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THE CONNECTION BETWEEN CHOSEN PHENOLIC COMPOUNDS OCCURRING IN WOOD AND THE RANGE OF TROPHIC ABILITIES OF BIRCH BRACKET (*PIPTOPORUS BETULINUS* (BULL.) P. KARST.)

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SYNOPSIS. The connections between some phenolic compounds occurring in wood of *Betula pendula* Roth. and trophic abilities of *Piptoporus betulinus* (Bull.) P. Karst.) were examined. 2-Furaldehyde, 2,6-Dimethoxyphenol, Vanillin, Anisic acid and Koniferol were used for artificial saturation of wooden samples based on vacuum method. Then the different samples were placed in the laboratory conditions on the mycelium of *P. betulinus* for 30, 60 and 90 days. The losses of their mass, compared with control samples impregnated only with distilled water, allowed to examine the true properties of tested substances. The experiment showed that all of them were able to stop the growth of the mycelium of *P. betulinus*. The most promising seemed to be Anisic acid and 2-Furaldehyde. The efficiency of their work was so obvious that there is a possibility to use them in the future for practical wood protection against decay caused by *P. betulinus* and maybe other species of fungi responsible for brown type of wood decomposition.

KEY WORDS: wood protection, *Betula pendula*, *Piptoporus betulinus*, 2-Furaldehyde, 2,6-Dimethoxyphenol, Vanillin, Anisic acid, Koniferol

INTRODUCTION

Birch bracket (*Piptoporus betulinus* (Bull.) P. Karst.) is a common species of conk occurring in boreal and sub-boreal zone of Europe, Asia and North America. It causes an intensive brown rot of wood. It belongs to group of classic monophagous species. According to KOTLABA (1984) it occurs nearly exclusively on woods from *Betula* genus (in 98.5% of cases). It was only sporadically reported from *Fagus sylvatica* L. and *Sorbus aucuparia* L. It should be expected that the main reason for its narrow trophic preferences may be the composition of birch wood, especially the presence or absence of some specified chemical substances that

could stimulate or inhibit the growth rate of mycelium of *P. betulinus*. According to present knowledge (Rayner and Boddy 1988, Theander and Lundgren 1989, Charlwood and Rhodes 1990, Davin et al. 1992, Wallace and Fry 1994, Kermasha et al. 1995, Obst 1998, Evensen et al. 2000) these substances should be searched between natural phenolic compounds occurring in trace quantities in wood of every tree species. Based on the results of chemical analysis of wood of *Betula pendula* Roth. obtained using chromatographic methods in laboratory of Section of Environment Chemistry of National Fund of Environment Protection in Warsaw, it was proved that there are at least 47 phenolic compounds in this material and 38 of them were able to be recognized (Zarzyński 2009 a). Comparing the level of each of these substances with the results of similar wood analysis (conducted) made parallel for 24 another tree species some chemical compounds were chosen occurring in the wood of *Betula pendula* in extremely small and relatively huge quantities (comparable with wood of another tested tree species). It was assumed that in the first group might be some potential inhibitors of *P. betulinus* mycelium's growth, and in the second group – some stimulators of this biochemical process. The inhibitors could be: 2-Furaldehyde, Furfyryl alcohol, 2--Propenamide,N-(aminocarbonyl), 2-Cyclopentene-1-on-2-hydroxy-3-methyl, 2,6--Dimetoxyphenol (Syringol), Vanillin, Guaiacylo acethone, Acethylobenzoic-2,5--dimethoxy acid, 2,6-Dimethoxy-4(propenyl)phenol, Acetosyringolne + ester of 4--Hydroxy-3-Methoxymethylo-Acetylosalicylic acid and Alpha-Lapachone. On the other hand, the potential stimulators could be: Cyclohexanone, Anisic acid, Koniferol and 1-(2,4,6-trihydroxyphenyl)-2-pentanone. Moreover, the comparative analysis between the contents of particular phenolic compounds in wood of several tree species and the rate of decomposition of this wood by mycelium of *P. betulinus* in laboratory conditions (where it shows much wider range of trophic abilities than in nature) was carried out by Zarzyński (2009 c). Based on the results some chemical substances which quantity level in wood of particular tree species increases (in statistically confirmed way) parallel to the range of the decomposition of this wood by mycelium of *P. betulinus* (i.e.: potential stimulators of its growth) were found. They were as follows: 2-Methoxy-6-vinylophenol, Methoxybenzenodiol, 3,4--Dimethoxybenzoic acid, 2 Isolapachol, 10-H-phenoxasilin-10,10-dimethyl, Alpha--Lapachone and 1-(2,4,6-trihydroxyphenyl)-2-pentanone. The chemical compounds that quantity level in wood of particular tree species decreases (in statistically confirmed way) parallel to the range of the decomposition of this wood by mycelium of *P. betulinus* (i.e.: potential inhibitors of its growth) were not found.

Among the substances listed above five phenolic compounds were chosen. Three of them might act as inhibitors of *P. betulinus* mycelium's growth, and two others are potential stimulators of this process. All of them were tested in laboratory conditions. Wooden samples impregnated by their solutions and then, they were placed on mycelium of testing fungus (controlled decomposition). The main aim of this experiment was to check the working mechanism of chosen phenolic compounds and – in case of potential mycelium's growth inhibitors – to test their usability for wood protection against decomposition by *P. betulinus* and other fungi species causing serious damages in forestry and wood management.

MATERIALS AND METHODS

Wood samples made from stored hardwood of *B. pendula* were used for laboratory tests. They were prepared in Section of Wood Science of Division of Wood Science and Wood Protection of Warsaw University of Life Science. Totally ca 150 samples were made. They were collected from one wood stem parallel to the grain. The size of each sample was 50 × 25 × 15 mm. Every sample was precisely measured using slide callipter accurate to 0.1 mm and then its dimension was calculated. After measuring, samples were dried during 72 hours in electric drying apparatus in the temperature of 105°C (first, they were initially dried during 24 hours in the temperature of 60°C) to the absolutely dry state. Then, they were weighed using laboratory scales exact to a 0.001 g and then their densities were calculated. To the experiment only the samples with similar value of this characteristic were qualified (samples of widely differed density were discarded, the allowable divergence of sample's density was 0.017 g/cm^3).

20 cm^3 of agar-maltose-wort medium (composition: Difco's agar – 20 g, Difco's maltose extract – 15 g, distilled water – 750 cm^3, wort – 250 cm^3) was poured to sterilized (autoclaved in temperature of 121°C for 30 min) 1500 cm^3 volume glass pots (Weck's pots). The wort used in all experiments came from Jabłonowo Brewery and was collected from the same part and has the same chemical composition, which means the medium could be considered as standardized. After 24 h inoculates of *P. betulinus* originated from the collection of pure mycelium from Section of Forest Phytopathology and Micology of Warsaw University of Life Sciences were grafted. Before using in this experiment the mycelium was passed on birch hardwood for 4 months for revitalization and full wood decaying abilities regaining. In every pot two inoculates were placed in the opposite edges of the pot's bottom.

After inoculation, flasks were put in the incubator in the temperature of 21°C. After another 14 days (the growth rate of mycelium of *P. betulinus* is relatively high, and all the surface of pot's bottom were covered), two wood samples impregnated with 0,1% water solutions of tested phenolic compounds (that were suspected to be natural inhibitors or stimulators of *P. betulinus* mycelium growth) were placed on glass rests and then were put in every flask on the growing mycelium. They were as follows: 2-Furaldehyde, 2,6-Dimethoxyphenol, Vanillin, Anisic acid and Koniferol. Comparison of quantities of these phenolic compounds identified in the wood of *B. pendula* and in the wood of 24 chosen tree species is showed in Table 1. The vacuum method of saturation was chosen with use of SHELLAB type 1425 vacuum dryer connected with BUCHI V-700 vacuum pump equipped with V-850 vacuum controller. The retentions of chemical substances introduced into the wood were calculated using the following formula:

$$R = (M_2 - M_1) \cdot C_p \cdot 0.01 \cdot V^{-1} \quad [\mathrm{g \cdot m^{-3}}]$$

where: R – retention of chemical compound in sample [g·m^{-3}],
M_1 – weight of sample before saturation [g],
M_2 – weight of sample after saturation [g],

V – sample's volume [m^3],
C_p – solution's percentage concentration [%].

Table 1. Quantities of phenolic compounds identified in the wood of *Betula pendula* in comparison with wood of 24 chosen tree species (according to Zarzyński 2009 a)

Species of tree	Contents of chosen phenolic compounds in the wood [µg/kg]				
	2-Furaldehyde	2,6-Dimethoxyphenol	Vanillin	Anisic acid	Koniferol
Abies alba Mill.	2.5	0.9	45.4	1.1	1.2
Acer pseudoplatanus L.	8.6	6.3	97.0	1.0	22.2
Alnus glutinosa (L.) Gaertn.	5.1	1.1	13.9	1.1	2.8
Aucoumea klaineana Pierre	8.8	6.9	84.7	0.7	42.2
***Betula pendula* Roth.**	**0.7**	**0.4**	**0.5**	**46.7**	**237.0**
Carpinus betulus L.	21.8	12.3	37.7	0.9	3.7
Chlorophora excelsa Benth. & Hook	7.1	3.1	31.2	0.6	28.2
Fagus sylvatica L.	8.9	8.8	39.2	0.8	7.2
Fraxinus excelsior L.	8.9	11.0	74.5	0.9	11.5
Hymnaea sp.	27.6	22.1	72.3	0.9	89.2
Intsia bakeri Prain	7.2	6.3	31.7	10.7	19.7
Larix decidua Mill.	2.3	0.8	39.6	0.8	0.8
Millettia laurentii De Wild.	7.5	3.9	38.0	0.5	43.3
Nauclea trillesii Merill	4.8	6.0	57.1	0.4	44.8
Picea abies (L.) H. Karst.	5.7	2.6	82.1	1.0	33.4
Pinus sylvestris L.	2.7	1.2	42.8	0.9	0.9
Populus tremula L.	3.5	2.0	40.3	0.7	0.7
Pterocarpus soyauxii Taubert	4.8	2.4	0.7	0.7	1.0
Quercus robur L.	18.2	1.7	48.1	0.8	6.4
Quercus rubra L.	11.0	2.4	34.5	0.6	21.0
Salix fragilis L.	5.8	2.0	63.5	1.0	1.9
Tabebuja sp.	6.1	2.4	63.7	211.2	33.1
Tilia cordata Mill.	5.4	3.9	35.8	1.1	11.6
Triplochiton scleroxylon K. Schum.	4.0	2.2	108.7	0.1	0.8
Ulmus laevis Pall.	5.6	1.4	0.2	0.6	22.9

Part of samples intended to be used as a control material were impregnated only by distilled water without any others chemical compounds. In one flask only the samples of the same variant were placed (control samples and samples impregnated by individual substances were not mixed).

Then all flasks were once again put into the incubator. The samples were exposed to fungus activity for 30, 60 or 90 days. For every variant of the experiment (different tested substances and times of exposure) 6 samples put in 3 flasks were

examined. After given time every sample was put out, cleaned from the rests of mycelium and once again dried and weighted exact to a 0.001 g. The loss of weight between first and second weighting showed the extent of wood decayed in every sample. Then it was described proportionally using following formula:

$$\Delta M = (M_0 - M_1) \cdot M_0^{-1} \cdot 100 \quad [\%]$$

where: ΔM – percentage weight loss of sample [%],
M_0 – weight of sample before the experiment [g],
M_1 – weight of sample after the experiment [g].

The comparison of the destruction range of wood from samples impregnated by particular tested phenolic compounds with wood from control samples (impregnated only by distilled water) allowed to confirm or reject the theory about potential properties of these substances to act as inhibitors or stimulators of growth of *P. betulinus* mycelium, which means – to stop or to accelerate the decay of *B. pendula* wood caused by this fungus.

To confirm the results on basis of one-way anova and multiple range test (LSD method) the differences among weight loss of wood samples impregnated by every of 5 tested phenolic compounds and control wood samples (impregnated only by water) were tested. It was carried out separately for 30, 60, 90 days of mycelium exposure. The analysis were done at the 95% confidence level.

RESULTS

In total, in all variants of the experiment, 108 wooden samples put in 54 pots were used. Their average retention and percentage loss of mass are shown in Table 2, and the results of statistical analysis in Table 3. After 30 days of exposure on mycelium of *P. betulinus* the least decomposed were wood samples impregnated by 2,6-Dimetoxyphenol (the average loss of mass of samples was 0.12%) and 2--Furaldehyde (0.15%). The most decomposed were wood samples impregnated by water (control samples) (0.79%) and Koniferol (0.73%).

In 60-days variant of the experiment the least decomposed were wood samples impregnated by Anisic acid (0.26%) and 2-Furaldehyde (0,32%). The most decomposed were control samples (10.31%) and samples impregnated by Koniferol (2.40%).

After 90 days of exposure on mycelium the least decomposed were wood samples impregnated by Anisic acid (0.40%) and 2-Furaldehyde (0.43%). The most decomposed were samples impregnated by distilled water (21.29%) and Koniferol (2.68%).

At the assumed confidence level, statistically important differences in the range of wood decomposition were found against samples impregnated by every tested phenolic compounds (the only exception was Koniferol in case of 30-days variant of experiment) and control samples impregnated only by distilled water.

Table 2. Average retention and percentage loss of mass of *Betula pendula* wood samples saturated by solutions of chosen phenolic compounds after 30, 60 and 90 days exposition on mycelium of *Piptoporus betulinus*

Tested phenolic compound	Average retention of tested compounds [g/m^3]	Average loss of mass of sample [%]		
		after 30 days	after 60 days	after 90 days
2-Furaldehyde	96.11	0.15	0.32	0.43
2,6-Dimethoxyphenol	99.46	0.12	0.58	0.77
Anisic acid	96.36	0.19	0.26	0.40
Vanillin	94.21	0.20	0.54	0.71
Koniferol	106.11	0.73	2.40	2.68
Control (water)	–	0.79	10.31	21.29

Table 3. Results of the variance of wood decay range between samples saturated by every of five tested phenolic compounds and control samples (saturated only by water) after 30, 60 and 90 days exposition on mycelium of *Piptoporus betulinus* (the grey color indicates statistically significant differences – by LSD tests at the 95% confidence level)

Tested phenolic compound	Coefficient of statistical significance of differences between mass losses of tested and control samples		
	after 30 days	after 60 days	after 90 days
2-Furaldehyde	−0.642597	−9.98967	−20.8523
2,6-Dimethoxyphenol	−0.664174	−9.73093	−20.5153
Anisic acid	0.59745	10.048	20.8903
Vanillin	0.59234	9.77264	20.5741
Koniferol	−0.0612089	−7.91042	−18.6062

The average retention of wood samples – depending on tested phenolic compound was between 94.21-106.11 g/m^3. The mean retention level was 98.45 g/m^3.

DISCUSSION

According to the results presented above, it can be clearly seen that all of the tested phenolic compounds naturally existing in wood show the abilities to stop the growth of *P. betulinus* mycelium in birch wood. Therefore, there is a probability that the narrow range of trophic preferences of this fungus (that in its natural environment occurs practically only on trees from *Betula* genus) is caused mostly by extremely low concentration (comparable to wood of other tree species) of natural phenolic compounds in birch wood, such as 2-Furaldehyde, 2,6-Dimetoxyphenol and Vanillin. These substances, artificially added in laboratory conditions to the

wood are practically able to stop the growth of the mycelium of *P. betulinus*. Their activity seems to be relatively permanent – it was clearly proved in all variants of the experiment including 90 days period of samples exposition on mycelium of testing fungus.

The laboratory tests of Anisic acid and Koniferol gave totally different results than it was expected before. These substances occur in wood of *B. pendula* in extraordinary huge quantities. The concentration of Anisic acid is in this wood more than 40 times higher than in wood of any other tested european tree species. The contents of Koniferol in birch wood is extremely high and it is nearly 3 times higher than in wood of any other known both european and exotic tree species (in case of european trees it is more than 6 times higher). Hence it was expected that these substances might act as development stimulators of *P. betulinus* mycelium (i.e.: as catalysts of its growth), and so being one of the reason of narrow trophic preferences of this fungus. However, laboratory tests showed that both of these phenolic compounds are able to decrease the growth of mycelium and to stop the wood decay caused by *P. betulinus*. This kind of activity was clearly visible especially in case of Anisic acid that protected the wood samples against decay nearly permanent in every time variant of the experiment maintaining the decomposition on practically imperceptible level.

The discovered properties of the tested phenolic compounds naturally existing in wood described above show that these substances may play complex and multiple roles in wood protection against decay caused by fungi. It is possible that some other still not-identified chemical components found in wood (only 38 phenolic compounds were identified in wood in total – Zarzyński 2009 a) might work as inhibitors or stimulators of growth of *P. betulinus* and other fungi species, too.

Obviously, some other reasons for *P. betulinus* trophic preferences might be also important. One of them might be the specific content of hemicelluloses in wood of *B. pendula* comparatively to the wood of other tree species (Kin 1980). The most important are polysaccharides: 4-O-Methyl-Glucoroxylan and Glucomannan. The main polysaccharide is 4-O-Methyl-Glucoroxylan acetate having molecule built from the rests of D-xylose, 4-O-Methyl-Glucorone acid and acetyl groups. Relatively high concentrations of these substances in wood of *F. sylvatica*, that is sporadically reported as natural host of this fungus might be the trace indicating their importance for *P. betulinus* mycelium development. However, this theory should be first confirmed by additional laboratory experiments.

Based on results presented in this and some other experiments (Zarzyński 2009 b, c, d, Zarzyński and Andres in press a, b) it seems that the decay of wood caused by fungi is a typical example of biochemical process conditioned mostly by chemical composition of wood being the natural medium for particular species of fungi. The main role play probably precisely specified phenolic compounds occurring in trace quantities in wood. However, these correlations have extremely complicated character and to know them better it would be necessary to carry out more research and additional experiments. These works seem to be very important, because the deep knowledge about the influence of particular phenolic compounds naturally existing in wood, on wood decaying fungi might be the best way to work-out some new effective and environment-friendly agents use-

ful for protection of wood allowing to decrease huge losses made by this group of organisms in forestry and wood management.

CONCLUSIONS

1. One of the reasons of narrow range of *Piptoporus betulinus* trophic preferences might be specific composition of birch wood that contains extremely small quantities (comparatively to the wood of other tree species) of some phenolic compounds like: 2-Furaldehyde, 2,6-Dimetoxyphenol (Syringol) and Vanillin.
2. All tested phenolic compounds having potential properties as *P. betulinus* mycelium's growth inhibitors (2-Furaldehyde, 2,6-Dimetoxyphenol and Vanillin) artificially introduced to the wood really work as inhibitors of this process efficiently protecting wood against decomposition.
3. Tested phenolic compounds (Anisic acid and Koniferol), that – theoretically – might work as stimulators of *P. betulinus* mycelium's growth artificially introduced to the wood of *Betula pendula* seem to work as inhibitors of this process efficiently protecting wood against decomposition.
4. It is possible that some phenolic compounds naturally existing in wood like: 2-Furaldehyde, Syringol, Vanillin, Anisic acid and Koniferol might be used in future for practical protection of wood against decomposition caused by fungi. The main advantages are high efficiency in stopping the growth rate of some species of wood decaying fungi and their natural origin which makes them safe for the environment.

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