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Dedicated to Professor Alina Skirgiełło on the occasion of her ninety-fifth birthday

Genetic diversity of *Heterobasidion* spp. in Scots pine, Norway spruce and European silver fir stands

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Investigations of genetic diversity of *Heterobasidion* spp. in Scots pine, Norway spruce and European silver fir stands indicated that almost all of identified genets occurred in those stands were small occupied only a single stump. In some cases two, three or even four genets could effectively exist in an individual stump. Genetic similarity of *H. annosum* s.s. genets varied from 0% to 62%, *H. parviporum* from 0% to 38% and *H. abietinum* from 0% to 55%. The oldest and biggest genet was found in laying fir log and overgrew the wood for at least 14 years. This genet belonged to *H. abietinum.* The size of genets was related to thinning operation, spore dispersal, age of stand or competition in wood colonization.

Key words: *Heterobasidion annosum* sensu stricto, *H. parviporum, H. abietinum,* genets, pine, spruce, fir

INTRODUCTION

Root and butt rot caused by *Heterobasidion* spp. is the most important disease in forests. In Europe there were described three species of *Heterobasidion – H. annosum* (Fr.) Bref sensu stricto, *Heterobasidion parviporum* Niemelä and Korhonen, *Heterobasidion abietinum* Niemelä and Korhonen (Niemelä, Korhonen 1998). In Poland the most economic losses causes *H. annosum* sensu stricto in Scots pine plantations growing on post-agricultural lands (Sierota 1987, 1995; Mańka 2005). Distribution of *Heterobasidion* species is related to the natural range of their main hosts. *Heterobasidion annosum* sensu stricto, which attack mainly Scots pine (*Pinus sylvestris* L.), occurs almost in whole Poland. *Heterobasidion parviporum* infected almost only Norway spruce (*Picea abies* (L.) Karst.) is noted in south and north-east part of Poland and *Heterobasidion abietinum* appeared on European silver fir (*Abies alba* Mill.) stumps or laying logs in south Poland. Those three species could

infect more than 200 species also deciduous tree as birch, beach or oak (Webb, Alexander 1985; Łakomy et al. 2000; Łakomy, Werner 2003; Łakomy, Cieślak 2006).

Heterobasidion spp. is dispersed mainly by basidiospores, which colonise freshly cut stumps or wounds (Redfern and Stenlid 1998). In colonized stand pathogens could spread vegetatively by mycelium transfer via roots contacts between colonized and healthy tissue (Stenlid, Redfern 1998). The affected stumps or trees became the source of spore production. Appearance of new genets in stands is related to new spore infection, thinning operations and pathogen's frequency and in particular basidiocarps on stumps or trees. Almost sixty percent genets of *Heterobasidion* sp. identified in final cutting stands had infected only one tree (Piri et al. 1990; Vasiliauskas, Stenlid 1998). However, bigger genets were also described, which occurs in 10 or more trees (Stenlid 1985; Piri, Korhonen 2001). Small size of genets might be resulted by lacking of roots contact and impossibility of mycelium transfer among trees and stumps. It could also indicate that the genets are young or very old and are not able for spreading. The small size of genets could also indicate a high competition among genets in a single stump.

The aim of this study was *i)* to enter the investigations of distribution of *Heterobasidion* spp. genets in Scots pine, Norway spruce and European silver fir stands in Poland and *ii)* to investigate the genetic similarity of genets.

MATERIALS AND METHODS

Three stands were chosen to this study: 53-year-old *Picea abies* stand (Suwałki Forest District 54°17'N, 22°51'E), 37-year-old *Pinus sylvestris* stand (Skwierzyna Forest District 52°33'N, 15°22'E) and 180-year-old *Abies alba* stand (Siemianice Experimental Forest of August Cieszkowski Agricultural University in Poznań, 51°01'N, 18°05'E). Norway spruce and Scots pine stands were growing as a first generation on post-arable soil. In fir stand two groups of stumps were chosen for this study (7 stumps and laying log and 5 stumps). The two groups were remote each other about 250 m. In each stand, stumps were investigated for presence of decay or *Heterobasidion* sp. basidiocarps. Wood from stumps was collected.

In laboratory the mycelium of *Heterobasidion* sp. were isolated from wood samples. The genets were identified with the aid of somatic incompatibility test (Stenlid 1985). Cultures were pairing in all possible combinations on 1.5% Malt Extract Agar (Merck, Germany). After 2-3 weeks pairings were marked to presence the demarcation line indicating different genets. Isolates representatives of each genet were identified to *Heterobasidion* species with the compatibility test (Korhonen 1978).

Genetic analysis. RAPD analysis were carried out using DNA, extracted as by Thompson and Henry (1995) from genotypes of *H. annosum* ss., *H. parviporum* and *H. abietinum.* DNA of each genotype was extracted from 10mm^2 mycelium, soaked for 15 min at 95°C in 120 µl of TPS buffer.

PCR reactions (12,5 µl) contained 10 ng of genomic DNA, 0,94 u Taq polymerase, 1.25 pmol of primer, 2mM of dNTP, 1.25 µl of BSA, 25 mM $MgCl_2$ and Tris-HCL. Six oligonucleotide primers (10 bases long) were used in each PCR amplification.

The cycling was performed as follows: 94°C/60 s followed by 10 cycles of amplification (94°C/5 s, 37°C/30 s, 72°C/30 s) and the next 35 cycles (94°C/5 s, 37°C/30 s, 72°C/30 s).

Amplification products were resolved by electrophoresis on gel consisting of 1,5% agarose and TBE buffer with ethidium bromide 10 μl/ 100 ml.

Genetic similarities among genotypes were estimated using Nei's equation (Nei and Li 1979).

RESULTS

Scots pine stand. Among analysed 34 stumps, 23 were colonized by *Heterobasidion annosum* s. s. In 80% stumps the pathogen was also present in root systems. On the base of somatic incompatibility test this area was colonized by 32 genets of *H. annosum* sensu stricto. Genets were very small. One genet occupied mainly only one stump. In five stumps there were found two different genets and in one stump – three.

Genetic similarity of genets varied from 0% to 62%. (fig.1). The genetic diversity of the *H. annosum* sensu stricto population was high. Only in 5% cases the genetic similarity between two genotypes was relatively high (40% – 62%) and in 1,5% was low (5%). In 24% cases the compared genets were genetically different. Genetic similarity of genets occupying the same stump varied from 0% – 54%. In stump where three genets colonized wood similarity among genets was 10%, 18% and 40%.

Norway spruce stand. In this stand 21 stumps were located in the plot. 15 stumps (71%) were colonized by *Heterobasidion parviporum.* 24 genets colonized this area. Genets were small and occupied only one stump at least. In two cases, four different

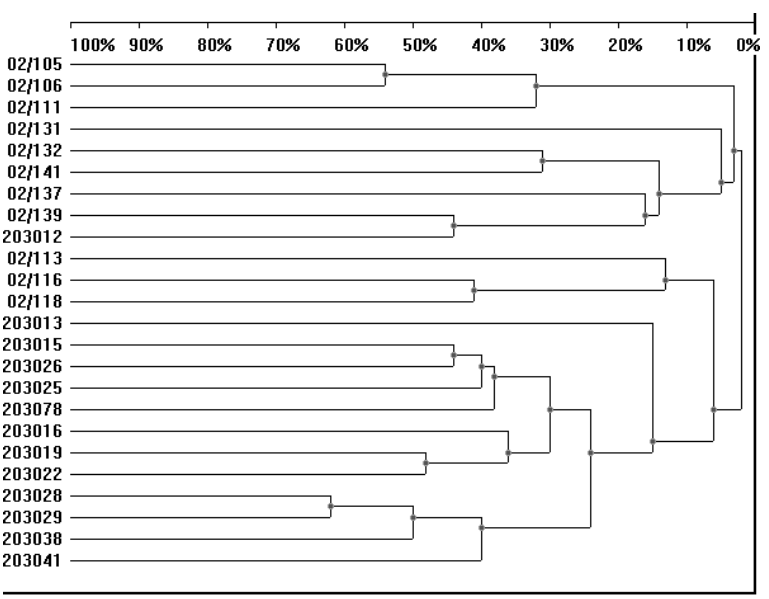

Fig.1. Dendrogram of similarity among genotypes of *H. annosum* sensu stricto.

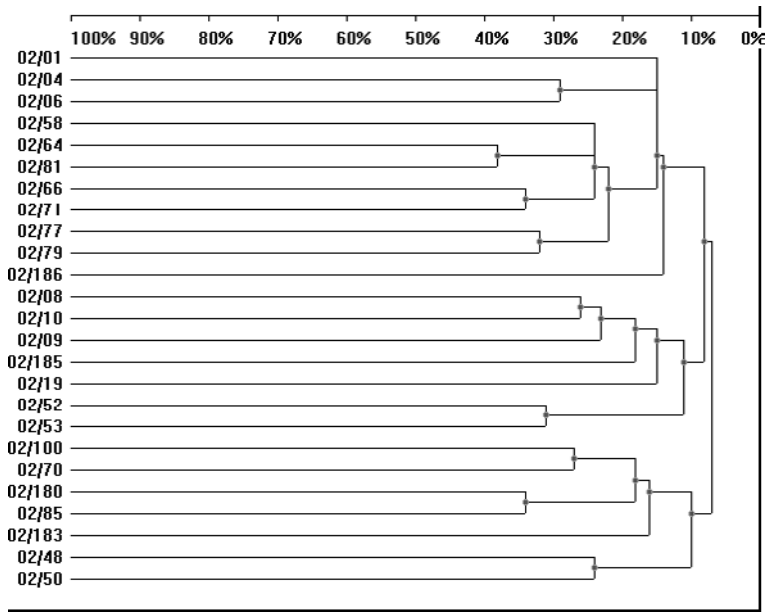

Fig.2. Dendrogram of similarity among genotypes of *H. parviporum.*

genets were found in stump, but in five cases 2 different genets colonized one stump.

Genetic similarity of genets varied from 0% to 38% (Fig.2). In 4% cases genetic similarity between two genets varied from 30% – 38%. The lowest genetic similarity between genets was 9%. Also in 9% genets were totally different. Similarity of genets that occupied the same stump varied from 4% to 35%. In stumps colonized by 4 genets, their similarity varied from 16% to 35%. There were no genetically different genets in one stump.

European silver fir stand. On the first plot *Heterobasidion abietinum* colonized laying log and only two stumps (29%) and on the second the pathogen occurred in

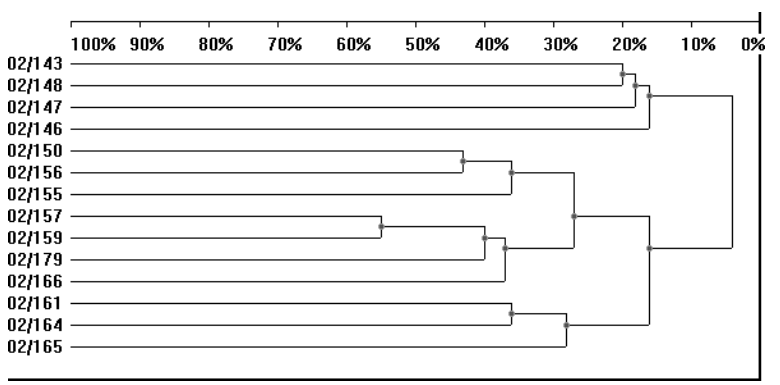

Fig.3. Dendrogram of similarity among genotypes of *H. abietinum.*

3 tumps (60%). On the base of somatic incompatibility stumps were colonized by 14 genets (7 in each plot). In the laying log three genets were localized. Two were small and occupied about 50 cm of stem. The third genet colonized wood on distance 7m of stem. Three stumps were colonized by two genets in each stump and in one stump grew four genets of *H. abietinum.* One stump was whole colonized by one genet.

Genetic similarity of genets varied from 0% to 55% (Fig. 3). The highest similarity (40% – 55%) was found in 5% of compared genets. In 13% cases the genets were genetically different. Similarity among genets colonized the same stump varied from 0,16% to 36%.

DISCUSSION

In this study all genets localized in Norway spruce and Scots pine stands were small. The most probable infection mode was done by basidiospores after thinning. Each stand was thinned at least three times (precommercial and commercial thinning). In Scots pine stands only after the last thinning all stumps were treated against *Heterobasidion annosum* s.s. with *Phlebiopsis gigantea* (Fr.: Fr.) Jülich., while stumps in the Norway spruce stand had never been treated with biopreparation. There were good conditions for stump infection for a long time. Both pine and spruce stand grew as a first generation of forest in post-arable soil. So the small size of genets might be resulted of relatively short time of *Heterobasidion* spp. existence in those stands. Although the vegetative spread of pathogens mycelia should be considered, even if genets were restricted to single stump.

Completely different situation was observed in *A. alba* stand. Lack of stumps in this stand was connected with age of trees and very limited treatment. Only dead or damaged by wind trees had been removed for last years. So almost all stumps were decayed. Those genets probably were older than in pine and spruce stands. Probably there were two reasons of small genet size – age of stumps and distance among stumps (2-15m). The root system around stumps had been decaying and diminishing for years and possible vegetative spread was also difficult. In addition size of genets colonizing the root system could also shrink. The proof of this idea is the size of genet in laying log. The stem was colonized after felling, because butt of stem was not colonized by *H. abietinum.* The biggest genet, which has been found, occupied wood on distance 7m. Considered the speed of mycelium growth in dead wood (50 cm per year) this genet exists for at least 14 years.

This observation improves earlier findings (Kowalski, Łakomy 1998) that root pathogen was able to infect the higher parts of stem after tree felling. Probably it was in connection with dead wood.

Kalio (1970) found that *Heterobasidion annosum* spores could spread even up to 500 km. The high genetic diversity of pathogen's genets and occurrence of many different or weakly similar genets could be explained by high pressure of spores from vicinity stands. This situation was also caused by lack of stump treatment against *Heterobasidion* sp. after thinning. The age of Norway spruce and Scots pine stands influenced the size of genets. At the beginning of stands colonization the vegetative spread via root systems must be limited.

The typical life span of *Heterobasidion* spp. mycelium in roots is still not known. However on the base of the average rapid mycelial growth in roots the largest area

occupied by a single genet was estimated on 50 m in diameter and the age of this genet would not be much more than 100 years (Stenlid and Redfern 1998). In investigated stands genets were very young and the oldest one found in fir stand has less than 20 years.

In coniferous stands a lot of *H. annosum* genotypes could exist in disease stand or gap (Chase, Urlich 1983; Garbelotto 1996; Harrington et al. 1998). Stenlid et al. (1998) found that size of genotypes could be large and one genotype could colonize even 15 trees. However Piri et al. (1990) and Piri (1996) indicated that genotypes in investigated stands colonized only 3 trees. Bodles et al. (2005) described genets in Sitka spruce stand, which had been never thinned before. They found that maximum number of trees affected by one genet was 6 and genet size varied from those occupied single tree to 22,5 m in length. The age of the largest genets, which were 22,5 m in length, 17,5 m and 9 m, they estimate on 45, 35 and 18 years, respectively. Swedjemark and Stenlid (1993) suggested that even if tree was colonized by two or more genets only one may eventually dominate in a single host tree and Woodward et al. (2005) partially supported that hypothesis. Łakomy, Boroda and Werner (2005, unpublished) observed that in some cases only one genet from two or more, which colonized the same stump characterize of highest growth rate in wood of living tree. This situation was noted for *H. annosum* ss., *H. parviporum* and *H. abietinum* genets existing in one stump (pine, spruce or fir). But at the other hand they also observed two dominant genets among three or four from the same stump. It might be result of competition between genets in wood colonization. However they also find no differences in wood colonization among two or three genets from a single spruce or even small pine stump.

Previous works indicated on existing variation in virulence between *Heterobasidion* species and also between isolates inside each species (Werner 1987, 1991; Stenlid, Swedjemark 1988; Swedjemark, Stenlid 1993; Werner, Łakomy 2001, 2002). Łakomy et al. (2005) suggested that damages might be related with number of active genets of *Heterobasidion* in stand. They showed that the variation of virulence among isolates belonged to the same species might be important for disease progress in stands. In their experiments the highest mortality caused both genets of *Heterobasidion annosum* genetically different and also in some degree similarity (30%).

Those three populations of *H. annosum* ss., *H. parviporum* and *H. abietinum* characterized of high genetic diversity. In pine and spruce stands this situation was resulted the high success in *Heterobasidion spp.* colonization of fresh stumps appearing after routine thinning operation. Those genets are young because both stands growth as a first generation on post agricultural soil. Moreover in spruce stands forest service had never treated stump s against *H. parviporum* and in pine stand this operation had been done too late – during last commercial thinning. At least twice stumps after precommercial thinning were open to spore infection. Different situation observed in fir stand was connected with trees' age (180-years-old). Some genets of *H. abietinum* must be old especially those which were present in old decayed stumps. In addition law number of stumps in stand was an important barrier to *H. abietinum* distribution.

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Zróżnicowanie genetyczne grzybów rodzaju *Heterobasidion* w drzewostanach: sosnowym, świerkowym i jodłowym

Streszczenie

Badania nad zróżnicowaniem genetycznym gatunków rodzaju *Heterobasidion* w drzewostanach sosnowym, świerkowym i jodłowym wskazały, że niemalże wszystkie genotypy występujące w badanych drzewostanach były małe i zasiedlały co najwyżej jeden pniak. W kilku przypadkach stwierdzono występowanie dwóch, trzech, a nawet czterech genotypów w jednym pniaku. Podobieństwo genetyczne genotypów *H. annosum* s. s. wynosiło od 0% do 62%, *H. parviporum* od 0% do 38%, a *H. abietinum* od 0% do 55%. Najstarszy i największy genotyp stwierdzono w leżącej kłodzie jodłowej, której drewno przerastał, przez co najmniej 14 lat. Ten genotyp należał do *H. abietinum*. Rozmiar genotypów był związany z intensywnością cięć pielęgnacyjnych w drzewostanach, rozprzestrzenianiem zarodników, wiekiem drzewostanów oraz konkurencją w zasiedlaniu drewna pomiędzy genotypami.