

GENETIC DIVERSITY OF *HETEROBASIDION ANNOSUM* SENSU STRICTO POPULATIONS IN CHOSEN SCOTS PINE STANDS WITH BEECH IN UNDERSTOREY

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Abstract. The aim of this study was to analyse genetic diversity of *H. annosum* s. s. population in selected Scots pine stands with planted beech as a second floor or understorey, in Bolewice and Tuczno Forest Districts. In these stands the 14 genets of *H. annosum* s. s. were recognised. The biggest genet occupied 84 m² and the smallest one colonized only one beech. The beech was attacked by pathogen similarly like Scots pine. The genetic diversity of *H. annosum* s. s. populations was high and the basidiospores played a major role in pathogen's spreading process in studied stands.

Key words: *Heterobasidion annosum* sensu stricto, genotypes, *Fagus sylvatica*, *Pinus sylvestris*, genetic diversity

INTRODUCTION

Heterobasidion spp. cause one of the most important diseases of forest trees [Heterobasidion... 1998, Mańka 2005]. Pathogens cause white pocket root and butt rot of mainly coniferous trees but also deciduous ones. The most important economic losses appear in Scots pine stands growing in the first and second generation on the post arable soil [Sierota 1995, 2001].

Heterobasidion annosum sensu lato was divided to three different species: *Heterobasidion annosum* (Fr.) Bref. sensu stricto, *H. parviporum* Niemelä et Korhonen, and *H. abietinum* Niemelä et Korhonen on the base of genetic studies [Niemelä and Korhonen 1998]. All *Heterobasidion* species occur in Poland [Łakomy and Werner 2004]. Occurrence of these species is connected with the natural range of its main hosts [Heterobasidion... 1998, Łakomy and Werner 2004]. There were only a few notes about *H. parviporum* occurrence out of natural range of Norway spruce (*Picea abies* Karst.) in Germany [Siepmann 1988, 1989] and in Denmark [Thomsen 1994]. *Heterobasidion* spp. infect over 200 coniferous and deciduous trees and shrubs.

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Heterobasidion annosum sensu stricto affect mainly Scots pine (*Pinus sylvestris* L.), but also other coniferous and deciduous trees. It has the widest hosts' range. *Heterobasidion parviporum* infected mainly Norway spruce and *H. abietinum* European fir (*Abies alba* Mill.).

Heterobasidion disease is permanent in infested stand and would not disappeared after clear cutting. Infected stumps and roots are the source of pathogen in the next stand [Sierota 2001, Mańka 2005]. In affected stand by *H. annosum* s. s. trees dying, what could case loss of stability of the stand. There is a need to forest converse in these stand. In most cases the beech is planted in understorey to converse Scots pine stands.

There was observed infection of young beeches (*Fagus sylvatica* L.) in conversed stands in north-west part of Poland. In some stands infection was very common [Łakomy and Cieślak 2006, 2008]. The aim of this work was the analysis of genetic diversity of *H. annosum* s. s. population in chosen Scots pine stands with the beech planted in understorey, growing on the arable soils in Bolewice and Tuczno Forest Districts.

MATERIALS AND METHODS

The study was realised in Scots pine stands with beeches in understorey. One stand was localized in Bolewice Forest District (Regional Directorate of the State Forests in Szczecin, 47-year-old Scots pine, 17-year-old beech, sandy soil, biological control of *H. annosum* s. s. after thinning) and two others in Tuczno Forest District (Regional Directorate of the State Forests in Piła, 55-year-old Scots pine, 13-year-old beech, sandy soil, biological control of *H. annosum* s. s. after thinning). In each stand an experimental plot was established, which covered some pine or birch stumps and several dead or dying beeches. In most cases *H. annosum* s. s. sporocarps were present around the root collar of trees and stumps. The situation plan of each plot was done. The wood of stumps, pathogen's sporocarps, wood from root collar of beeches were taken to the laboratory to isolate the mycelium of *H. annosum* s. s. Inoculums from woods were put on 1% malt extract agar (Merck) with two antibiotics (neomycin and streptomycin). The growing mycelium was isolated on 1% malt extract agar in probes after 3-4 days.

Identification of *Heterobasidion* spp.

Identification of *Heterobasidion* isolates was done with the aid of mating tests using homokaryotic testers of *H. annosum* s. s., *H. parviporum* and *H. abietinum* [Korhonen 1978].

Genets

Genets were distinguished with the aid of somatic compatibility tests. Inoculums of heterokaryotic mycelium were put at the centre of Petri dishes 1 cm from each other on the 1.5% malt extract agar. Three weeks after inoculation the results of somatic compatibility tests were analysed. Isolates were compatible if the mycelium were joined and they belonged to the same genet. In opposite situation, when isolates were not compatible, between mycelium appeared the zone without or with sparse mycelium [Korhonen 1978, Heterobasidion... 1998].

RESULTS

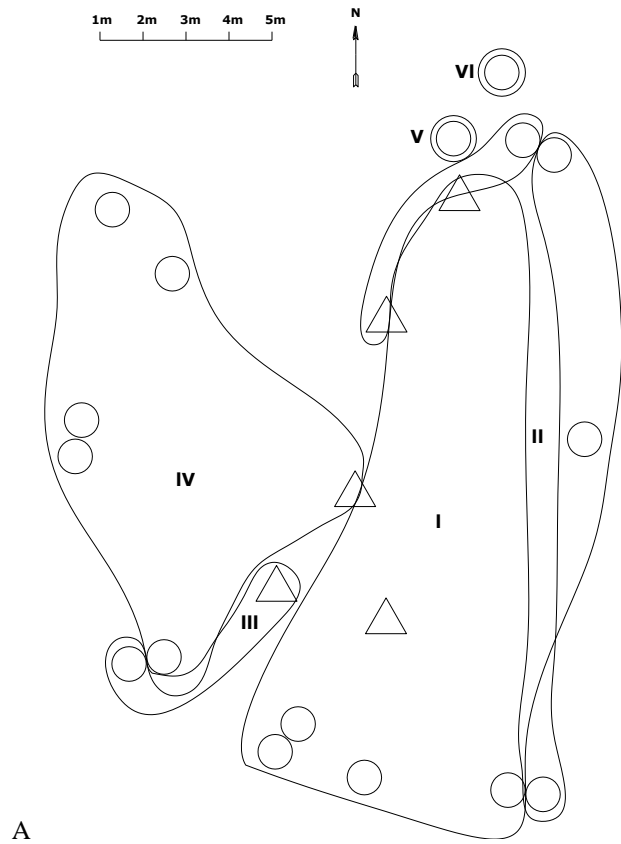
Heterobasidion mycelium was isolated from the wood of 36 beeches (60% of analysed trees) and 10 pine stumps (91% of analysed stumps). Pathogen's cultures were not isolated from 24 dead trees and one stump. All tested isolates belonged to the *Heterobasidion annosum sensu stricto*. There were recognised six genets (Table 1) on the first plot (Tuczno Forest District). The biggest genet covered 84 m² and colonized six trees and three stumps. Second genet covered 14 m² and colonized two trees and two stump. Third genet occurred on the area of 5 m² and infected one tree and one stump. Fourth genet – second in the size – 48 m² and infected five trees and one stump. Genets fifth and sixth were the smallest and each colonized only one beech (Fig. 1 A). On the second plot three genets were found (Table 1). The biggest one covered the area of 66 m² and infected five trees and two stumps. Second genet colonized one stump and third one beech (Fig. 1 B). On the third plot five genets were recognized (Table 1). The first genet colonized three trees and cover area of 8 m². Second genet colonized also three trees and cover 4 m². The third genet was smaller – infected two trees and cover area of 3.5 m². Fourth and fifth genets were found in a simple stump.

DISCUSSION

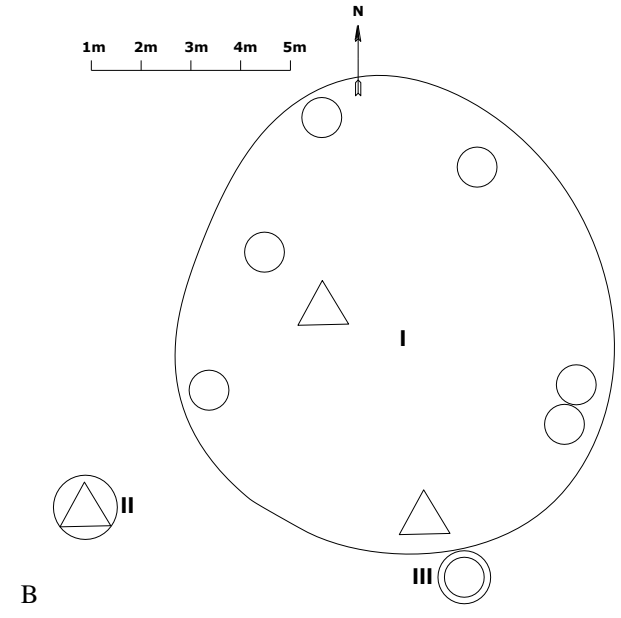
Genetic diversity of *Heterobasidion annosum sensu lato* was the aim of many studies. Most of these investigations were done in coniferous stands. Stenlid et al. [1998] found, that genets size could be big and one genet could colonize over 15 trees. However, Piri et al. [1990] and Piri [1996] showed, that genets in the analysed stand were rather small and colonized only three trees. In *Abies concolor* Lindl. stand the *H. annosum* coours similarly – gap was colonized by some genets [Garbelotto 1996]. Bodles et al. [2005] described genets in Sitka spruce (*Picea sitchensis* Carr) stand, which had never been thinned. They found that the biggest genet colonized six trees. In the study of Łakomy and Cieślak [2008] the early infection of *Fagus sylvatica* was analysed. In north-western part of Poland they observed common infection of young beeches by *H. annosum s. s. Fagus sylvatica* was planted in these stands as the understorey, which could be the next generation after Scots pines were damaged by pathogen. In the studied stands by Łakomy and Cieślak [2008] the pathogen was present at least in 60% of the analysed stumps. *Heterobasidion annosum s. s.* killed from 3% to 13% beeches per year, but the real number of infected beeches could be from 3% to 10% higher. The youngest beech (4-year-old) died three years after planting, however the oldest one was 17-year-old.

All isolates found in the analysed stands in this study belonged to *H. annosum s. s.* The most genetically different pathogen's population was found in the first stand, where six genets were recognised. The biggest genet cover area of 84 m² and infected six trees, and the smallest one infected only one beech. In addition one stump was colonized by two genets.

In the second stand the genetic difference of pathogen's population was lower. The studied area was colonized by three genets, and the biggest one infected six stumps and two other were localized only in one stump each. In the third stands five genets were found, but their size was small and covered at least three beeches.



A



B

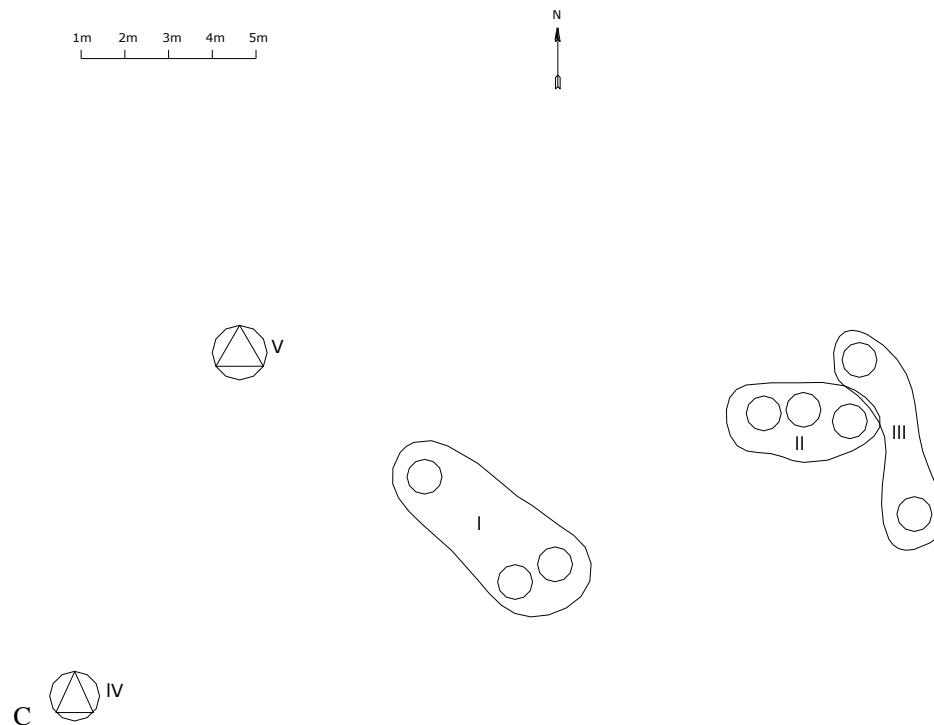


Fig. 1. Graphic schedules of genets' areas on the plot: A – 1, B – 2, C – 3; ○ – dead beeches, Δ – stumps

Rys. 1. Graficzny schemat wielkości genotypów na powierzchni: A – 1, B – 2, C – 3; ○ – zmarłe buki, Δ – pniaki

Considering the possibility of *H. annosum* in colonizing dead wood and spreading through the roots contacts there is the chance to calculate the age of genets [Rishbeth 1951, 1957, Slaughter and Parmeter 1995, Bendz-Hellgren et al. 1999]. On the base of knowledge that the mycelium grows in average 50 cm per year the biggest genets had been spreading through 12-15 years in roots systems. Probably before spreading between roots systems the mycelium had colonized for several years the root system of the stump – elementary source of pathogen. The infestation by pathogen started in 25-30-year old stand. So the stumps after the first commercial thinning should be treated against root rot pathogens.

Werner and Łakomy [2001] showed that stand infestation and trees mortality depend more on properties of pathogen than on resistance of the hosts. Some authors suggested that the size of damages in the stands might depend on genetic diversity of *H. annosum* population [La Porata et al. 1997, Werner and Łakomy 2001].

Łakomy [unpublished 2005] found, that genetic diversity of *Heterobasidion* spp. population was very high and showed that basidiospores of *Heterobasidion* spp. had an important role in spreading and infection process in European fir, Norway spruce and Scots pine stands – what was proved in this study.

Table 1. Genets occurring in studied plots
Tabela 1. Genotypy występujące na powierzchniach badawczych

Plot number Numer powierzchni	Genets Genotypy	Occurrence Występowanie	
		beeches buki	stumps pniaki
1	I	6	1
	II	2	1
	III	1	1
	IV	5	1
	V	1	0
	VI	1	0
2	I	6	2
	II	0	1
	III	1	0
3	I	3	0
	II	3	0
	III	2	0
	IV	0	1
	V	0	1

CONCLUSIONS

The results of this study conducted in 2007 and 2008 allowed to draw conclusions:

1. *Fagus sylvatica* is infected by *Heterobasidion annosum* sensu stricto as heavy as coniferous trees.
2. Genetic diversity of pathogen in studied stands proved that thinning and clear cutting stumps and basidiospores are very important in Scots pine infestation by *H. annosum* s. s. in spite of using biological control.

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**ZRÓŻNICOWANIE GENETYCZNE
POPULACJI *HETEROBASIDION ANNOSUM* SENSU STRICTO
W WYBRANYCH DRZEWOSTANACH SOSNOWYCH
Z PODSADZENIEM BUKA**

Streszczenie. Celem pracy była analiza zróżnicowania genetycznego populacji *Heterobasidion annosum* s. s. w wybranych drzewostanach sosnowych z podsadzeniem buka zlokalizowanych w Nadleśnictwie Bolewice i Tuczo. W tym celu pobierano drewno pniakowe oraz korzeni i szyi korzeniowej porażonych buków. W laboratorium dokonano izolacji grzybni i identyfikacji patogena. Zróżnicowanie genetyczne populacji ustalono na podstawie testu somatycznej kompatybilności grzybni. Wykazano, że 36 buków (z 60 zamarłych) i 10 pniaków (z 11 analizowanych) było zasiedlonych przez *H. annosum* s. s. Na badanych powierzchniach stwierdzono 14 genotypów patogena. W Nadleśnictwie Tuczo na powierzchni 1 występowało sześć genotypów. Największy genotyp zasiedlał powierzchnię 84 m² (sześć buków i cztery pniaki sosnowe), a najmniejszy zasiedlał system korzeniowy jednego buka (17-letniego). Na powierzchni 2 (Nadl. Tuczo) stwierdzono tylko trzy genotypy, a największy zasiedlał powierzchnię 66 m² (sześć buków i dwa pniaki sosnowe). Na powierzchni 3 (Nadl. Bolewice) genotypy zasiedlały mniejszą powierzchnię w porównaniu z tymi stwierdzonymi w Nadleśnictwie Tuczo. Na tej powierzchni stwierdzono pięć genotypów, a największy zasiedlał powierzchnię 8 m², natomiast najmniejszy tylko system korzeniowy pojedynczego buka (13-letniego). Badania przeprowadzone na wybranych powierzchniach w 2007 i 2008 roku pozwoliły na wyciągnięcie następujących wniosków: 1. *Fagus sylvatica* jest tak samo mocno porażony przez *Heterobasidion annosum* sensu stricto jak drzewa iglaste. 2. Zróżnicowanie genetyczne populacji patogena w badanych drzewostanach świadczy o dużym znaczeniu pniaków potrzebujących i zrębnych oraz zarodników podstawkowych w procesie zasiedlania drzewostanów sosnowych przez *H. annosum* s. s., mimo prowadzonej ochrony biologicznej.

Słowa kluczowe: *Heterobasidion annosum* sensu stricto, genotypy, *Fagus sylvatica*, *Pinus silvestris*, zróżnicowanie genetyczne

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