THE VIRULENCE OF HETEROBASIDION PARVIPORUM POPULATION FROM NORWAY SPRUCE STAND IN SUWAŁKI FOREST DISTRICT

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Abstract. The aim of this study was to determine the virulence of H. parviporum genets with known genetic similarity and representing pathogen’s population, which infested a part of Norway spruce stand. Genets caused average 47% mortality of spruce seedlings in infection experiment in vitro. The mortality rate varied from 16% to 80%. In infection experiment in vivo the mycelium of H. parviporum caused the wood necrosis on distance from 0.8 to 7.78 mm. 56% of genets of investigating population characterized by high virulence, 22% by average and 22% by low virulence.

Key words: Heterobasidion parviporum, genets, virulence, Norway spruce

INTRODUCTION

Heterobasidion root and butt rot disease is one of the most important forest trees diseases in Poland and boreal zone. Heterobasidion spp. cause white pocket root and butt rot. The rot column could spread several meters in stem height [Korhonen et al. 1998, Sierota 2001, Mańka 2005]. In Poland occur three Heterobasidion species – H. annosum (Fr.) Bref. sensu stricto, H. parviporum Niemelä et Korhonen i H. abietinum Niemelä et Korhonen [Łakomy et al. 2000, Łakomy and Werner 2003]. The occurrence of these species cover the range of its main plant hosts – Pinus sylvestris L. for H. annosum s. str., Picea abies (L.) H. Karst. for H. parviporum and Abies alba Mill. for H. abietinum [Łakomy and Werner 2003]. The host list of Heterobasidion is very wide and includes more than 200 species of trees, shrubs and heathers [Webb and Alexander 1985]. Heterobasidion annosum s. str. attacks the largest number of host species. By contrast H. parviporum is a narrow specialized species, which occurs mainly on Norway spruce and H. abietinum on European fir, rarely attacks larch (Larix decidua Mill.) [Wagn 1980, Łakomy and Werner 2003, Łakomy and Cieślak 2008].

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Different inoculation method were used to characterize the virulence of pathogens and resistance of its hosts. Including the roots inoculations, in almost all experiments the mycelium was inserted into the wound. The ability of mycelium growth in alive wood and appearance of wood necrosis were the criteria for detection of pathogens’ aggressiveness [Dimitri 1969 a, b, Delatour 1982, Stenlid and Swedjemark 1988, Werner and Łakomy 2002 b]. Werner and Łakomy [2002 a] found that seedlings could be used in vitro experiments for determination of *Heterobasidion* spp. virulence. Previously, there were used *Heterobasidion* spp. isolates that belonged to different populations in studies concerning its virulence [Werner 1987, 1991, Stenlid and Swedjemark 1988, Werner and Łakomy 2002 a, b]. Łakomy et al. [2007] displayed the results of population studies of *Heterobasidion* spp. virulence in Poland.

The aim of this study was to describe the virulence of *H. parviporum* isolates representing different genotypes of determined relationships and belonged to the population that existed in a part of Norway spruce stand.

**MATERIAL AND METHODS**

Nine isolates were used in this experiment, which represented different genotypes of *H. parviporum* population, and which colonized stumps in Norway spruces stand in Suwałki Forest District (110-year-old, 54°34‘N; 23°01‘E). The genetic relationships of these genotype were described in previous study (Table 1) [Łakomy et al. 2011].

| Table 1. Genetic relationship (%) of *H. parviporum* population [Łakomy et al. 2011] |
|---|---|---|---|---|---|---|---|---|
| Genotypes Genotyp | 8/3 | 8/4 | 9/1 | 10/1 | 11/1 | 12/1 | 13/2 | 14/1 |
| 8/1 | 36 | 68 | 36 | 45 | 45 | 45 | 0 | 45 |
| 8/3 | 36 | 62 | 36 | 36 | 36 | 0 | 36 |
| 8/4 | 36 | 45 | 45 | 45 | 0 | 45 |
| 9/1 | 36 | 36 | 36 | 0 | 36 |
| 10/1 | 68 | 68 | 0 | 56 |
| 11/1 | 56 | 0 | 56 |
| 12/1 | 0 | 36 |
| 13/2 | 0 |

**Infection experiment in vitro.** Norway spruce seedlings were growing in pure cultures on the medium containing macro- and microelements [Ingestand 1979]. After a month the seedlings were infected by putting the mycelium close to their root collar. Observations of seedlings growth, appearance of disease symptoms, seedlings mortality were started after inoculation. Each combination was duplicated 20 times. After 9 months the genotypes virulence was determined on the base of seedlings mortality [Werner and Łakomy 2002 a].
Infection experiment *in vivo*. 3-year-old Norway spruce seedlings were used in this study. The seedlings were planted in pots three months before the inoculation. Pots were put in an open area and watered as desired. The inoculum consisted of a small piece of wood (5 mm long and 2 mm in diameter) colonized by pathogen mycelium. The inoculum was inserted in the hall made by drill just under the root collar. Next wounds were banded by laboratory film. Each combination was duplicated 10 times. For control Norway spruce seedlings were inoculated with sterile wood pieces. After four months the seedlings were examined on the presence of necrosis caused by pathogen’s colonization and rot process [Werner and Łakomy 2002 b].

The virulence of genotypes was calculated on the base of the ability of alive wood colonization, causing the wood necrosis and seedlings mortality.

The analysis of variance (ANOVA/MANOVA) and HSD Tukey test were done (Statistica v. 6).

RESULTS

Nine months after inoculation, genotypes caused mortality of Norway spruce seedlings on average 47%. The isolate 13/2 was characterized by the highest mortality. This isolate caused 80% of seedlings mortality. For other genets the mortality varied from 16% to 65% (Fig. 1). The analysis of variance showed the significant differences in mortality caused by different genotypes of *H. parviporum* (Table 2). In 42% cases genotypes differ in virulence and caused seedlings mortality (Table 3). Three genets 8/1, 13/2 and 14/1 differed in comparison to other in most cases pairings, and genets 8/1 and 14/1 caused the lowest mortality (appropriately 20% and 16%). Average genetic similarity of genets, which caused the significant different mortality was 22%, but on the other hand in 45% these differences were connected with unrelated genotypes.

![Fig. 1. Mortality of Norway spruce seedlings caused by *H. parviporum* genotypes](image)

Rys. 1. Śmiertelność siewek świerka pospolitego powodowana przez genotypy *H. parviporum*
Four months after the inoculation the mycelium of *H. parviporum* spread in wood of Norway spruce stems on average distance of 7.78 mm. The weakness mycelium development in the wood was observed for genotype 14/1 (distance 0.8 mm). The mycelium of genet 10/1 overgrew the wood on average distance of 11.8 mm (Fig. 2). The analysis of mycelium spread divided genotype into three groups, which mycelium spread in the wood on different distance (Table 4, p < 0.05). Looking into the genetic similarity, differences in genotypes aggressiveness occurred in all similarity groups. Average genetic similarity of isolates differed (p < 0.05) in mycelium growth in the Norway spruce wood was 36.5% (Table 5, Fig. 2). In the examined *H. parviporum* population five genotypes were characterized by the high virulence, two average and two low virulence (Fig. 3).
The virulence of *Heterobasidion parviporum* population from Norway spruce ...

Fig. 2. Average of necrosis length in Norway spruce stems
Rys. 2. Średnia długość nekrozy w strzałkach świerka pospolitego

Table 4. Analysis of variance of necrosis in Norway spruce stems caused by *H. parviporum* genotypes
Tabela 4. Analiza wariancji dla nekrozy strzałek świerka pospolitego powodowanej przez genotypy *H. parviporum*

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p</th>
<th>Test F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among groups</td>
<td>580.1778</td>
<td>8</td>
<td>72.52222</td>
<td>12.87377</td>
<td>1.57E-08</td>
<td>2.208518</td>
</tr>
<tr>
<td>Inside groups</td>
<td>202.8</td>
<td>36</td>
<td>5.633333</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td>782.9778</td>
<td>44</td>
<td></td>
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</tbody>
</table>

Table 5. Results of HSD Tukey test for comparison of necrosis of Norway spruce stems caused by *H. parviporum* genotypes
Tabela 5. Wyniki testu HSD Tukeya dla porównania nekrozy siewek świerka pospolitego powodowanej przez genotypy *H. parviporum*

<table>
<thead>
<tr>
<th>Genotypes Genotyp</th>
<th>8/3</th>
<th>8/4</th>
<th>9/1</th>
<th>10/1</th>
<th>11/1</th>
<th>12/1</th>
<th>13/2</th>
<th>14/1</th>
<th>Control Kontrola</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/1</td>
<td>0.0077</td>
<td>0.10115</td>
<td>0.08912</td>
<td>0.00641</td>
<td>0.18719</td>
<td>0.0158</td>
<td>0.01097</td>
<td>0.03985</td>
<td>0.14975</td>
</tr>
<tr>
<td>8/3</td>
<td>0.012099</td>
<td>0.03393</td>
<td>0.30168</td>
<td>0.01818</td>
<td>0.21364</td>
<td>0.5</td>
<td>5.83E-05</td>
<td>0.00072</td>
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<tr>
<td>8/4</td>
<td>0.38712</td>
<td>0.0175</td>
<td>0.35303</td>
<td>0.01211</td>
<td>0.00237</td>
<td>8.52E-05</td>
<td>0.00096</td>
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<tr>
<td>9/1</td>
<td>0.03123</td>
<td>0.29040</td>
<td>0.06632</td>
<td>0.02127</td>
<td>0.001302</td>
<td>0.00606</td>
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<td></td>
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</table>

*Silvarum Calendanum Ratio et Industria Lignaria* 10(3) 2011
Table 5 – cont. / Tabela 5 – cd.

<table>
<thead>
<tr>
<th>Genotypes Genotyp</th>
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<th>8/4</th>
<th>9/1</th>
<th>10/1</th>
<th>11/1</th>
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<tbody>
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<td>10/1</td>
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<td>0.13813</td>
<td>0.27996</td>
<td>0.000507</td>
<td>0.00201</td>
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<td></td>
<td></td>
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<tr>
<td>11/1</td>
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<td>0.01426</td>
<td>0.00467</td>
<td>0.02067</td>
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</tr>
<tr>
<td>12/1</td>
<td>0.09606</td>
<td>1.2E-06</td>
<td>4.58E-05</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13/2</td>
<td>4.12E-07</td>
<td>1.78E-07</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14/1</td>
<td>0.00834</td>
<td></td>
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<td></td>
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</table>

DISCUSSION

Stands might be colonized by *Heterobasidion* spp. in different degree. This is related to the importance of *Heterobasidion* root rot for stability of infected stands. Stenlid [1985] found that Norway spruce stands were colonized by pathogen in 12% and 17%. Rieger [1995] observed increase of infection from 7.5% to 33% during decade. On the other hand Stanlid and Wästerlund [1986] found infection of 77% of Norway spruces in the stand. Łakomy et al. [2001] analysed the stands destroyed by windstorms and
displayed that from 56% to 100% of the damaged trees had previously been colonized by pathogen. Łakomy et al. [2011] analysed colonization of *H. annosum* s. str. and *H. parviporum* in ten stands – five Scots pine and five Norway spruce stands. Scots pine stands were infested in 42% – 100%, whereas infestation of Norway spruce stands was lower and estimated from 28% to 35%. Trees or stumps may be colonized by one or more *Heterobasidion* genets [Stenlid 1985, Piri et al. 1990, Piri 1996, Łakomy et al. 2007, Piri and Korhonen 2007, Dalke and Łakomy 2009]. Bodles et al. [2005] showed, that 45-year-old Sitka spruce stand (*Picea sitchensis* (Bong.) Carrière), that grew without thinning, was severely infected by *H. annosum* s. str. They found 25 pathogen’s genotypes, and the biggest one spread on the distance of 22.5 m. At the other hand Dalke and Łakomy [2009] displayed bigger size of genets of *H. annosum* s. str. in Scots pine stand with beech undersorey. The biggest genotype cover area of 84 m² and occurred in three stumps and six young beeches. Stenlid and Redfern [1998] found that the biggest *H. annosum* sensu lato genet occupied area of 50 m in diameter. The age of this genet might be estimated to approx. 50 years, because there is assumption that *H. annosum* overgrows dead wood in average 50 cm per year [Rishbeth 1951, 1957, Slaughter and Parmeter 1995, Bendz-Hellgren et al. 1999]. Łakomy et al. [2011] found that the biggest genotype occurred on the area of 160.2 m² (about 28 m in diameter) and probable age of this genet was estimated to 28-years. They also showed a high genetic variability of the studied *H. annosum* s. str. populations expressed by low similarity among genotypes. Populations of *H. parviporum* were more similar. In all cases they found genets very closely related in 70-77% and also totally genetically different. Stenlid [1985] found that pathogen genotypes might be big and occupy even 15 trees in Norway spruce stands. However, Piri et al. [1990] estimated genets that colonized on the average, each three tree. In *Abies concolor* (Gordon et Glend) Lindl. ex Hildebr. stand [Garbelotto 1996] the longest distance between colonized by the same genet trees was 6 meters.

Pathogens may be varied in pathogenicity and aggressiveness between each other [Stenlid and Swedjemark 1988, Swedjemark et al. 1999, Werner and Łakomy 2002 b]. The health status of trees and damages caused by decay development in Norway spruce stems depended on virulence of existing pathogen’s population in this stand. The aggressiveness and pathogenicity of genets belonging to different populations were tested in several studies [Stenlid and Swedjemark 1988, Swedjemark et al. 1999, Werner and Łakomy 2002 b]. Łakomy et al. [2005] investigating stumps from gap in Norway spruce stand found that 19 genotypes of *H. parviporum* differed in virulence. Forty two percent of genets caused mortality of less than 20% of seedlings in vitro. Moreover, 32% of genets were highly pathogenic (mortality over 50% of seedlings). In this study the pathogenicity of *H. parviporum* population was higher. Only 22% of genets caused mortality less than 20% of seedlings, and 55% were highly pathogenic. These genets were both highly pathogenic and aggressive that was demonstrated by spread of necrosis in Norway spruce stems. Werner and Łakomy [2002 a, b] obtained similar results but they used only one (the same) isolate in both experiments in vivo and in vitro. The diversity of aggressiveness among isolates of *H. parviporum* was found in several studies [Stenlid and Swedjemark 1988, Swedjemark et al. 1999, Werner and Łakomy 2002 b]. The knowledge about virulence of *Heterobasidion* is very important in making a forecast of *Heterobasidion* spreading in a stand. This study showed that population that occurred in stand in the Suwalki Forest District could colonize the stand rather fast, because most of genets were highly virulent. La Porta et al. [1997] and Łakomy et al. [2005] suggested that size of damages in stand probably depended on genetic diversity.
of pathogen’s population. In addition Łakomy and Werner [unpublished] showed that sometimes the pathogenicity might be related with genetic diversity. Pathogen’s populations differ on the base of genetic variability, areas occupied by genets and stand’s age.

The recognition of genetic diversity, genets size and their virulence is very important in prediction of *Heterobasidion* root rot, economic damages and organizing the protection objectives. That is why the study on *Heterobasidion* populations, their virulence, spreading in stands, ability of trees infestation and host resistance should be continued.

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**REFERENCES**


WIRULENCA POPULACJI HETEROBASIDION PARVIPORUM
Z DRZEWOSTANU ŚWIERKOWEGO NADLEŚNICTWA SUWAŁKI

Streszczenie. Celem badań było określenie wirulencji izolatów H. parviporum reprezentujących różne genotypy o określonym pokrewieństwie i należące do populacji patogena zasiedlającej część drzewostanu świękowego. Do badań wykorzystano dziewięć izolatów, o określonym podobieństwie genetycznym, reprezentujących odrębne genotypy populacji H. parviporum zasiedlające pniaki w drzewostanie świerka pospolitego w Nadleśnictwie Suwałki. Wykonano dwa doświadczenia infekcyjne. Doświadczanie infekcyjne in vitro przeprowadzono na pożywie agarowej zawierającej mikro- i makroskładniki. Po miesiącu wzrostu siewki były inokulowane grzybnią patogena. Patogeniczność określano na podstawie śmiertelności siewek po 9 miesiącach wzrostu. W doświadczeniu infekcyjnym in vivo użyto 3-letnich sadzonek świerka pospolitego posadzonych w donicach trzy miesiące przed założeniem doświadczenia. Strzałki świerkowe inokulowano grzybnią w ranę wykonaną nad szystą korzeniową. Po 4 miesiącach drewno strzałek analizowano podkątem nekrozy powstałej w wyniku zasiedlenia i rozkładu drewna powodowanego przez patogena. Wirulencję genotypów określało na podstawie zdolności grzybni do zasiedlania żywego drewna oraz powodowanej śmiertelności siewek świerka pospolitego. Śmiertelność siewek wahała się od 16% do 80%. Analiza wariancji wykazała istotne różnice w śmiertelności siewek świerka pospolitego powodowanej przez poszczególne genotypy H. parviporum. Grzybnia poszczególnych genotypów patogena przerosła drewno strzałek sosnowych na średnią odległość od 0,8 mm do 11,8 mm. Różnice w agresywności genotypów występowały pomiędzy izolatami we wszystkich grupach pokrewieństwa. W badanej populacji H. parviporum pięć genotypów charakteryzowało się wysoką wirulencją, dwa średnią, a dwa niską.

Słowa kluczowe: Heterobasidion parviporum, genotypy, wirulencja, świerk pospolity

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