypami pozyskanymi z sosen i buków. Izolaty wykazujące największe tempo wzrostu w martwym drewnie charakteryzowały się we wcześniejszych badaniach najsilniejszą agresywnością. W populacji patogena występuje zróżnicowanie cech poszczególnych genotypów warunkujące tempo ich rozprzestrzeniania w środowisku oraz dynamikę procesu chorobowego. Zróżnicowanie tych cech w populacji patogena wpływa na nierównomierność porażenia i tempo rozwoju choroby w poszczególnych drzewostanach. Genotypy o wysokim potencjale patogeniczności wykazują także wysoki poziom właściwości saprotroficznych. Wydaje się, że najbardziej agresywne genotypy patogena wykazują największe tempo kolonizacji drzewostanu.