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THE CONNECTION BETWEEN CHOSEN PHENOLIC COMPOUNDS OCCURRING IN WOOD AND THE RANGE OF TROPHIC ABILITIES OF QUININE FUNGUS (*LARICIFOMES OFFICINALIS* (VILL.) KOTL. ET POUZAR)*

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Abstract. Quinine fungus (*Laricifomes officinalis*) is a typical monophagic species decaying in Central Europe nearly exclusively hardwood of trees from *Larix* genus. The main reason of these narrow trophic preferences is probably the specific chemical composition of Larch wood and, especially, the lack of some phenolic compounds that might play an important role as natural inhibitors of Quinine fungus growth. To improve this theory five different phenolic compounds selected basing on results of previous investigation and quantity analysis of phenolic compounds in wood of 25 different tree species were tested in laboratory conditions. They were as follows: 3',5'-dimethoxyacethophenone, furanone, 2,6-dimethoxy-4(propenyl)phenol, syringe aldehyde and 1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl. Wood samples saturated by solutions of this substances were exposed on the mycelium of *L. officinalis*. Results of this experiment compared with the range of control sample's decay showed that all the tested phenolic compounds caused the decrease of the range of wood decomposition by *L. officinalis*. It means that they work as natural growth inhibitors of this fungus and probably are one of the reasons of its specific trophic preferences.

Key words: wood decay, *Laricifomes officinalis*, 3',5'-dimethoxyacethophenone, furanone, 2,6-dimethoxy-4(propenyl)phenol, syringe aldehyde, 1,4-buthanodiamine-2,3--dimethoxy N,N,N',N'tetramethyl

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INTRODUCTION

Quinine fungus (*Laricifomes officinalis* (Vill.) Kotl. et Pouzar) is one of the rarest tree fungi (conks) occurring on the area of Poland. There are only five localities known with 27 fruit bodies of this species growing on 17 trees [Piętka and Szczepkowski 2004]. It shows an interesting case of that fungus extermination caused by human activity connected with planned harvesting of its fruit bodies. The reason of this were wide medical properties of *L. officinalis* well-known and often described as well in old [among others: Syreński 1613, Kluk 1808] as in present medical and mycological literature [Muszyński 1954, Knopf 1984, Semerdžieva and Veselský 1986].

Laricifomes officinalis is distributed widespread in the sub-boreal zone. It occurs in almost all Europe, Canada, United States, Russia, Mongolia, India, China, Korea, Japan and Morocco [Ryvarden and Gilbertson 1993, Chlebicki 2001, Chlebicki et al. 2003]. It is an example of an extremely interesting species of fungi also because of its very narrow range of trophic preferences. In Europe, excluding Spain it is able to use as a host practically only trees from *Larix* genus [Domański et al. 1967, Chlebicki 2001]. In Spain and outside Europe it is found also on trees from the following genus: *Abies*, *Cedrus*, *Picea*, *Pinus*, *Pseudotsuga* and *Tsuga* [Kotlaba 1984, Ryvarden and Gilbertson 1993]. The answer to the question why the great majority of European population of *L. officinalis* is not able to destroy in nature wood of trees except of *Larix* genus is still a mystery. It is suspected that the reason of so narrow range of their trophic preferences is quite specific chemical composition of Larch wood, and especially the lack of some chemical compounds that could play an important role as natural growth inhibitors of this fungus. Basing on present knowledge these substances should be looked for against group of phenolic compounds naturally occurring in wood [Charlwood and Rhodes 1990, Davin et al. 1992, Evensen et al. 2000, Kermasha et al. 1995, Obst 1998, Rayner and Boddy 1988, Theander and Lundgren 1989, Wallace and Fry 1994]. The aim of this work was a trial of identification some of them on the basis of the results of chemical investigation and quantity analysis of Larch wood and laboratory experiments with wood samples saturated with solutions of chosen phenolic compounds. Better knowledge of biochemical fundaments of *L. officinalis* trophic abilities range might be a key to improve in future methods of its artificial inoculation (trials of its active protection were already made – Piętka and Grzywacz 2005) and might help to protect this extremely rare and original fungus against extinction.

As a comparative materials the results of chemical analysis of wood made by chromatography methods in laboratory of Section of Natural Environment Chemistry of National Fund of Environment Protection in Warsaw were used [Zarzyński 2009 a]. Basing of them a group of five phenolic compounds occurring in Larch wood in extremely tiny quantities (comparing with the wood of other tree genus) was selected. It was assumed that against them might be natural inhibitors of *L. officinalis* growth being the main reason of narrow trophic abilities range of European (including Polish) populations of this fungus. To verify this theory all these chemical compounds were classified to further laboratory experiments.

MATERIALS AND METHODS

Basing on the comparison of the chemical analysis results of wood from 25 different tree species five phenolic compounds occurring in the wood of *Larix decidua* Mill. in extremely tiny quantities (comparing to the wood of other tested tree species) were found [Zarzyński 2009 a]. They were as follows:

- 3',5'-dimethoxyacethophenone – occurs in wood of *L. decidua* in concentration of 0.8 μg/kg (this is the smallest concentration of this substance in wood for all 25 tested tree species) when average concentration for wood of all 25 tested tree species equals 15.30 μg/kg
- Furanone – occurs in wood of *L. decidua* in concentration of 1.5 μg/kg (the smallest concentration in group of all tested tree species). The average concentration for 25 tested tree species equals 6.54 μg/kg
- 2,6-dimethoxy-4(propenyl)phenol – occurs in wood of *L. decidua* in concentration of 0.8 μg/kg (the smallest concentration in group of all tested tree species after *Betula pendula* Roth. – 0.4 μg/kg), when average value for wood of all 25 tested tree species is 4.56 μg/kg
- Syringe aldehyde – occurs in wood of *L. decidua* in concentration of 15.0 μg/kg (the smallest concentration in group of all tested tree species after *Abies alb*a Mill. – 10.4 μg/kg and *Betula pendula* – 11.0 μg/kg). The average value for the wood of all 25 tested tree species equals 112.19 μg/kg
- 1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl – occurs in wood of *Larix decidua* in concentration of 2.2 μg/kg (the smallest concentration in group of all tested tree species after *Populus tremula* L. – 1.0 μg/kg, *Triplochiton scleroxylon* K. Schum. – 1.4 μg/kg, *Alnus glutinosa* Gaertn. – 1.7 μg/kg, *Chlorophora excelsa* Benth. et Hook – 1.8 μg/kg, *Pinus sylvestris* L. 1.9 μg/kg and *Salix fragilis* L. 2.1 μg/kg). The average concentration for wood of all 25 tested tree species is 15.55 μg/kg.

Water solution of phenolic compounds described above in concentrations of 0.1% were used in the following experiment for wood samples saturation.

For laboratory tests wood samples made from stored hardwood of *L. decidua* were used. 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. 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 shape. Immediately after putting out they were weighed on the laboratory scales exact to a 0.001 g and then their densities were calculated. To the experiment were qualified only samples with similar value of this characteristic (samples of widely differed density were discarded).

To sterilized (autoclaving in temperature of 121°C for 30 minutes) 1500 ml volume glass pots 20 ml of agar-maltose-wort medium (composition: Difco's agar – 20 g, Difco's maltose extract – 15 g, distilled water – 750 ml, wort – 250 ml) was poured. 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 recognised as standardized. After 24 hours inoculates of *L. officinalis* origi-

nated from the collection of pure mycelium from Section of Forest Phytopathology and Micology of Warsaw University of Life Sciences were grafted. The mycelium were collected by Piętka [Piętka and Szczepkowski 2004] with the agreement of the Chef of Polish Nature Conservancy Department (*L. officinalis* is strictly protected in Poland). Its authenticity was proofed by DNA tests made by PCR-RFLP method [Piętka and Grzywacz 2005]. Before using in this experiment the mycelium was passed on Larch hardwood during the period of four months for revitalization and full wood decaying abilities regaining.

After inoculation flasks were put in the incubator in the temperature of 21°C. After another 21 days in every flask on the growing mycelium two wood samples saturated of 0.1% solutions of tested phenolic compounds (that were suspected to be natural inhibitors of *L. officinalis* mycelium growth) placed on glass rests were put. The saturation was conducted in the laboratory of Section of Wood Protection of Division of Wood Sciences and Wood Protection of Warsaw University of Life Sciences. The vacuum method of saturation were 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:

$$R = (M_2 - M_1) \cdot C_p \cdot V^{-1}, \text{ g} \cdot \text{m}^{-3},$$

where:

R – retention of chemical compound in sample, $g \cdot m^{-3}$,
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, %.

Part of samples intended to be used as a control material were saturated only by distilled water without any others chemical compounds.

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) six samples put in three flasks were examined. After the assumed time every sample was put out, cleaned from the remains of mycelium and once again dried and weighed exact to a 0.001 g. The loss of weight between first and second weighing showed the extent of wood decayed in every sample. Then it was described proportionally using the following formula:

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

where:

ΔM – percentage sample weight loss, %,
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 saturated by particular tested phenolic compounds with wood from control samples (saturated only by distilled water) allowed to improve or reject the theory about potential properties of these substances to inhibit growth of *L. officinalis* mycelium, which means – to stop the decay of *L. decidua* wood caused by this fungus.

Totally, in all variants of the experiment 108 wooden samples put in 54 pots were used. On basis of one-way anova and multiple range test (LSD method) the differences among weight loss of wood samples saturated by every of five tested phenolic compounds and control wood samples (saturated only by water) were tested. It was carried out separately for 30, 60, 90 days of mycelium exposure. The analysis were done at 95% confidence level.

RESULTS

Laboratory experiments results of controlling decay of wood samples saturated by solutions of chosen phenolic compounds (with their average retention level) were showed in Table 1. Table 2 shows the 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) for every period of exposure on mycelium of *L. officinalis*.

The average retention of every tested chemical compounds was from 97.13 to 117.21 $g \cdot m^{-3}$. Basing the results after 30 days of the exposure on the mycelium the most decomposed wood samples were control samples saturated only by distilled water (the average loss of their mass was 0.39%). In case of wood samples saturated by five tested phenolic compounds the average losses of their mass were smaller (0.23--0.36%), but the differences turned out to be not statistically significant.

Table 1. Average retention and mass loss of *Larix decidua* wood samples saturated by solutions of chosen phenolic compounds after 30, 60 and 90 days of exposition on mycelium of *Laricifomes officinalis*

Tabela 1. Średnia retencja oraz ubytek masy próbek drewna *Larix decidua* nasyconego roztworami wybranych związków fenolowych po 30-, 60- i 90-dniowej ekspozycji na grzybni *Laricifomes officinalis*

Tested phenolic compound Testowany związek fenolowy	Average retention of wood samples Średnia retencja próbek g/m^3	Average mass loss of wood samples, % Średni ubytek masy próbek, %		
		30 days 30 dni	60 days 60 dni	90 days 90 dni
3',5'-dimethoxyacethophenone 3',5'-dimetoksyacetofenon	107.11	0.24	0.45	1.09
Furanone Furanon	97.13	0.36	0.77	1.01
2,6-dimethoxy-4(propenyl)phenol 2,6-dimetoksy-4-(propenylo)fenol	112.35	0.33	0.80	0.97
Syringe aldehyde 4-hydroksy-3,5-dimetoksy benzaldehyd	117.21	0.32	0.76	0.92
1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl 1,4-butanodiamina-2,3-dimetoksy N,N,N',N'tetrametyl	102.36	0.23	0.47	0.87
Control (water) Kontrola (woda)	–	0.39	0.79	3.59

Table 2. 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) for every period of exposition on mycelium of *Laricifomes officinalis* (the grey color indicates statistically significant differences – by LSD tests at the 95% confidence level)

Tabela 2. Wyniki analizy statystycznej istotności różnic ubytku masy drewna pomiędzy próbkami drewna nasyconymi każdym z pięciu testowanych związków chemicznych a próbkami kontrolnymi dla poszczególnych okresów ekspozycji próbek na grzybni *Laricifomes officinalis* (kolor szary oznacza zależność istotną pod względem statystycznym – test NIR przy poziomie ufności 95%)

Tested phenolic compound Testowany związek fenolowy	Water – Woda		
	30 days 30 dni	60 days 60 dni	90 days 90 dni
3',5'-dimethoxyacethophenone 3',5'-dimetoksyacetofenon	–0.151349	**–0.341976**	**–2.50504**
Furanone Furanon	0.0310529	0.0284012	**2.58413**
2,6-dimethoxy-4(propenyl)phenol 2,6-dimetoksy-4-(propenylo)fenol	–0.0626018	0.000455608	**–2.6217**
Syringe aldehyde 4-hydroksy-3,5-dimetoksy benzaldehyd	0.0737976	0.0325438	**2.67513**
1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl 1,4-butanodiamina-2,3-dimetoksy N,N,N',N'tetrametyl	–0.166095	**–0.324868**	**–2.72092**

After 60 days of exposure on the mycelium the most decomposed samples were control samples (the average loss of their mass was 0.79%). The wood from samples saturated by tested substances being potential inhibitors of *L. officinalis* growth was destroyed slower (except of 2,6-dimethoxy-4(propenyl)phenol – 0.80%). The wood from samples saturated by solutions of other tested phenolic compounds were decomposed much slower, especially 3',5'-dimethoxyacethophenone (0.45%) and 1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl (0.47%). In case of these samples statistically significant differences between them and control samples saturated only by water were found.

After 90 days of exposure on the mycelium the average loss of *L. decidua* wood from control samples was 3.59%. In case of samples saturated by solutions of tested phenolic compounds this value oscillated between 0.87 and 1.09%. The slowest range of wood decompositions were shown for samples saturated by solutions of 1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl (0.87%) and syringe aldehyde (0.92%). Statistically significant differences between the average losses of mass of control samples and samples saturated by all the tested substances were found.

DISCUSSION

Basing on the results showed above it is clearly seen that all phenolic compounds naturally existing in wood of tested European, introduced and exotic tree species used in this experiment have the abilities to decreasing growth of mycelium of *L. officinalis*

and wood decay caused by this fungus. So, there is a high probability that one of main reason of occurring this rare species in Central Europe exclusively on trees from *Larix* genus is extremely low concentration in their wood of substances like: 3',5'-dimethoxyacethophenone, furanone, 2,6-dimethoxy-4(propenyl)phenol, syringe aldehyde, and 1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl. Relatively low quantities of these phenolic compounds found in wood of the majority of other tree species being – together with *Larix* species – natural hosts of *L. officinalis* in Spain and outside Europe [Zarzyński 2009 a] seem to additionally improve this theory.

It should be stressed that all the tested phenolic compounds were artificially applied to Larch wood in quantities exceeding many thousand times their natural concentration in this material, however, the quantities were relatively small (the concentrations of the solutions used for wood saturation were only 0.1%). In case of 3',5'-dimethoxyacethophenone the quantity of artificially introduced chemical substance was ca 260 000 times higher than its natural concentration in wood of *L. decidua* (the average mass of air-dry Larch wood used in the experiment was ca 566 kg/m^3). For others tested substances their quantities applied to wood was higher then their natural concentration accordingly: 115 000 times (furanone), 250 000 times (2,6-dimethoxy-4(propenyl)phenol), 13 800 times (syringe aldehyde) and 82 000 times (1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl) [Zarzyński 2009 a].

The most important natural inhibitor of *L. officinalis* mycelium growth seems to be 1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl. This chemical substance applied to wood in relatively small (but exceeding over 82 000 times its natural concentration) quantities decreasing its decay caused by this fungus in clearly seen way improved by statistically significant differences showed against control samples. Similar effects are demonstrated by 3',5'-dimethoxyacethophenone. Other tested phenolic compounds seem to have the inhibiting properties not before than in later phase of wood destruction and it could be clearly seen only after 90 days of exposure on mycelium of *L. officinalis*.

The influence of 1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl on the rate of *L. officinalis* mycelium growth seem to be indirectly improved by the results of some previous experiments, too [Zarzyński 2009 b]. In laboratory conditions stored wood samples made from 25 different both European and exotic tree species were exposed on mycelium *L. officinalis*. Most of these species have never been reported as natural (even only occasional) hosts of this fungus. The experiment showed, that in *in vitro* condition the wood from *Fraxinus excelsior* L. was destroyed faster than wood from any other tree species. Samples made from wood of *L. decidua* and other coniferous species being potential natural hosts of *L. officinalis* were destroyed much slower. The wood of *F. excelsior* contains a lot of 3',5'-dimethoxyacethophenone, furanone, 2,6-dimethoxy-4(propenyl)phenol and syringe aldehyde but it is obviously not the barrier for *L. officinalis*. Only the concentration of 1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl in *F. excelsior* wood is relatively small (2.4 μg/kg) and could be compared with its quantity in wood of *L. decidua*. It might be another proof that this phenolic substance could play an important role in process of biochemical wood decomposition made by mycelium's hyphae of *L. officinalis* and may be a key to solve the mystery of specific trophic preferences showed by this unique fungus. On the other hand low concentration of 1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl is typical for many other tree species but their wood is never decayed by *L. officinalis* nor in nature or in laboratory conditions. So, it seems that some phenolic compounds occur-

ring in wood may work synergistically and have an effect on the mycelium growth of this and other fungi only were existing together and in strictly determined proportions.

It is not impossible, that trophic preferences and abilities of wood destroying presented by *L. officinalis* might be connected not only with lack in Larch wood some of phenolic compounds but also with other chemical factors. There are some facts in literature [Chadžaeva and Ušakov 1974] suggesting that wood decay caused by this fungus is connected with concentration in wood some hemicelluloses, especially arabinogalactan. This is a polymer substance built from simple carbohydrates occurring not only in wood from *Larix* genus, but also in some fruits, vegetables, wheat, coco nuts and red wine. It is used as a supplement of human diet and scientific researches are made about possibilities of using this substance in preventive treatment and therapy of some sickness [Larch... 2000]. The concentration of arabinogalactan in wood of *L. decidua* is very variable and oscillating between 4.5 even to 29.7% [Kin 1980]. It might be – together with co-existence of some phenolic compounds – the main key to the wood resistance (or their absence) against destroying by mycelium of *L. officinalis*, but to improve or reject this theory it is necessary to make another laboratory tests and experiments for research on the mechanism of trophic preferences of this original and already nearly extinct species of fungi.

CONCLUSIONS

One of reasons of narrow range of trophic preferences of Middle-European populations of *L. officinalis* is probably the specific chemical composition of wood of trees from *Larix* genus and, especially, the lack of some phenolic compounds like: 3',5'--dimethoxyacethophenone, furanone, 2,6-dimethoxy-4(propenyl)phenol, syringe aldehyde, 1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl.

Wood of *L. decidua* artificially saturated by water solutions of 3',5'-dimethoxyacethophenone, furanone, 2,6-dimethoxy-4(propenyl)phenol, syringe aldehyde and 1,4--buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl was destroyed in laboratory conditions much slower than wood from control samples saturated only by distilled water.

Comparing natural levels of particular phenolic compounds in wood with the results of laboratory tests it seems that main role in growth inhibition of mycelium of *L. officinalis* plays 1,4-buthanodiamine-2,3-dimethoxy N,N,N',N'tetramethyl probably working synergistically with other natural phenolic compounds occurring in wood. To investigate all these connections further laboratory tests and experiments should be made.

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ZWIĄZEK POMIĘDZY WYSTĘPOWANIEM W DREWNIE WYBRANYCH SUBSTANCJI FENOLOWYCH A ZAKRESEM ZDOLNOŚCI TROFICZNYCH MODRZEWNIKA LEKARSKIEGO (*LARICIFOMES OFFICINALIS* (VILL.) KOTL. ET POUZAR)

Streszczenie. Modrzewnik lekarski (*Laricifomes officinalis*) jest grzybem typowo monofagicznym rozkładającym na terenie Europy Środkowej wyłącznie drewno twardzielowe drzew rodzaju *Larix*. Prawdopodobnym wytłumaczeniem takich preferencji troficznych jest specyficzny skład drewna modrzewiowego, a w szczególności niedostatek w nim niektórych związków chemicznych mogących być inhibitorami wzrostu tego grzyba. W celu udowodnienia tej teorii przebadano w warunkach laboratoryjnych pięć związków o charakterze fenolowym wytypowanych na podstawie przeprowadzonej wcześniej jakościowo-ilościowej analizy chemicznej drewna 25 różnych gatunków drzew. Były to: 3',5'--dimetoksyacetofenon, furanon, 2,6-dimetoksyfenol, syringe aldehyde i 1,4-butanodiamina-2,3-dimetoksy N,N,N',N'tetrametyl. Nasycone nimi próbki drewna modrzewiowego zostały poddane w warunkach laboratoryjnych kontrolowanemu rozkładowi na grzybni *L. officinalis*. Wyniki doświadczenia, w porównaniu z tempem rozkładu próbek kontrolnych, pozwoliły na stwierdzenie, że wszystkie z badanych związków fenolowych wpływają na spowolnienie tempa rozkładu drewna przez *L. officinalis*, czyli są naturalnymi inhibitorami wzrostu jego grzybni i mogą być jednym z czynników warunkujących wąską specjalizację troficzną tego gatunku.

Słowa kluczowe: rozkład drewna, *Laricifomes officinalis*, 3',5'-dimetoksyacetofenon, furanon, 2,6-dimetoksyfenol, 4-hydroksy-3,5-dimetoksy benzaldehyd, 1,4-butanodiamina--2,3-dimetoksy N,N,N',N'tetrametyl

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