

SUSCEPTIBILITY OF *LARIX DECIDUA* MILL. TO *HETEROBASIDIUM* spp. IN AN INFECTION EXPERIMENT

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ABSTRACT

The aim of this study was to investigate the susceptibility of young *Larix decidua* seedlings to *H. annosum*, *H. parviporum* and *H. abietinum* based on an infection experiment. Two-year-old European larch seedlings were inoculated with five strains of each *Heterobasidion* species: *H. annosum*, *H. parviporum* and *H. abietinum*. The extent of necrosis above and below the inoculum point was the basis for analyses of susceptibility of larch seedlings. Necrosis caused by *Heterobasidion* spp. differed depending on the pathogens species, with the longest found in larch stems infected by *H. annosum*. Moreover, the variation in necrosis size was found among all the used strains. Each *Heterobasidion* species was represented with highly and less aggressive isolates, but in the case of *H. annosum* high pathogenicity was most frequent. *Larix decidua* could be a potential host of all European *Heterobasidion* species.

Keywords: *Heterobasidion annosum*, *Larix decidua*, susceptibility, infection

INTRODUCTION

Heterobasidion is one of the most important genera of fungi for forestry in the Northern hemisphere (Dai and Korhonen, 1999; Gremmen, 1970; Korhonen et al., 1998; Peace, 1962). Species of *Heterobasidion* cause root and butt rot mainly in coniferous trees. The greatest damage was observed in former farmland planted with pine or spruce species in the first and second generations of the stands (Sierota, 1987; 2013). The hosts of *Heterobasidion* spp. were described and listed for more than 200 species of both coniferous and deciduous trees (Capretti et al., 1994; Łakomy and Werner, 2003; Wagn, 1980; Webb and Alexander, 1985). In Europe *H. abietinum* infected mostly the *Abies* genus and it was also noted *Larix*, *Picea* and *Fagus*. Hosts of

H. parviporum are mainly spruce species, but also firs. The widest range of hosts was described for *H. annosum*. This species attacks mostly genera of coniferous species, but it was also noted some deciduous species including *Betula pendula*, *Quercus* spp., *Carpinus betulus*, *Fagus sylvatica* and *Alnus* spp. *Heterobasidion* spp. showed the host specialization to the tree species (Greig et al., 2001; Łakomy and Werner, 2003; Stenlid and Swedjemark, 1988; Swedjemark et al., 1999). Susceptibility of various tree species to *H. annosum* s.l. was investigated in many studies to study intraspecific variation in the host preference. Various inoculation methods and tree age variants (2–100 years old) were used in these analyses (Delatour, 1982; Dimitri, 1963;

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1969a; 1969b; Stenlid and Swedjemark, 1988; Swedjemark et al., 1999; Werner and Łakomy, 2002a; Werner et al., 2005). Werner and Łakomy (2002b) used seedlings in an *in vitro* experiment and on the basis of their mortality rates they concluded that this method could be useful for testing the pathogenicity of *Heterobasidion* to pine and spruce hosts. In most infection experiments the main hosts included Scots pine, Norway spruce or European fir. Only in few cases susceptibility of other species was tested under greenhouse or outdoor conditions (Delatour, 1982; Dimitri, 1963; Stenlid and Swedjemark, 1988; Swedjemark et al., 1999; Werner and Łakomy, 2002a; 2002b).

The aim of this study was to investigate susceptibility of young *Larix decidua* seedlings to *H. annosum*, *H. parviporum* and *H. abietinum* on the basis of an infection experiment.

MATERIALS AND METHODS

Two-year-old trees of *Larix decidua* from a forest nursery (Siemianice Experimental Forest of the Poznań University of Life Sciences, Poland) were planted individually in 10 l pots in March. Each pot was filled with a pathogen-free sphagnum peat-perlite mixture (1:1). The bottom of each pot had openings to allow for adequate drainage. In order to protect the seedlings

from the environment throughout the experiment the pots were placed in a greenhouse. The roof of the greenhouse shielded seedlings from natural precipitation, while the open sides provided ventilation keeping the temperature close to the ambient temperature and preventing seedling overheating. Seedlings were watered twice weekly during the growing season. Fifteen strains of *Heterobasidion*: five each of *H. annosum*, *H. parviporum* and *H. abietinum* were selected for the study as specified in Table 1. A round hole of 3 mm in diameter was made with a sterile drill in stems of each plant (about 5 cm above the root collar) three months after planting. Beech dowels colonised by *Heterobasidion* spp. mycelium were inserted into the wounds, which next were protected with Parafilm. The sterile beech dowels were used in the control. Each *Heterobasidion* sp. strain × host treatment was replicated fifteen times. The experiment was of the randomised block design (Werner and Łakomy, 2002a). Four months after inoculation the plants were removed from soil and the extent of necrosis above and below the inoculum point was measured. Stems selected at random were surface sterilised in 2% HgCl₂ and divided into 5-mm thick discs using a sterile knife. Discs were incubated in Petri dishes under humid conditions and then analysed for the anamorph of *H. annosum*: *Spiniger meineckellus* (Olson) Stalpers.

Table 1. The origin of *Heterobasidion* spp. isolates
Tabela 1. Pochodzenie izolatów *Heterobasidion* spp.

Lp. No	Fungus species Gatunek grzyba	Isolate code Kod izolatu	Location – Lokalizacja	Host – Gospodarz
1	2	3	4	5
1	<i>H. annosum</i>	0/2	Skwierzyna (52°32'N, 15°21'E)	<i>Pinus sylvestris</i> stump – pniak
2	<i>H. annosum</i>	03/4	Skwierzyna (52°32'N, 15°21'E)	<i>Pinus sylvestris</i> stump – pniak
3	<i>H. annosum</i>	03/07	Łobez (53°40'N, 15°38'E)	<i>Pinus sylvestris</i> stump – pniak
4	<i>H. annosum</i>	Sk4	Skwierzyna (52°32'N, 15°21'E)	<i>Pinus sylvestris</i> dying tree – drzewo zamierające
5	<i>H. annosum</i>	T2/1	Tuczno (53°16'N, 16°11'E)	<i>Fagus sylvatica</i> dying tree – drzewo zamierające

Table 1 – cont. / Tabela 1 – cd.

1	2	3	4	5
6	<i>H. parviporum</i>	17004	Świerklańiec (50°25'N, 19°01'E)	<i>Picea abies</i> living tree – żywe drzewo
7	<i>H. parviporum</i>	17017	Nowy Targ (49°25'N, 20°05'E)	<i>Picea abies</i> living tree – żywe drzewo
8	<i>H. parviporum</i>	H6/1/1	Henryków (50°38'N, 17°02'E)	<i>Picea abies</i> stump – pniak
9	<i>H. parviporum</i>	Suw06	Suwałki (53°59'N, 23°02'E)	<i>Picea abies</i> stump – pniak
10	<i>H. parviporum</i>	Suw1	Suwałki (54°16'N, 22°51'E)	<i>Picea abies</i> stump – pniak
11	<i>H. abietinum</i>	17021A	Ojcowski National Park Ojcowski Park Narodowy (50°12'N, 19°40'E)	<i>Abies alba</i> lying log – leżąca kłoda
12	<i>H. abietinum</i>	20057	Roztoczański National Park Roztoczański Park Narodowy (50°31'N, 23°27'E)	<i>Abies alba</i> lying log – leżąca kłoda
13	<i>H. abietinum</i>	94137	Węgierska Góra (49°42'N, 19°15'E)	<i>Abies alba</i> dead tree – martwe drzewo
14	<i>H. abietinum</i>	96056	Nowy Targ (49°25'N, 20°05'E)	<i>Picea abies</i> stump – pniak
15	<i>H. abietinum</i>	96070	Węgierska Góra (49°42'N, 19°15'E)	<i>Abies alba</i> stump – pniak

RESULTS

The necrosis caused by *Heterobasidion* spp. varied depending on the pathogens species ($p = 0.000595$). The longest discoloration areas were found in larch stems infected with *H. annosum* (on average 5.23 mm). *Heterobasidion parviporum* and *H. abietinum* mycelia spread in stems over a similar distance of 3.47 and 3.5 mm, respectively, whereas the discoloration was approx. 33% shorter than in the case of *H. annosum*. In the control stems the discoloration spread over a distance of 1.23 mm (Fig. 1). The length of necrosis caused by *H. annosum* differed significantly from those found in the control and larch stems infected with *H. parviporum* and *H. abietinum* (Table 2). Moreover, the discoloration areas caused by *H. parviporum* and *H. abietinum* were almost three times longer than in the control stems; however, due to the wide maximum and minimum ranges there were no significant differences between those groups of specimens

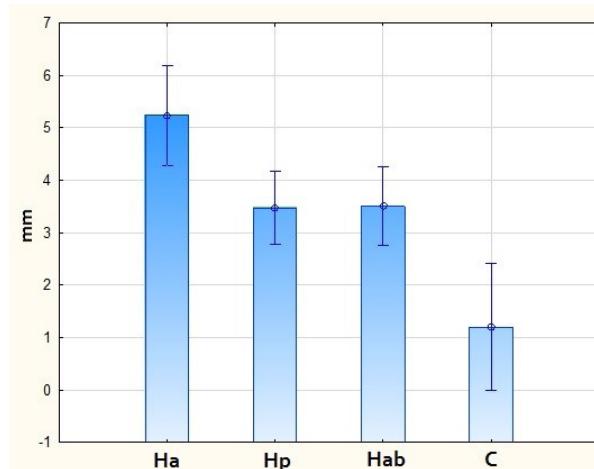


Fig. 1. The length of necrosis caused by *H. annosum* (Ha), *H. parviporum* (Hp), *H. abietinum* (Hab) and in control stems of larch (C), mm

Rys. 1. Długość nekrozy spowodowanej przez *H. annosum* (Ha), *H. parviporum* (Hp), *H. abietinum* (Hab) i w strzałkach kontrolnych modrzewi (C), mm

Table 2. Results of the Duncan test comparison for the length of necrosis caused by *H. annosum*, *H. parviporum* and *H. abietinum* in larch wood ($p < 0.05$)

Tabela 2. Wyniki testu Duncana porównania długości nekrozy powodowanej przez *H. annosum*, *H. parviporum* i *H. abietinum* w drewnie modrzewi ($p < 0,05$)

Combination Kombinacja	Ha	Hp	Hab	C
Ha	0.001987	0.002376	0.001854	
Hp	0.001987	0.955443	0.076483	
Hab	0.002376	0.955443	0.072586	
C	0.001854	0.076483	0.072586	

Ha – *H. annosum*, Hp – *H. parviporum*, Hab – *H. abietinum*.

($p = 0.076$ and $p = 0.074$, respectively; Table 2). Analyses of susceptibility of larch to individual isolates showed that inside each *Heterobasidion* species there were isolates that caused significant necroses in stem wood and others causing limited necrosis size in comparison to the control (Table 3). Only one isolate of *H. abietinum* (Hab96070) caused a comparable length of discoloration as in the control. Mycelium growth of other isolates caused a discoloration in wood at least two times longer than in the control (3 isolates), three times longer (3 isolates) and greater (significant differences in comparison to the control). The discoloration in stems varied from 1.36 mm (Hab96070) to 6.88 mm (HaSk4). Statistically significant discoloration of wood was caused by four isolates of *H. annosum*

Table 3. The homogenous group based on the Duncan test

Tabela 3. Grupy jednorodne określone na podstawie testu Duncana

Isolate code Kod izolatu	Necrosis length Długość nekrozy	1	2	3	4	5	6
Control – Kontrola	1.20	****					
Hab96070	1.35	****	****				
Hp17004	2.50	****	****	****			
Hab96056	2.74	****	****	****	****		
Hp6/1/1	2.79	****	****	****	****		
HpSuw06	3.65	****	****	****	****	****	
Ha03/4	3.75	****	****	****	****	****	
Hp17017	3.79	****	****	****	****	****	
Hab20057	4.00	****	****	****	****	****	****
Ha03/07	4.19		****	****	****	****	****
Hab94137	4.40			****	****	****	****
HpSuw1	4.68			****	****	****	****
Hab17021A	5.11			****	****	****	****
Ha0/2	5.61				****	****	****
HaT2/1	5.90					****	****
HaSk4	6.88						****

Ha – *H. annosum*, Hp – *H. parviporum*, Hab – *H. abietinum*.

The grey shadow indicates significant differences in the length of necrosis in comparison to control seedlings, $p < 0.05$.

Kolor szary wskazuje na istotność różnic długości nekrozy w porównaniu z sadzonkami kontrolnymi, $p < 0,05$.

(Ha03/07 – 4.19 mm, Ha0/2 – 5.51 mm, HaT2/1 – 5.9 mm, HaSK4 – 6.88 mm), two isolates of *H. abietinum* (Hab94137 – 4.4 mm, Hab17021A – 5.11 mm) and only one isolate of *H. parviporum* (HpSuw1 – 4.68 mm; Table 3). Reisolation of *Heterobasidion mycelium* confirmed the presence of the pathogen in 42% samples of necrotic tissue regardless of the pathogen species.

DISCUSSION

Heterobasidion annosum is the species with the widest range of host organisms in comparison to *H. abietinum* and *H. parviporum*. It was shown in a previous study that *H. annosum* could colonise in the same way pine, spruce and fir, as opposed to *H. parviporum* and *H. abietinum*, which as host-specialised species mainly colonised spruce and fir, respectively (Stenlid and Swedjemark, 1988; Werner and Łakomy, 2002a; 2002b). Also in our experiment *H. annosum* was the most aggressive species towards larch. Moreover, four isolates instead of five caused a significant discolouration of larch wood in comparison to the control. Only one isolate caused three times longer discolouration than in the control. The same phenomenon was observed in the case of two isolates of *H. parviporum* and one isolate of *H. abietinum*. A 2-fold longer discolouration was found when analysing stems of larch infected with two isolates of *H. parviporum* and one isolate of *H. abietinum* in comparison to those found in the control stems. However, despite the considerable diversity in necrosis length in some cases non-significant differences were found between infected and controls stems. Probably the mycelium died immediately after inoculation in some larch stems in the experiment combination, which influenced the results of the statistical analysis. However, in other inoculated larches with extensive discolouration of wood *Heterobasidion* was reisolated. An interesting finding was connected with the fact that one of the most aggressive isolates of *H. abietinum* was collected 24 years ago from a fir stump and its aggressiveness was maintained for a long time after collection. In a previous study (Werner and Łakomy, 2002b) this isolate was less aggressive to pine and spruce seedlings and highly aggressive to European fir in comparison to other tested isolates of three *Heterobasidion* species. Larch

turned out to be the species as susceptible as fir to those isolates.

Larch is noted as a susceptible species, when it was planted on the sites infested by *Heterobasidion annosum*. Rönnberg and Vollbrecht (1999) found three plantations of hybrid *Larix x eurolepis* highly infested by *H. annosum*. The previous stand consisted of Norway spruce infected in 75%. The incidence of *H. annosum* was 7%, 33% and 70% in 2-, 3- and 5-year old plantations. The most interesting finding is that none of the infested trees showed disease symptoms. Other investigations also showed that thinning could increase the incidence of butt rot in European and Japanese larch (Vollbrecht et al., 1995) or in hybrid larch when the plantations were established after a Norway spruce stand infested by *H. annosum* (Larsson-Stern et al., 1996 after Rönnberg and Vollbrecht, 1999). Greig et al. (2001) investigated experimental plots established in sites highly infested by *H. annosum* and planted with different tree species in randomised blocks. They found a relatively high incidence of decay in larch stems during the first thinning (age 21–22 years) in comparison to other species. In one site the incidence of decay in stems was 59% in *Larix decidua*; 51% in *P. menziesii*; 37% in *Chamaecyparis lawsoniana*; 33% in *Abies amabilis* and 21% in *Tsuga heterophylla*. They found negligible incidence rates of the disease in *A. procera*, *Abies grandis* and *Pinus sylvestris*. In addition the mean height of colonization by *H. annosum* within the diseased trees was 2.1 m for *L. decidua*, 1.4 m for *P. sitchensis* and 1.3 m for *P. abies*.

Kurkela (1988) reported mortality of Siberian larch seedlings planted after a Scots pine stand infected by *H. annosum*. Piri (2003) analysed infestation by *Heterobasidion* in the previous Norway spruce generation and in the present generation consisting of exotic trees, i.e. lodgepole pine and Siberian larch. That author isolated both *H. parviporum* and *H. annosum* from Siberian larch and concluded that regeneration of infested sites with exotic tree species does not eradicate *H. parviporum* from the site. Both tree species became infected from old spruce stumps. However, damage caused by *H. parviporum* remains lower in subsequent lodgepole pine and Siberian larch stands than in a subsequent Norway spruce stand.

An inoculation experiment conducted by Swedjemark and Stenlid (1995) showed that both Japanese

and hybrid larches are highly susceptible both to *H. annosum* and *H. parviporum*, which partly corresponds to the results of this study. In our experiment we confirmed high susceptibility of European larch to *H. annosum* and moderate susceptibility to *H. parviporum* and *H. abietinum*. In turn, Kraj and Kowalski (2010) found infection of *H. annosum* on European larch four times more frequently than by *H. parviporum*. They showed no infection of larch by *H. abietinum*.

In conclusion *Larix decidua* is more susceptible to infection caused by *H. annosum* than *H. parviporum* and *H. abietinum*. Inside each pathogen species variability of virulence exist. Specimens of each *Heterobasidion* species were characterised by high and low virulence in relation to larch. *Larix decidua* could be a potential host of all European *Heterobasidion* species.

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PODATNOŚĆ *LARIX DECIDUA* MILL. W STOSUNKU DO *HETEROBASIDIUM* SPP. NA PODSTAWIE DOŚWIADCZENIA INFEKCYJNEGO

ABSTRAKT

Celem pracy było określenie podatności sadzonek *Larix decidua* w stosunku do grzybów rodzaju *Heterobasidion* na podstawie doświadczenia infekcyjnego. W doświadczeniu wykorzystano dwuletnie sadzonki modrzewia, pochodzące ze szkółki leśnej. Sadzonki inkulowano grzybinią pięciu izolatów każdego z gatunków *Heterobasidion*: *H. annosum*, *H. parviporum* oraz *H. abietinum*, wprowadzając patogeny w postaci drewna zasiedlonego przez grzybnię, w wywiercony w strzałce otwór. Kontrolę stanowiły sadzonki z wprowadzonymi w ranę sterlynymi fragmentami drewna. Podstawą określania podatności była długość nekrozy pojawiającej się w strzałkach drzew, w góre i dół od miejsca inkulacji będącej efektem rozwijającej się w drewnie grzybni patogenów. Długość nekrozy powodowana przez grzyby rodzaju *Heterobasidion* różniła się w zależności od gatunku patogenu. Najdłuższą nekrozę stwierdzono w strzałach inkulowanych przez *H. annosum* (5,23 mm). Grzybnia *H. parviporum* i *H. abietinum* rozprzestrzeniła się w strzałkach na podobną odległość, mianowicie 3,47 mm i 3,5 mm, i była krótsza o około 33% od nekrozy spowodowanej przez *H. annosum*. W kontroli nekroza wokół rany osiągnęła odległość 1,23 mm. Stwierdzono także zróżnicowanie podatności modrzewi na rozwój grzybni poszczególnych izolatów gatunków *Heterobasidion*. W obrębie każdego gatunku występowały izolaty zarówno o małej, jak i dużej agresywności wobec modrzewi, aczkolwiek najczęściej izolatów o dużej agresywności stwierdzono w obrębie *H. annosum*. Konkludując, można stwierdzić, że modrzew europejski może być potencjalnym gospodarzem wszystkich europejskich gatunków grzybów rodzaju *Heterobasidion*.

Słowa kluczowe: *Heterobasidion annosum*, *Larix decidua*, podatność, infekcja