

DESCRIPTION OF *HETEROBASIDION ANNOSUM* SENSU STRICTO POPULATION OCCURRING IN SCOTS PINE STANDS IN CZŁOPA AND PODANIN FOREST DISTRICTS. I. MYCELIUM DEVELOPMENT IN ALIVE WOOD

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Abstract. The aim of this work was 1) to investigate the genetic diversity of *H. annosum* s. str. populations, 2) to recognize genets ability of their aggressiveness to Scots pine. The genetic similarity among genets varied from 0% to 74%. Totally different genets were found in 17.9% of cases. The lowest similarity occurred among 20.7% of genets and it was calculated on 18%. Six months after inoculations of Scots pine stems by *H. annosum* s. str. the distance of wood colonization by mycelium was measured. Wood was colonized to a distance varying from 0.52 cm to 2.06 cm. The most aggressive genets were isolated from stumps, and the less aggressive from trees' stems. The infections in stands caused by *H. annosum* s. str. basidiospores and appearance of a new pathogen's organisms, which might distinguish by high aggressiveness, could increase a damages in these stands. There is still a need to use a biological control against root rot disease to reduce spreading of a new generations of pathogens.

Key words: *Heterobasidion annosum* sensu stricto, aggressiveness, genets, Scots pine

INTRODUCTION

Heterobasidion annosum (Fr.) Bref. sensu stricto causes butt and root rot of forest trees in the whole boreal zone [Korhonen et al. 1998, Sierota 2001, Mańka 2005]. *Heterobasidion annosum* s. str. is the most common species among three *Heterobasidion* species occurring in Poland and in addition due to the most important economic losses [Łakomy et al. 2000, Łakomy and Werner 2003]. Pathogen causes the most serious damages in Scots pine stands growing in the first and second generation on the post agricultural lands [Sierota 1987, 1995, 2001]. Pathogen's spread in stands is possible

thanks to basidiospores, which colonize stumps and create new organisms in population [Redfern and Stenlid 1998]. The spread of this organisms follows the vegetative mycelium growing among root contacts from diseased to healthy roots [Stenlid and Redfern 1998]. In this way the pathogen's population colonize new tree-hosts. The *H. annosum* s. str mycelium growth rate in dead wood could reach 50 cm per year (in average 25 cm), whereas the growth of mycelium in alive wood is slower, because up to 10 cm per year [Rishbeth 1951, 1957, Slaughter and Parmeter 1995, Bendz-Hellgren et al. 1999]. The genetic diversity of pathogen's population might be different. From organisms very close related to those among which there is no similarity [Łakomy et al. 2007]. The area occupied by individual genotypes is also different. The genets could occur in a part of stump wood (one stumps could be colonized by several genets) or in several stumps (10-15) localized on a considerable area (even in diameter of 50 m) [Stenlid 1985, Piri et al. 1990, Stenlid and Redfern 1998, Vasiliauskas and Stenlid 1998, Piri and Korhonen 2001, Łakomy et al. 2007, 2011].

The aim of this work was 1) to investigate the genetic diversity of *H. annosum* s. str. populations, 2) to recognize genets ability of their aggressiveness to Scots pine.

MATERIAL AND METHODS

Genetic diversity of population. Different genets were selected to this study, which represented three *H. annosum* s. str. populations and occupied two 45-year-old Scots pine stands (plots A, C) and 17-year-old trees planted in the gap (B), localized in the Człopa Forest District and in addition one population isolated from 13-year-old Scots pine plantation (D) localized in the Podanin Forest District. These stands were growing on the post agricultural lands. Pathogen isolates were collected from wood of stumps and roots or from wood of root collars of young Scots pine. Identification of 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 was described using DNA extraction. Amplification profile was obtained in PCR using M13 microsatellite (5'-AGGGTGGCGGTTCT-3') [Karlsson 1993] with the aid of transilluminator Vilber Lourmat ECX-12.M and universal system of gel documentation POLYDOC (version 1.0).

The rate of growth in Scots pine stems. Thirty four pathogen genotypes were used in this study. Inoculation experiment was carried out on 4-year-old Scots pine plantation. Pathogen's inoculum, which consisted of woody stick overgrown by mycelium of *H. annosum* s. str., 5 mm in length and 2 mm in diameter, was put in the whole made by the drill, close to the root collar of tree. After that this place was wrapped with the laboratory film. Each combination was multiplied 10 times. The control trees were inoculated with sterile woody stick. Six months after inoculation trees were excavated. The wood of stems was analysed on the presence of discoloration and decay caused by pathogen [Werner and Łakomy 2002]. The length of discoloration was measured. The small pieces of inoculum were put on 1% malt extract agar with benomyle and antibiotics in Petri dishes. After 4-5 days the growing mycelium was observed with the aid of microscope on presence of conidial sporulation stage of *Spiniger meineckellus* (A.J. Olson) Stalpers, which proved that this discoloration was caused by *H. annosum* s. str.

The basic hypothesis was verified in different experiments. The multifactorial analysis of variance was used (ANOVA/MANOVA) and HSD Tuckey test. The Statistica v. 6 (2003) [Bobrowski 1980, Kala 2002] was used for this purpose.

RESULTS

The analysis of genetic similarity indexes obtaining in binary system allowed to separate two groups of similarity. The most related genets showed the 74% of similarity (27% of genets). In 20.7% cases, it was found the lowest similarity between genotypes estimated on 18%. The genetic different genets were found in 17.9% of pairings (Fig. 1). In next part of paper the only genotype ACZ3 would take under consideration from plot A, because of DNA isolation failure of other isolates. The relationship among many genotypes from plot B, C and the only from plot A was very close. The highest degree of genetic similarity (74%) was found between genotypes BCZ1 and CCZ11, which occurred in stumps 350 meters far from each other. Similar strong relationship was found for genets BCZ6 and CCZ11 (63%), BCZ5 and CCZ11 (55%) as well as ACZ3 and BCZ2, BCZ4 (48%).

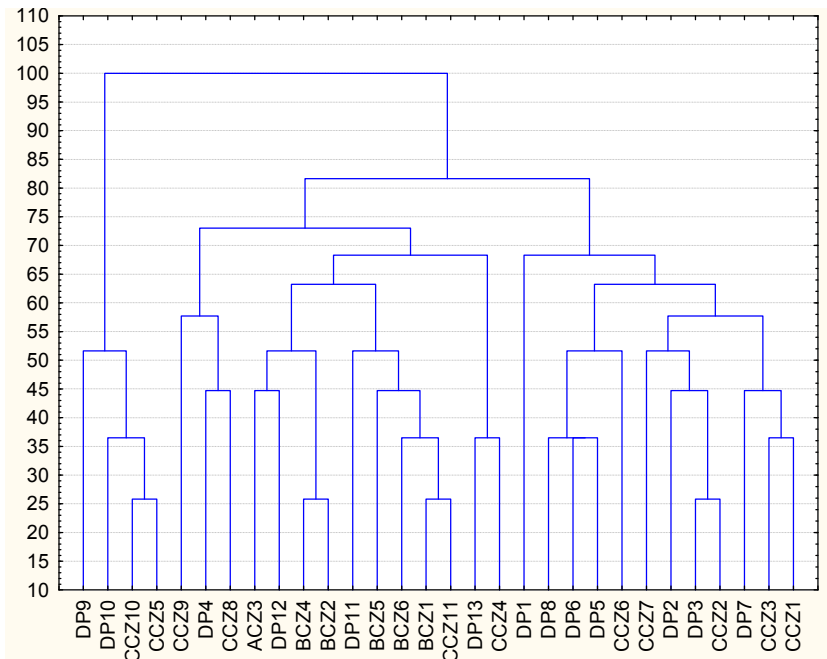


Fig. 1. Diagram of similarity among individual genotypes of *Heterobasidion annosum* s. str.

Rys. 1. Dendrogram podobieństwa genetycznego poszczególnych genotypów *Heterobasidion annosum* s. str.

The relative high genetic similarity was confirmed between pathogen's populations occurred in stands of Podanin and Człopa Forest Districts, that was distant 55 km far from each other. The highest genetic similarity was found between populations occurred in plot C (Człopa Forest District) and D (Podanin Forest District). Genets CCZ2 and DP3 were genetically similar in 74%, genets CCZ5, CCZ10 and DP10, CCZ4 and DP13 in 63%, genets CCZ8 and DP4, CCZ2 and DP3, CCZ1, CCZ3 and DP7 in 55%, whereas genets CCZ7 and DP3, CCZ6 and DP5, DP6 and DP8 in 48%. Equally high genetic similarity was found for some genets from plot B (Człopa Forest District) and D (Podanin Forest District) – DP11 and BCZ3, BCZ6 and BCZ11 (48%).

The discoloration range in 4-year-old Scots pine stems caused by growing mycelium of individual *H.annosum* s. str. genets is shown in Table 1. The genotype DP8 overgrew the pine wood (in average 2.06 cm) the most intensively, whereas the genotype DP14 the less intensively – in average 0.52 cm. Both genets were isolated from stem wood of 13-year-old Scots pine (Podanin Forest District). Other genotypes spread in the pine wood from 0.6 cm (DP13) to 1.97 cm (DP12). Tested genets from plot A (Człopa Forest District) overgrew the stems wood in average 15% more intensively than genets isolated from plot B, in 5% from plot C and in 20% from plot D (Podanin Forest District). Genets from plot A overgrew stems wood from 1.25 cm (ACZ2) to 1.94 cm (ACZ3), genets from plot B from 1.11 cm (BCZ2) to 1.76 cm (BCZ3), from plot C from 1.04 cm (CCZ7) to 1.94 cm (CCZ1), and from plot D from 0.52 cm (DP14) to 2.06 cm (DP8). Only six genotypes (17%) derived from plot D colonized stems wood on distance below 1 cm. Considering only the plot D, these genotypes made 46% of genets from this plot. The analysis of mycelium colonization up and down of stems' wood it was found that the genotype BCZ3 (1.01 cm) overgrew stems most intensively in the up direction, and genets DP14 the less intensively (0.24 cm). Genets ACZ3 (1 cm), DP8 (1.11 cm) and DP12 (1.10 cm) colonized wood most intensively in root direction but the genotypes DP14 overgrew the wood the less intensively (0.28 cm). Mycelium grew faster most often in top than in roots direction. This situation was found in 65% of cases. Genotypes isolated from plot D (Podanin Forest District) in 79% of cases grew more intensively in roots than in top direction (Table 1). The analysis of variance showed the significant

Table 1. Average range of stems' wood discoloration infected by isolates belonged to individual genotypes of *H. annosum* s. str. six months after inoculation

Tabela 1. Średni zasięg przebarwienia drewna strzałek sosnowych zakażonego przez izobaty należące do poszczególnych genotypów *H. annosum* s. str., sześć miesięcy po inokulacji

Genotype Genotyp	Length of discoloration caused by <i>H. annosum</i> s. str., cm Długość przebarwienia spowodowanego przez <i>H. annosum</i> s. str., cm		
	up – w górę	down – w dół	total – razem
1	2	3	4
ACZ1	0.87	0.59	1.46
ACZ2	0.76	0.49	1.25
ACZ3	0.94	1.00	1.94
BCZ1	0.67	0.47	1.14
BCZ2	0.64	0.47	1.11

Table 1 – cont. / Table 1 – cd.

1	2	3	4
BCZ3	1.01	0.75	1.76
BCZ4	0.60	0.58	1.18
BCZ5	0.79	0.56	1.35
BCZ6	0.70	0.59	1.29
CCZ1	0.73	0.48	1.94
CCZ2	0.71	0.50	1.21
CCZ3	0.84	0.76	1.60
CCZ4	0.79	0.59	1.38
CCZ5	0.99	0.80	1.79
CCZ6	0.98	0.85	1.83
CCZ7	0.57	0.47	1.04
CCZ8	0.70	0.57	1.27
CCZ9	0.70	0.58	1.28
CCZ10	0.79	0.57	1.36
CCZ11	0.87	0.63	1.50
DP1	0.86	0.92	1.78
DP2	0.45	0.44	0.89
DP3	0.48	0.49	0.97
DP4	0.57	0.55	1.12
DP5	0.64	0.86	1.50
DP6	0.60	0.70	1.30
DP7	0.68	0.89	1.57
DP8	0.95	1.11	2.06
DP9	0.70	0.82	1.52
DP10	0.51	0.44	0.95
DP11	0.28	0.35	0.63
DP12	0.87	1.10	1.97
DP13	0.28	0.32	0.60
DP14	0.24	0.28	0.52
Control Kontrola	0.20	0.16	0.36

differences ($p < 0.05$) among the rate of overgrow the Scots pine stems by individual genotypes of *H. annosum* s. str. (Table 2). Results of HDS Tuckey test (Table 3) showed that only genets, that colonized wood the less intensively (DP13, DP14) significantly differ ($p < 0.05$) of those that overgrew stems' wood the most intensively (DP12, DP8). In the other cases the rate of Scots pine stems wood colonization by genotypes from A, B, C and most from plot D was similar ($p > 0.05$).

Table 2. The analysis of variance of discoloration length of stems' wood infected by *H. annosum* s. str. genets, $\alpha = 0.05$

Tabela 2. Analizy wariancji dla długości przebarwienia drewna strzałek sosnowych zakażonych przez genotypy *H. annosum* s. str., $\alpha = 0,05$

	SS	Degree Stopnie	MS	F	p
Genotype Geotyp	61.3242	34	1.8037	9.969	0.00
Error Błąd	56.9940	315	0.1809		

Table 3. Significant equally groups of ability of stems wood discoloration of Scots pine infected by individual genotypes of *H. annosum* s. str. determined by HSD Tukey test, $\alpha = 0.05$

Tabela 3. Grupy statystycznie jednorodne dla zdolności przebarwienia drewna sosny zwyczajnej zakażonego przez poszczególne genotypy *H. annosum* s. str. ustalone na podstawie testu HSD Tukeya, $\alpha = 0,05$

Genotype Genotyp	Discoloration Przebarwienie cm	1	2	3	4	5	6	7	8	9
1	2	3	4	5	6	7	8	9	10	11
Control Kontrola	0.360000	****								
DP 14	0.520000	****	****							
DP 13	0.600000	****	****	****						
DP 11	0.630000	****	****	****	****					
DP 2	0.890000	****	****	****	****	****				
DP 10	0.950000	****	****	****	****	****	****			
DP 3	0.970000	****	****	****	****	****	****			
CCZ 7	1.040000	****	****	****	****	****	****	****		
BCZ 2	1.110000		****	****	****	****	****	****	****	
DP 4	1.120000		****	****	****	****	****	****	****	
BCZ 1	1.140000		****	****	****	****	****	****	****	
BCZ 4	1.180000		****	****	****	****	****	****	****	
CCZ 2	1.210000		****	****	****	****	****	****	****	
ACZ 2	1.250000			****	****	****	****	****	****	****
CCZ 8	1.270000			****	****	****	****	****	****	****
CCZ 9	1.280000			****	****	****	****	****	****	****
BCZ 6	1.290000			****	****	****	****	****	****	****
DP 6	1.300000			****	****	****	****	****	****	****

Table 3 – cont. / Tabela 3 – cd.

1	2	3	4	5	6	7	8	9	10	11
BCZ 5	1.350000				****	****	****	****	****	****
CCZ 10	1.360000					****	****	****	****	****
CCZ 4	1.380000					****	****	****	****	****
ACZ 1	1.460000					****	****	****	****	****
CCZ 11	1.500000					****	****	****	****	****
DP 5	1.500000					****	****	****	****	****
DP 9	1.520000					****	****	****	****	****
DP 7	1.570000					****	****	****	****	****
CCZ 3	1.600000					****	****	****	****	****
BCZ 3	1.760000						****	****	****	****
DP 1	1.780000							****	****	****
CCZ 5	1.790000							****	****	****
CCZ 6	1.830000							****	****	****
ACZ 3	1.940000								****	****
CCZ 1	1.940000								****	****
DP 12	1.970000								****	****
DP 8	2.060000									****

On the base of results of wood discoloration of stems inoculated with different genotypes we can say that there is no connection between the colonization ability of alive wood of Scots pines and genetic relationship. The correlation analysis showed, that trend line occurred between the studied categories was not significant ($p > 0.05$).

DISCUSSION

The high genetic diversity was found inside the populations of *H. annosum* s. str occurred in plot C and D, that was also shown as a low genetic relationship among genets. This fact shows that basidiospores were a very important factor of *Heterobasidion* spread and stumps colonization in these stands after thinning. On the other hand high genetic diversity of pathogen's population on plot D, where the Scots pine plantation was infested proved that previous stands was similarly sever infected by *H. annosum* s. str., because all infections were caused though roots contact (e.g. old stumps) from infected to healthy roots. Dying trees in rows might be infected though roots systems from one to another or roots of all trees in the same row contacted with an long and old colonized by pathogen stump's root. The interesting findings was to occur 74% of genetic similarity between two genets remote about 55 km, and with simultaneous lack of similarity between genets occurred several meters far from each other. It is well known that basidiospores are resistant to influence of unfavourable environmental factors and could spread

on distance from 70 km to 500 km [Kallio 1970]. According to geographical location and direction of winds the probable scenario was that basidiospores from the Człopa Forest District were transferred by wind to the stand in the Podanin Forest District. Łakomy et al. [2007] showed the high genetic diversity of *H. annosum* s. str. population in Scots pine stand, *H. parviporum* Niemelä et Korhonen in Norway spruce stand and *H. abietinum* Niemelä et Korhonen in European Fir stand. In these stands the highest genetic similarity varied from 40 to 60% and was concerned with 5% of genets, however 24% of genets were not related. This study showed, that on the very small plot D, in Scots pine stands, the 14 genets of *H. annosum* s. str. colonized 87 trees. In 8.8% of cases there were no genetic relationship among the studied genets. Previously studies showed that each *Heterobasidion* species has main plant-host preferences, but also that the existed diversity of aggressiveness among isolates of the same species and among isolates belonged to different species [Stenlid and Swedjemark 1988, Werner 1991, Swedjemark and Stenlid 1993, Stenlid 1994, Werner and Łakomy 2001, 2002]. In this study the significant differences in aggressiveness of different genotypes of *H. annosum* s. str. was proved. This study concerned the mycelium growth rate in pine stems. The correlation between the genetic similarity and studied features was not found. The genetic relationships did not condition the pathogen's aggressiveness ($p > 0.05$). However, La Porta et al. [1997] and Werner and Łakomy [2002] suggested, that the scale of damages might be dependent on genetic diversity of *H. annosum* s. str.

The rate of mycelium spread in the wood, after infection of root of alive tree, varied, because it has to brake a defensive barriers, decompose of phenol compounds, which are released in defensive reactions of trees. This action influenced on the slower spread rate of pathogen in wood, estimated on 10 cm per year [Stenlid and Redfern 1998]. This study, on the base of yearly growth rate of genets in alive wood, showed that theoretically mycelium might spread from 1.96 cm to 6.18 cm per year. Mycelium of genets isolated from stumps (plot A and C) spread significantly faster than mycelium of genets isolated from alive trees (Plot B and D). It could be explained as a results of host-passage [Mańka 2005]. The pathogen's mycelium had to brake the defensive reactions of alive trees and might lost their high aggressiveness, whereas mycelium spread in dead wood might increase their aggressiveness. This study could proved this hypothesis, because several collected genotypes differed in their ability of colonization alive wood in Scots pine stems. The more varied pathogen population, the ability was stronger.

Stumps infections by basidiospores and appearance of new pathogen's organisms with high aggressiveness result in increase of damages in stands. Hence it is very important to use a biological control treatment against *Heterobasidion* as a prevention application.

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CHARAKTERYSTYKA POPULACJI *HETEROBASIDION ANNOSUM* SENSU STRICTO WYSTĘPUJĄCYCH W DRZEWOSTANACH SOSNOWYCH NADLEŚNICTW CZŁOPA I PODANIN. I. ROZWÓJ GRZYBNI W ŻYWYM DREWNI

Streszczenie. Celem pracy było zbadanie: 1) różnicowania genetycznego populacji, 2) zdolności poszczególnych genotypów pod kątem ich agresywności w stosunku do sosny zwyczajnej. Do badań wybrano izolaty należące do różnych genotypów, reprezentujące trzy populacje *H. annosum* s. str.: zasiedlające dwa 45-letnie drzewostany sosnowe (powierzchnie A, C), 17-letnie drzewa posadzone w luce (B) w Nadleśnictwie Człopa oraz jedną populację z 13-letniej uprawy sosnowej w Nadleśnictwie Podanin (D). Drzewostany rosły na gruntach porolnych. Podobieństwo genetyczne populacji określono, stosując ekstrakcje DNA. Profil amplifikacji uzyskano w PCR, używając mikrosatelity M13 (5'-AGGGTGGCGTTCT-3'). Doświadczenie infekcyjne, polegające na inokulacji strzałek sosnowych grzybnią patogena, przeprowadzono w 4-letniej uprawie sosnowej. Analiza indeksów podobieństwa genetycznego, uzyskanych w systemie binarnym, umożli-

liwiła wyodrębnienie dwóch grup podobieństw w obrębie badanych genotypów. Najbliżej spokrewnione osobniki charakteryzowały się podobieństwem 74-procentowym, natomiast najniższe podobieństwo genetyczne określono na poziomie 18%. Całkowity brak podobieństwa charakteryzował 17,9% genotypów. Stwierdzono także znaczne podobieństwo genetyczne pomiędzy populacjami zlokalizowanymi w drzewostanach nadleśnictw Człopa i Podanin odległymi od siebie o 55 km. Zasięg przebarwienia drewna sosnowego w strzałkach 4-letnich sosen, powstałego w wyniku przerastania grzybni poszczególnych genotypów *H. annosum* s. str., wahał się od 0,52 cm do 2,06 cm. Jedynie genotypy, które najwolniej przerastały drewno strzałek sosnowych różniły się w sposób istotny od genotypów najsilniej przerastających drewno ($p < 0,05$). Z analizy wyników rozmiarów przebarwienia strzałek sosnowych inokulowanych grzybnią różnych genotypów patogena nie można stwierdzić zależności pomiędzy podobieństwem genetycznym a zdolnością genotypów do przerastania drewna żywych sosen.

Słowa kluczowe: *Heterobasidion annosum sensu stricto*, agresywność, genotypy, sosna zwyczajna

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