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RESEARCH REPORTS

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WOOD DECOMPOSITION BY *PORODAEDALEA PINI* STRAINS FROM DIFFERENT REGIONS OF POLAND

The results of studies on heartwood decomposition caused by Porodaedalea pini *in pine, spruce and larch are presented. It was found that spruce wood was decomposed to the greatest extent, while pine and larch less so. No relationship was found between the geographical origin of the isolate and its wood decomposition capacity.*

Keywords: white pocket rot, weight loss, *Porodaedalea pini*, wood decomposition

Introduction

Red ring rot (*Porodaedalea pini* (Brot.) Murrill., previously *Phellinus pini*) is a fungus frequently found in pure and mixed pine stands and in tree plantings partly composed of pines. *Porodaedalea pini* sensu lato has been documented as occurring on a wide variety of coniferous and some hardwood hosts, including the following genera: *Abies*, *Acer*, *Betula*, *Calocedrus*, *Cedrus, Chamaecyparis*, *Crataegus*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, *Taxus*, *Thuja* and *Tsuga* [Gilbertson, Ryvarden 1986; Larsen, Cobb-Poulle 1990]. The range of *P. pini* covers the whole of Europe, northern Asia and North America [Gilbertson, Ryvarden 1987]. In Poland, this fungus infests approx. 8% of Scots pine wood [Szewczyk 2008]. *Porodaedalea pini* decomposes heartwood (affecting stands starting from age class II), forming white pocket rot as a result [Ezhov, Konyushatov 2001].

The older the stand, the greater the number of infested pines. It is estimated that in a 100-year-old stand, approx. 15–35% of the pines are infested, while in a 160-year-old stand, it may be as much as 100% of the trees. The range of rot

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increases with time and in old stands the development of rot may exceed the increment in volume of non-infested wood tissue [Szewczyk 2008]. The mycelium of *P. pini* actively overgrows the infested wood tissue. It has a unique type of wood decomposition. Lignin and cellulose are decomposed more or less at the same time; however, the content of the cellulose in the softwood is almost two-fold greater than that of lignin. The fungus causes the formation of numerous holes (pockets) filled with white cellulose, visible to the naked eye over the dark brownish red background [Boyce 1961; Ważny 1968]. Losses are very high, since this parasite is a common species and destroys the lower sections of stems, which are the most commercially valuable [Szewczyk 2008]. The aim of this study was to determine the decomposition capacity of *P. pini* in the heartwood of pine, spruce and larch in relation to the geographic origin of the isolates.

Materials and methods

Investigations into the wood-decomposing capacity of *P. pini* were conducted using 26 isolates, collected in the years 2008–2010, from living *Pinus sylvestris* trees. Their sporophores were collected in forests of north-west Poland located 20–600 km apart. Axenic, dikaryotic (n + n) cultures were obtained from the fresh sporophore context material taken from above the hymenium with a sterile scalpel and plated on Goldfarb's selective medium (malt agar 15 g L^{-1}, prochloraz 1 mg L^{-1}, benomyl 1 mg L^{-1}, thiobendazole 1 mg L^{-1}, streptomycin 1 mg L^{-1}, rose Bengal 1 mg L^{-1}). Fungi were incubated at 24°C in darkness. After 7–14 days, they were transferred to 1.5% malt medium (malt extract 15 g L^{-1}, peptone 5 g L^{-1}, agar (Difco) 15 g L^{-1}) and maintained at 4°C in darkness. Previously the isolates had been subjected to an analysis of the phylogenetic variation [Szewczyk et al. 2014]. Tests were conducted on the heartwood of three native species, i.e. Scots pine, Norway spruce and European larch. To ensure the greatest possible similarity of the wood samples, the wood of a given species was all taken from the same specimen. Analyses of the wood decomposition rate were conducted in accordance with the specifications of the Polish standard [PN-EN 350-1:2000]. The prepared wood samples of 5.0 × 2.5 × 1.5 cm were dried for 72 hrs in an electric drier at 105°C until they were absolutely dry. They were then weighed on a laboratory balance, accurate to 0.01 g, and samples of comparable weight were selected for analysis. The weight of each wood sample was recorded. Kolle flasks containing 2% maltose culture medium (2% maltose extract, 2% agar), sterilized in an autoclave at 121°C for 30 min, were inoculated with the *P. pini* mycelium. After 7 days, six wood samples, previously soaked in sterile water, were placed in each flask to verify whether the mycelium used in the test was live and uncontaminated with other organisms. The mycelium of each isolate was tested on each wood species in a separate flask. Six replicates of each isolate were tested on the wood of each tree species. After 6 months of incubation at 21°C, the samples were again surface-cleaned to remove the mycelium, dried once more in an electric drier for 72 hrs at 105°C until absolutely dry and then weighed on a laboratory balance accurate to 0.01 g. The weight data were subjected to a two-way (isolate and species as factors) analysis of variance (ANOVA) in order to test the null hypotheses that there were no individual effects of the isolate and species, and no isolate × species interactions on the wood decomposition. The means, standard deviations and coefficients of variation (cv) were calculated [Kozak et al. 2013]. Values for the least significant differences (LSD) were estimated and on their basis, homogeneous groups were established for comparisons of the isolates in terms of their wood-decomposing capacities. All the calculations in the statistical analyses were performed using the GenStat 15 statistical software package.

Results and discussion

All the isolates showed the capacity to decompose the heartwood of spruce, pine and larch, but to different extents. The one-way ANOVA showed significant differences between the isolates and between the wood samples from the different tree species (table 1). There was also significant interaction ($P < 0.001$) showing that the different isolates decomposed the wood from the different tree species to different extents. The greatest wood decomposing capacity, averaged over all the wood types, was recorded for isolate 23/2011 (2.04 g weight loss). It caused the most decomposition in the spruce wood. This isolate came from forests in the Rudka Forest District and it was the only isolate from that part of Poland, which was also the most easterly location. Isolate 6/2011, from stands in the Kutno Forest District, ranked second in terms of wood decomposition, with most decomposition (2.64 g weight loss) found in the pine wood. The isolate ranked next in terms of its decomposing ability was 24/2011, collected from a stand in the Mieszkowice Forest District, the most westerly area included in this study. The isolates that decomposed the heartwood to the greatest extent came from extreme locations, and a common characteristic of these isolates was that they were collected from fruit bodies growing on relatively young trees (55–70 years).

Table 1. One-way analysis of variance showing effects of isolates and wood from different tree species, and isolate × wood-type interaction

Independent variables	Sum of squares	Degrees of freedom	Mean square	Variance ratio (F)	Probability (*P*)
Isolate number	25.8222	25	1.0329	4.032	<0.001
Wood type	64.5580	2	32.2790	125.996	<0.001
Isolate number × Wood type	27.0522	50	0.5410	2.112	<0.001

The smallest weight loss was caused by isolate 27/2011 (1.09 g). Isolates from the same locations decomposed the wood to different extents (in the case of the isolates from Zielonka and Mieszkowice). In contrast, the three isolates (16/2010, 2/2011, 10/2011) from Góra Śląska were very similar in terms of decomposing ability. Statistical analysis showed seven homogeneous groups (table 2).

Table 2. Mean loss in weight [g] and standard deviation [SD] of wood from different tree species decomposed by individual isolates of *P. pini*

Species	Larch		Scots pine		Spruce		Mean	
isolates	mean	SD	mean	SD	mean	SD	mean	SD
10/2011	1.01	0.118	1.396	0.052	1.906	0.544	1.43defg	0.482
14/2011	0.72	0.406	1.670	0.145	1.11	0.424	1.16fg	0.517
16/2010	0.61	0.087	1.390	0.225	1.842	0.429	1.28efg	0.586
16/2011	1.08	0.292	1.718	0.690	2.556	0.229	1.78abcd	0.751
2/2011	0.72	0.246	1.468	0.680	2.066	0.349	1.42efg	0.711
21/2011	1.02	0.452	2.092	0.621	2.43	0.580	1.84ab	0.807
23/2011	1.17	0.356	2.150	0.837	2.81	0.302	2.04a	0.864
24/2011	1.05	0.171	1.884	0.455	1.334	0.640	1.42defg	0.558
27/2011	0.66	0.462	1.554	0.209	1.076	1.157	1.09g	0.772
28/2011	0.78	0.713	1.890	0.148	1.168	0.877	1.28efg	0.772
29/2011	0.73	0.254	1.518	0.101	1.64	0.464	1.29efg	0.506
30/2011	0.89	0.227	1.334	0.368	1.878	0.523	1.37fg	0.551
31/2011	0.92	0.277	1.916	1.097	2.676	0.340	1.83ab	0.974
37/2011	0.84	0.660	1.566	0.341	1.32	0.524	1.24efg	0.575
39/2011	0.91	0.185	1.402	0.465	1.66	0.933	1.32efg	0.651
4/2010	0.80	0.128	1.322	0.395	2.516	0.333	1.54bcde	0.795
4/2011	0.81	0.554	1.568	0.137	1.816	0.189	1.39efg	0.546
45/2011	0.96	0.458	1.616	0.040	1.756	0.537	1.44cdefg	0.519
46/2011	2.03	1.557	1.19	0.244	2.368	0.891	1.86ab	1.095
47/2011	0.90	0.280	1.658	0.334	1.972	0.546	1.51bcdef	0.595
5/2011	1.20	0.247	1.79	0.370	1.656	0.592	1.54bcde	0.474
51/2011	0.78	0.149	1.624	0.497	1.982	0.578	1.46cdefg	0.666
52/2011	1.34	0.318	1.894	0.285	2.182	0.478	1.80abc	0.498
6/2011	1.10	0.440	2.644	0.220	2.162	0.582	1.96a	0.782
7/2010	0.60	0.104	1.58	0.152	1.636	0.176	1.27efg	0.509
8/2010	0.77	0.183	1.054	0.806	1.978	0.700	1.26efg	0.787
Species	0.942C	0.5037	1.65B	0.5292	1.904A	0.7041		
$LSD_{0.05}$	Isolate: 0.364; Species: 0.1235; Isolate × Species: 0.6299							

LSD – least significant difference at $P = 0.05$
Means followed by the same letters are not significantly different

The most numerous group was composed of 10 isolates and caused a weight loss of 1.245 to 1.421 g during wood decomposition. These isolates came from different regions, indicating that the geographical origin of *P. pini* isolates had no effect on their decomposing ability. This is in line with the theory of the genetic structure of the pathogen's population, which is typically composed of a range of many different physiological races, with a few dominant ones [Burdon 1993]. The area from which the isolates originated (100 000 km^2) may, however, have been too small to allow identification of the effects of isolate origin on decomposing ability.

Most weight loss during decomposition occurred, on average, in the spruce (1.90 g), while the least weight loss was in the larch (0.94) (table 2). Larch wood is considered to be more durable wood than the moderately durable pine and spruce wood. The basic macrostructural characteristic of wood is its density, a value expressed as a synthesis of many traits of wood tissue structure. Although there is no marked correlation between density and natural stability of wood [PN-EN 350-2[2000]], many properties of wood, including resistance to fungal decomposition, depend on density [Szczepkowski 2010]. The decomposition could also have been influenced by wood density, which according to Zenkteler and Woźniak [1965] in spruce is 0.47 g cm^{-3}, in pine 0.52 g cm^{-3}, and in larch 0.59 g cm^{-3}. The heartwood of larch is also characterised as having the lowest pH value among the three species (pH 4.27); the pH of pine heartwood is 4.58, and that of spruce is the highest among these three species at 5.04 [Zenkteler, Woźniak 1965]. Wood decomposition by *P. pini* is also proportional to cellulose content in heartwood, which in spruce amounts to 57.04%, in pine to 51.9% and in larch to 34.00% [Nikitin 1962; Levin et al. 1978], as well as to lignin content, which in spruce is 27.14%, pine 26.73% [Fengel, Wegner 1989] and larch 21.6% [Tsvetaeva et al. 1958]. Weight loss in the spruce wood samples caused by most of the test isolates, expressed as %, exceeded the lignin content of the wood. This suggests that both wood components were decomposed. Blanchette [1982] reported that *P. pini* first causes decomposition of the lignin, as also stated by Otjen et al. [1987]. Mańka [2005] later stated that both of these wood components decomposed at a uniform rate. Studies on wood decomposition by other fungal species showed pine and spruce wood exposed to the action of *Coniophora cerebella* and *Merulius lacrymans* lost 24–55% of their minerals; the loss of elements was greatest in spruce wood, and less in pine [Ważny 1968]. In the present study, the decomposition rate was found to be relatively high, ranging from 6.62% (larch) to 42.19% (spruce). In contrast, Zarzyński [2009] reported that the heartwood of different tree species was decomposed by *P. pini* to a very limited extent under laboratory conditions, and suggested the fungus had potential for development only in live trees. This seems rather unlikely since the fungus develops in heartwood, which is composed of dead elements. Most probably the mycelium needs adequate moisture content for its development, as evidenced by the occurrence of live fruit bodies on lying pine logs [Nowińska et al. 2009]. The results of the present experiments may also have been influenced by the method of sample preparation, since the wood was wetted after drying and weighing, which would have simulated natural conditions and probably promoted colonisation by the mycelium. The primary causes for the differences in the rate of wood decomposition in a given tree species by the mycelium of a specific fungal species may comprise many factors, the most important being the origin of the mycelium strain and the provenance of the wood (both geographical and within the trunk) [Zarzyński, Andres 2010]. Heartwood extractives play a key role in natural durability, beside lignification and growth characteristics [Zabel, Morrell 1992]. Gierlinger et al. [2004] proved then the concentration of phenolics was strongly correlated with decay resistance in the wood of larch. The isolates of *P. pini* used in the present experiment came mostly from the northern part of Poland and the isolate with the greatest wood decomposition capacity came from north-eastern Poland [Szewczyk et al. 2014]. The presence of seven homogeneous groups, shown by statistical analysis, shows that the geographic origin of *P. pini* isolates had no effect on the wood-decomposition trait.

Conclusions

The heartwood of the larch was the most resistant of the three tree species to decomposition by *Porodeadeala pini*. There was no correlation between the geographical origin of the *P. pini* isolates and their wood decomposition capacity. Younger stands in which *P. pini* has been reported should be reconstructed in order to avoid greater losses.

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List of standards

PN-EN 350-1:2000 Trwałość drewna i materiałów drewnopochodnych. Naturalna trwałość drewna litego. Wytyczne dotyczące zasad badania i klasyfikacji naturalnej trwałości drewna (Durability of wood and wood-based products – Natural durability of solid wood. Guide to the principles of testing and classification of the natural durability of wood)

PN-EN 350-2:2000 Trwałość drewna i materiałów drewnopochodnych. Naturalna trwałość drewna litego. Wytyczne dotyczące naturalnej trwałości i podatności na nasycanie wybranych gatunków drewna mających znaczenie w Europie (Durability of wood and wood-based products – Natural durability of solid wood. Guide to natural durability and treatability of selected species of importance in Europe)