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Degradation of lipophilic wood extractive constituents in *Pinus sylvestris* by the white-rot fungi *Bjerkandera sp.* and *Trametes versicolor*

J. Dorado, T. A. van Beek, F. W. Claassen, R. Sierra-Alvarez

Abstract The white-rot fungi *Trametes versicolor* and *Bjerkandera spp.* are among the most frequent decomposers of angiosperm wood in forest ecosystems and in wood products in service. Wood extractives have a major impact on wood properties and wood utilization. This work evaluated the ability of two white-rot fungal strains (*Bjerkandera sp.* strain BOS55 and *T. versicolor* strain LaVec94-6) to degrade the main lipophilic extractive constituents in Scots pine (*Pinus sylvestris* L.). The time course of wood decay and wood extractive degradation was monitored in stationary batch assays incubated for eight weeks. The strains tested eliminated high levels of total resin, 34 to 51% in two weeks. Wood triglycerides were the most readily degraded extractive components (over 93% elimination in only two weeks). Free fatty acids and resin acids, which are potential fungal inhibitors, were also rapidly decomposed by the fungal strains. Sterols were used more slowly, nonetheless, the fungal degradation of this extractive fraction ranged from 50 to 88% after four weeks.

Introduction

Wood extractives, commonly known as resin or pitch, are lipophilic substances in wood consisting mainly of triglycerides, fatty and resin acids, waxes, fatty alcohols, sterols and steryl esters (Fengel and Wegener 1989). The chemical structure of typical lipophilic extractives in Scots pine wood is illustrated in Figure 1. Although extractives are a minor component, often constituting less than 10% of the total dry matter, they have a major contribution to the characteristics of wood.

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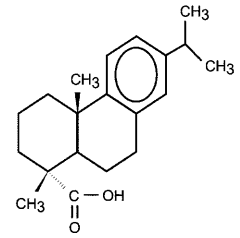
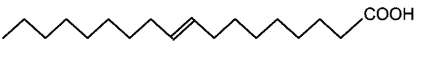


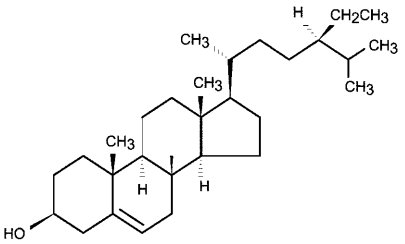
Dehydroabietic acid

(RESIN ACID)



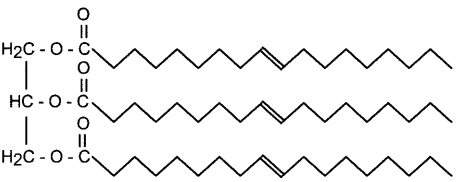
Oleic acid

(LONG CHAIN FATTY ACID)



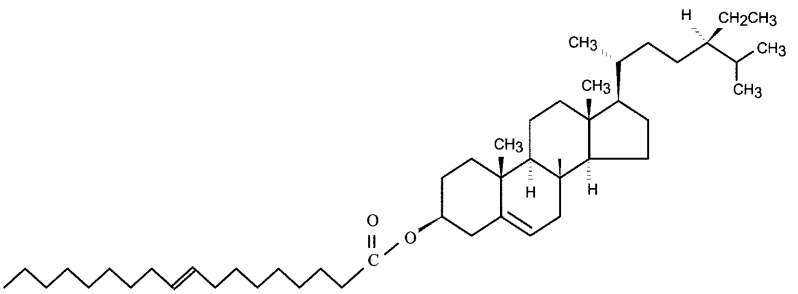
β-Sitosterol

(STEROL)



Triolein

(TRIGLYCERIDE)



β-Sitosteryl oleate

(STERYL ESTER)

Fig. 1. Chemical structure of typical extractive constituents in Scots pine wood

Extractives give wood its color, its odor and, to some extent, its physical properties (Imamura 1989). Of considerable significance is the role of wood extractives in protecting wood, both in the living tree and in wood products, from biological attack (Hart 1989). Extractives can also have an important effect on wood processing, where they may cause technical (Hillis and Sumimoto 1989) and environmental problems (Leach and Thakore 1976; O'Connor et al. 1992).

Biodegradation of extractives in freshly felled wood is generally attributed to the biological activity of sapstain fungi (Eaton and Hale 1993; Zabel and Morrell

1992). These microfungi are earlywood colonizers that are capable of assimilating the easily available carbon and nitrogen nutrients present in parenchyma cells, resin ducts and other woody tissues. Recent studies indicate that white-rot basidiomycetes are also very effective in the degradation of wood extractives (Dorado et al. 1998; Gutiérrez et al. 1998). Very little information is, however, available regarding the ability of white-rot fungi to decompose specific wood extractive constituents.

This research investigated the biodegradation of the various individual extractive constituents in Scots pine (*Pinus sylvestris* L.) wood by two different white-rot fungi, *Bjerkandera* sp. strain BOS55 and *Trametes versicolor* strain LaVec94-6. These fungal strains were selected as outstanding resin degraders in a previous screening program (Dorado et al. 1998). Improved understanding of the degradative ability of these fungi is of importance since *Bjerkandera* spp. and *T. versicolor* are among the most common and widely distributed fungal species colonizing decayed wood in forest ecosystems. As an example, *T. versicolor* and *Bjerkandera adusta* are, respectively, the second and fifth most widespread fungal species in The Netherlands (Nauta and Vellinga 1995). Both species are also among the six most frequently recorded fungal species in The Netherlands. Furthermore, *T. versicolor* is one of the most frequent degraders of hardwood timbers in service (Eaton and Hale 1993). New insights on the degradation of extractives by white-rot fungi can also facilitate the application of these microorganisms in biotechnological methods for wood deresination. The potential of fungal biotechnology to minimize extractive-related problems in the forest products sector is receiving increasing interest in recent years (Messner 1998).

Materials and methods

Microorganism and culture conditions

The white-rot strains *Bjerkandera* sp. strain BOS55 and *Trametes versicolor* strain LaVec94-6 were a gift from Dr. J.A. Field from the Division of Industrial Microbiology (Wageningen Agricultural University, Wageningen, The Netherlands). Strains were maintained on peptone yeast extract slants (per liter: 20 g glucose, 5 g peptone, 2 g yeast extract, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 5H_2O$, 15 g agar) and kept refrigerated at 4 °C until used. Agar plugs (6-mm diameter) from the leading edge of the mycelium grown on glucose malt extract plates (per liter: 15.0 g agar, 10.0 g of glucose and 3.5 g of malt extract) were used as inoculum for the experiments. All experiments were incubated statically under an air atmosphere at a temperature of 27 °C and a relative air humidity of 70%.

Wood

Freshly felled logs of Scots pine were supplied by the pulp and paper mill Parenco Newsprint BV (Renkum, The Netherlands). Logs were debarked, sapwood was separated from heartwood, and stored immediately at −20 °C to prevent biodegradation of wood extractives. Prior to each experiment, sapwood meal (particle size 0.425–1 mm) was prepared by milling and sieving sapwood chips.

Fungal treatment

Experiments were conducted in 500 mL, pre-sterilised loosely capped serum flasks supplied with 10 g oven dry (o.d.) wood. Distilled water was added to adjust the wood moisture to 70% (on wet wood weight basis). Flasks were inoculated with six mycelium-agar plugs. Abiotic controls (not inoculated) were run in

parallel. Wood moisture was frequently monitored and, if required, water losses were compensated by addition of sterilised water. Cultures were incubated during a total period of 8 weeks. All the experiments were performed in triplicate. Wood mass losses and total extractive losses were determined after 1, 2, 4, 6 and 8 weeks of incubation. Reduction of individual extractive constituents was determined in control and fungal treated samples incubated for 1, 2 and 4 weeks.

Analytical techniques

Total losses of wood mass were calculated gravimetrically from the difference of the initial dry mass and the dry mass of the treated wood sample. Dry mass was calculated indirectly by determining the moisture content (103 °C, overnight) in a wood sub-sample in order to avoid thermal modification of the wood constituents.

The total extractive content in wood was determined by Soxhlet extraction of dried wood samples (60 °C, overnight) with distilled acetone for 6 h. The acetone extract was concentrated and evaporated to dryness by rotatory evaporation at 45 °C, and then the extract was quantified gravimetrically. Wood extractive compounds were analysed by high-pressure liquid chromatography with evaporative light scattering detection (HPLC-ELSD) (Claassen et al. 1998). This analytical procedure enabled direct determination of individual extractive constituents in the free long chain fatty acid, sterol (triterpene alcohols), steryl ester (long chain fatty acid esters), diglyceride (fatty diacylglycerols) and triglyceride (fatty triacylglycerols) fraction. All resin acids (diterpene carboxylic acids) with the exception of dehydroabietic co-eluted as a single peak. Major constituents expected in the resin acid peak are pimaric, palustric, levopimaric, isopimaric, abietic and neoabietic acids, that are known to occur in the resin acid fraction of Scots pine (Fengel and Wegener 1989).

Results and discussion

Table 1 presents the average results obtained upon detailed chemical analysis of the extractives (acetone extracts) from Scots pine sapwood. The total extractive content was high and ranged from 4.5 to 4.7% of the o.d. weight of the wood. Triglycerides were the dominant fraction, representing 54% of the identified lipids. Free long chain fatty acids, resin acids, diglycerides and sterols were present in lower amounts. Steryl esters, which are generally very minor components in the extractives of Scots pine (Saranpää and Nyberg 1987), were not detected in the fresh wood samples.

Wood decay by *T. versicolor* and **Bjerkandera** sp. caused high eliminations of the total resin content amounting to 51% and 34%, respectively, in only two weeks. Considerably lower reductions of total extractives were generally attained with selected sapstain fungi in the genus *Ophiostoma* sp. in studies using loblolly pine (*Pinus taeda*) (Farrell et al. 1993; Fischer et al. 1994), spruce (*Picea abies*) (Fischer et al. 1994), radiata pine (*Pinus radiata*) (Kay et al. 1998) and aspen (*Populus tremuloides*) (Rocheleau et al. 1998), even when longer treatment times were applied. *Ophiostoma* spp. are among the most common sapstain fungi found in wood (Zabel and Morrell 1992). The effectiveness of the two white-rot fungi evaluated in our study to degrade softwood resin also compares favourably with that reported for other white-rot basidiomycetes. Research with the white-rot fungi *Ceriporiopsis subvermispora* showed a 34% reduction in the extractives from loblolly pine wood chips after four weeks (Fischer et al. 1994). In a related study, a 10 to 61% resin reduction in loblolly pine logs treated with the white-rot fungus

Table 1. Average chemical composition of the acetone extracts (expressed as % of the total oven dry wood) from Scots pine sapwood

Extractive constituent	Content (%)
Triglycerides[a]	
OOO	0.12 ± 0.03
LOO	0.37 ± 0.12
LLO	0.44 ± 0.13
LLL	0.06 ± 0.01
Other triglycerides	0.23 ± 0.04
Diglycerides[a]	
OO	0.04 ± 0.01
LL	0.07 ± 0.01
Fatty acids	
Oleic acid	0.33 ± 0.14
Linoleic acid	0.17 ± 0.08
Linolenic acid	0.03 ± 0.02
Sterols	
Campesterol	0.01 ± 0.02
β-Sitosterol	0.02 ± 0.01
Resin acids	
Dehydroabietic acid	0.14 ± 0.02
Other resin acids	0.21 ± 0.04
Steryl esters	ND[b]
Total acetone extract	4.58 ± 0.08

[a] Codes used to referred to the esterified fatty acids in the fatty acyl-glycerols are as follows: O = oleate; L = linoleate
[b] ND = Not detected

Phlebiopsis gigantea was determined eight to ten weeks after inoculation (Behrendt and Blanchette 1997). The maximum extent of resin elimination attained by the two white-rot strain evaluated here varied between 54 to 62% in eight weeks. These high resin removal efficiencies were accompanied by total losses of wood mass of 6% for *Bjerkandera* sp. and 12% for *T. versicolor*.

Figures 2 and 3 show the degradation of the major compound classes in the acetone extractives of Scots pine by wood pretreatment with *Bjerkandera* sp. and *T. versicolor*, respectively, as a function of time. Comparison of these results indicates that *Bjerkandera* sp. generally degraded the various identified extractive constituents at a somewhat higher rate compared to *T. versicolor*.

Although triglycerides were the most abundant extractive fraction in the Scots pine wood, these compounds were nearly depleted by both fungi after only one week of incubation. The susceptibility of triglycerides to fungal degradation was apparently not affected by their substitution pattern, and all the triglyceride constituents detected were rapidly decomposed. The rate of free fatty acid degradation was also high, and as much as 78 to 87% of this fraction was removed in two weeks. Actual eliminations of free fatty acids are considerably higher, when assimilation of the fatty acids released from the fungal hydrolysis of trylglicerides and diglycerides is considered. All the detected free fatty acid constituents, namely oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acid, were rapidly degraded regardless of their degree of saturation. Dehydroabietic acid and other

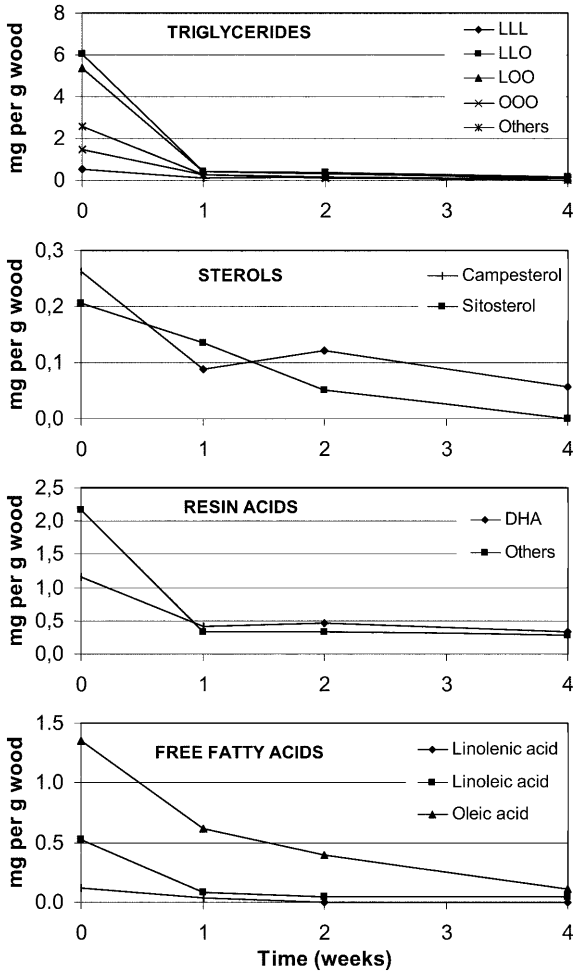


Fig. 2. Degradation of the major compound classes in the acetone extractives of Scots pine wood by pretreatment with the white-rot fungus *Bjerkandera* sp. strain BOS55 as a function of time. Abbreviations: Triglycerides.- Codes used to refer to the esterified fatty acids are as follows: O = oleate; L = linoleate. Resin acids.- DHA = Dehydroabietic acid

resin acid constituents were extensively degraded by *Bjerkandera* sp. and *T. versicolor* during the first week of incubation, resulting in exceptionally high removal rates ranging from 60 to 78%. Surprisingly, resin acid degradation did not increase further with incubation time, and a residual amount of total resin acids ranging 0.62 to 0.76 mg g^{-1} o.d. wood was still detected in wood samples treated for four weeks. Finally, sterols were decomposed more slowly. Nonetheless, β-sitosterol was almost completely removed after four weeks of incubation. Campesterol, another sterol detected in Scots pine, was eliminated by *Bjerkandera* sp., but resisted degradation by *T. versicolor*.

In spite of the very high extractive levels present in the Scots pine wood used in this study, the extent of degradation observed for free fatty acids and resin acids after only one to two weeks of incubation with the white-rot strains generally exceeded values reported for *O. piliferum* and other sapstain fungi (Chen et al.

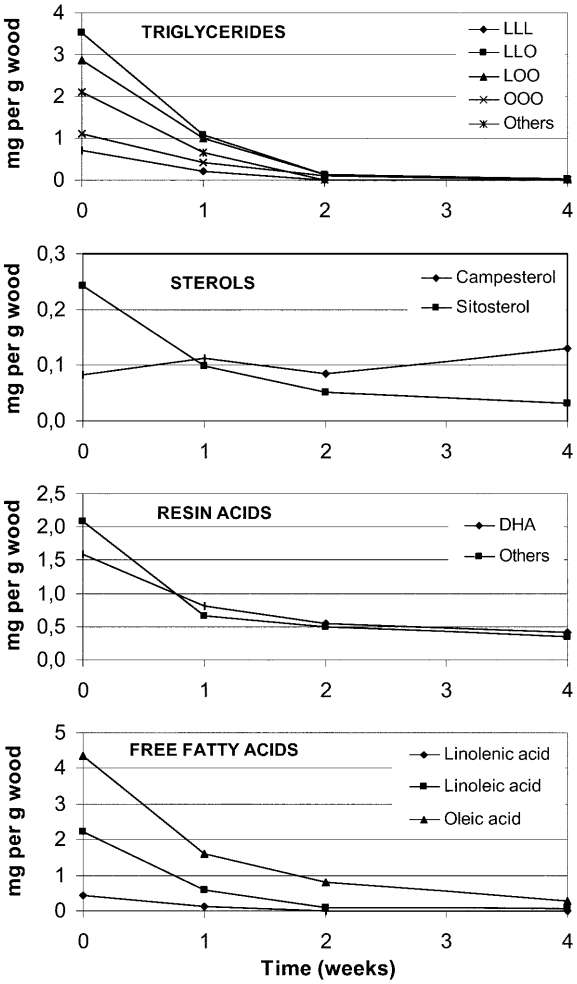


Fig. 3. Degradation of the major compound classes in the acetone extractives of Scots pine wood by pretreatment with the white-rot fungus *Trametes versicolor* strain LaVec94-6 as a function of time. Abbreviations: Triglycerides.- Codes used to refer to the esterified fatty acids are as follows: O = oleate; L = linoleate. Resin acids.- DHA = Dehydroabietic acid

1994; Wang et al. 1995). Likewise, the sterol eliminations reported in studies with sapstain fungi are by comparison low (Gutiérrez et al. 1998; Rocheleau et al. 1998). In contrast, triglycerides that were rapidly degraded by *Bjerkandera* sp. and *T. versicolor*, appear to be also readily decomposed by sapstain fungi (Breuil 1997). Based on the results obtained in this study, it is not possible to conclude whether the observed elimination of wood extractives should be attributed to the mineralization or simply to the biotransformation of these compounds by the white-rot fungi. Literature data provide evidence that some wood-inhabiting fungi can utilize the glycerol and fatty acids liberated after triglyceride hydrolysis as carbon source (Zheng et al. 1994). Little is known, however, about the ability of wood-inhabiting fungi to assimilate other lipophilic extractive constituents such as resin acids, sterols and steryl esters.

The results obtained indicate that white-rot fungi are very effective in the degradation of the various classes of lipophilic extractive constituents found in Scots pine wood. In particular, the degradative ability of *Bjerkandera* sp. and *T. versicolor* towards lipophilic constituents appears to exceed that of well studied sapstain fungi.

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