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BRIEF ORIGINAL

# Fate of organic solvent-soluble extractives and arabinogalactan during brown rot degradation of siberian larch heartwood

**Tiina Belt[1] · Anni Harju[2] · Martti Venäläinen[2] · Petri Kilpeläinen[1]**

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**Abstract**
The decay resistance of durable heartwoods is primarily due to heartwood extractives, but some extractives have been found to be degradable by wood decaying fungi. We investigated the degradation of heartwood extractives in Siberian larch by the brown rot fungi *Coniophora puteana* and *Rhodonia placenta* and found that neither fungus caused the degradation of flavonoids, the primary organic solvent-soluble extractives in the larch samples. However, both fungi caused the gradual depletion of arabinogalactan, the polysaccharide extractive found in larch heartwood.

## 1 Introduction

The heartwoods of some tree species have increased resistance to degradation by wood decaying fungi, primarily due to the deposition of antifungal and antioxidant extractives during the heartwood formation process (Taylor et al. 2002). The quantities and biological activities of extractives are essential to the decay resistance of heartwoods, but the stability of the extractives is also likely to play a role. We recently reported that high levels of heartwood degradation by the brown rot fungus *Rhodonia placenta* were linked to the degradation of heartwood extractives in the early stages of decay in Scots pine (Belt et al. 2022). Here, we extend our investigation of extractive degradation in brown rot decay to Siberian larch, another moderately durable softwood species. The decay resistance of larch heartwood is thought to be due to phenolic extractives (Gierlinger et al. 2004; Venäläinen et al. 2006; Füchtner et al. 2020), primarily flavonoids such as taxifolin (Füchtner et al. 2020). The heartwood also contains very large amounts of arabinogalactan (AG), a polysaccharide whose role in decay resistance is poorly understood (Gierlinger et al. 2004; Venäläinen et al. 2006). The degradation of flavonoids and other organic solvent-soluble extractives as well as AG by the brown rot fungi *Coniophora puteana* and *R. placenta* was studied in two different decay tests. First, larch heartwood samples obtained from one tree were exposed to brown rot in a stacked-sample decay test as in Belt et al. (2022) to give a series of samples in different stages of decay. Then, increment core samples from five different trees were exposed to the same fungi for different durations to confirm the findings of the first test. The contents of organic solvent-soluble extractives and AG were measured after decay in both tests to determine the fate of extractives over the course of brown rot degradation.

## 2 Materials and methods

### 2.1 Decay test 1 – stacked-sample test

Decay test 1 was part of the decay test reported by Belt et al. (2022). Sample blocks with dimensions of 12 mm (radial) × 8 mm (tangential) × 8 mm (longitudinal) were prepared from fresh Siberian larch heartwood as well as Scots pine sapwood to act as the nondurable reference. The larch heartwood samples were obtained from one log cut from one 70-year-old Siberian larch tree growing in an experimental forest in eastern Finland (felled in 2020). The log was stored frozen and protected from light and desiccation until use. The samples were prepared from one thick disc as described in Belt et al. (2022). In an attempt to minimise radial variations in heartwood extractive composition, all samples were cut from the same annual ring position in outer heartwood.

✉ Tiina Belt
Tiina.belt@luke.fi

[1] Production Systems Unit, Natural Resources Institute Finland, Viikinkaari 9, Helsinki 00790, Finland

[2] Production Systems Unit, Natural Resources Institute Finland, Vipusenkuja 5, Savonlinna 57200, Finland

The Scots pine sapwood reference samples were the same as those reported in Belt et al. (2022). All prepared samples were dried at 60 °C for 24 h to determine their initial mass and then sterilised by ionising radiation (25–50 kGy dose).

The decay test was conducted in test tubes over malt extract agar inoculated with *C. puteana* (strain BAM Ebw. 15) or *R. placenta* (strain BAM 113). Each tube ($N=5$) received seven heartwood or sapwood blocks stacked on top of each other over a piece of plastic netting. The tubes were plugged with cotton wool and incubated at 85% RH at room temperature for 19 weeks, until the visible fungal mycelium of one species reached the top of the topmost block in one tube. After the test the samples were wiped to remove adhering mycelium and then dried overnight in a fume hood, followed by 24 h at 60 °C to determine their decayed mass. Further details on sample preparation and the decay test are given in Supplementary Figure S1.

### 2.2 Decay test 2 – time-series test

Increment cores 5 mm thick were obtained from five 71-year-old Siberian larch trees growing in the same experimental forest in eastern Finland (sampled in 2021). A total of eight cores were obtained from each tree at regular intervals (from 120–145 cm to 145–170 cm) and stored frozen in plastic tubes until use. Core pieces 3 cm in length were cut from the outer heartwood portion of each core, from a fixed radial position 3 mm from the visible heartwood-sapwood border. The core pieces were split lengthwise along the grain to produce two halves: one half for the decay test and the other to act as an undecayed extractive content control. The core halves were dried at 60 °C for 24 h, after which the decay test halves were weighed to determine their initial mass and then sterilised by ionising radiation (25–50 kGy dose).

The decay test was conducted on petri dishes containing 30 ml of malt extract agar inoculated with one plug of mycelium from the growing edges of *C. puteana* or *R. placenta* cultures maintained on malt extract agar. Each plate received one larch core half separated from the agar by a piece of plastic netting. The plates were sealed with Parafilm and incubated at 85% RH at room temperature. Samples were removed from the decay test after 10, 20, 27 and 36 days of incubation. At each timepoint, two samples from each tree (one exposed to *C. puteana* and one exposed to *R. placenta*) were removed so that the cores from each tree acted as the timepoints and the five sampled trees acted as the replicates. The samples were wiped to remove adhering mycelium and then dried overnight in a fume hood, followed by 24 h at 60 °C to determine their decayed mass. Further details on sample preparation and the decay test are given in Supplementary Figure S1.

### 2.3 Extractive content analysis

All decayed samples from decay test 1 and the decayed and control samples from decay test 2 were individually ground to a fine powder in a laboratory mill. Organic solvent-soluble extractives were analysed as in Belt et al. (2022). Briefly, 10 mg of each sample was extracted with 2 mL of methanol (with 30 µg/mL of heptadecanoic acid as internal standard) for 30 min. A 750 µL aliquot of the extract was then recovered, evaporated to dryness under $N_2$ flow, and trimethylsilylated with 100 µL of N, O-bis(trimethylsilyl) trifluoroacetamide (BSTFA), 50 µL of chlorotrimethylsilane (TMCS) and 20 µL of pyridine at 70 °C for 60 min. The samples were analysed by GC–MS on a HP 6890/HP 5973 system with a HP-5 column (30 m; 0.25 inner diameter, 0.25 µm film thickness). The oven temperature program was 18 °C/min from 170 to 220 °C (3 min), 10 °C/min from 220 to 260 °C, and 5 °C/min from 260 to 300 °C. Mass spectra were recorded at 70 eV in 30–700 m/z range. Example total ion chromatograms are shown in Supplementary Figure S2.

For the analysis of AG, the powdered samples were extracted using cold water (Venäläinen et al. 2006). A 20 mg portion of sample was extracted using 0.8 mL of ultrapure water for 1.5 h with continuous shaking at room temperature. A 500 µL aliquot of the extract was collected and freeze-dried for carbohydrate monomer quantification by acid methanolysis. The samples were methanolysed using 2 mL of 2 M HCl in anhydrous methanol for 3 h at 100 °C, then cooled to room temperature and neutralised with 200 µL of pyridine. Internal standard solution was added (1 mL of methanol with 0.1 mg/mL of sorbitol and 0.1 mg/mL of resorcinol), the solvent evaporated under $N_2$ flow, and the samples trimethylsilylated with 100 µL of pyridine, 150 µL of hexamethylsilazane (HMDS), and 70 µL TMCS at room temperature overnight. Monosaccharides were quantified by GC-FID using a Shimadzu GC-2010 system equipped with a HP-1 column (25 m; 0.2 mm inner diameter, 0.11 µm film thickness). Hydrogen was used as the carrier gas (1.08 mL/min), and the oven temperature program was set to 4 °C/min from 100 to 175 °C, followed by 12 °C/min to 290 °C (5 min). The AG content of the samples was estimated as the sum of arabinose and galactose obtained by cold water extraction and methanolysis.

## 3 Results and discussion

The mass losses of the larch heartwood samples and Scots pine sapwood references due to *C. puteana* and *R. placenta* in decay test 1 are shown in Fig. 1a as a function of sample position. The sapwood references were strongly degraded by *C. puteana*, and there was a linear increase in average

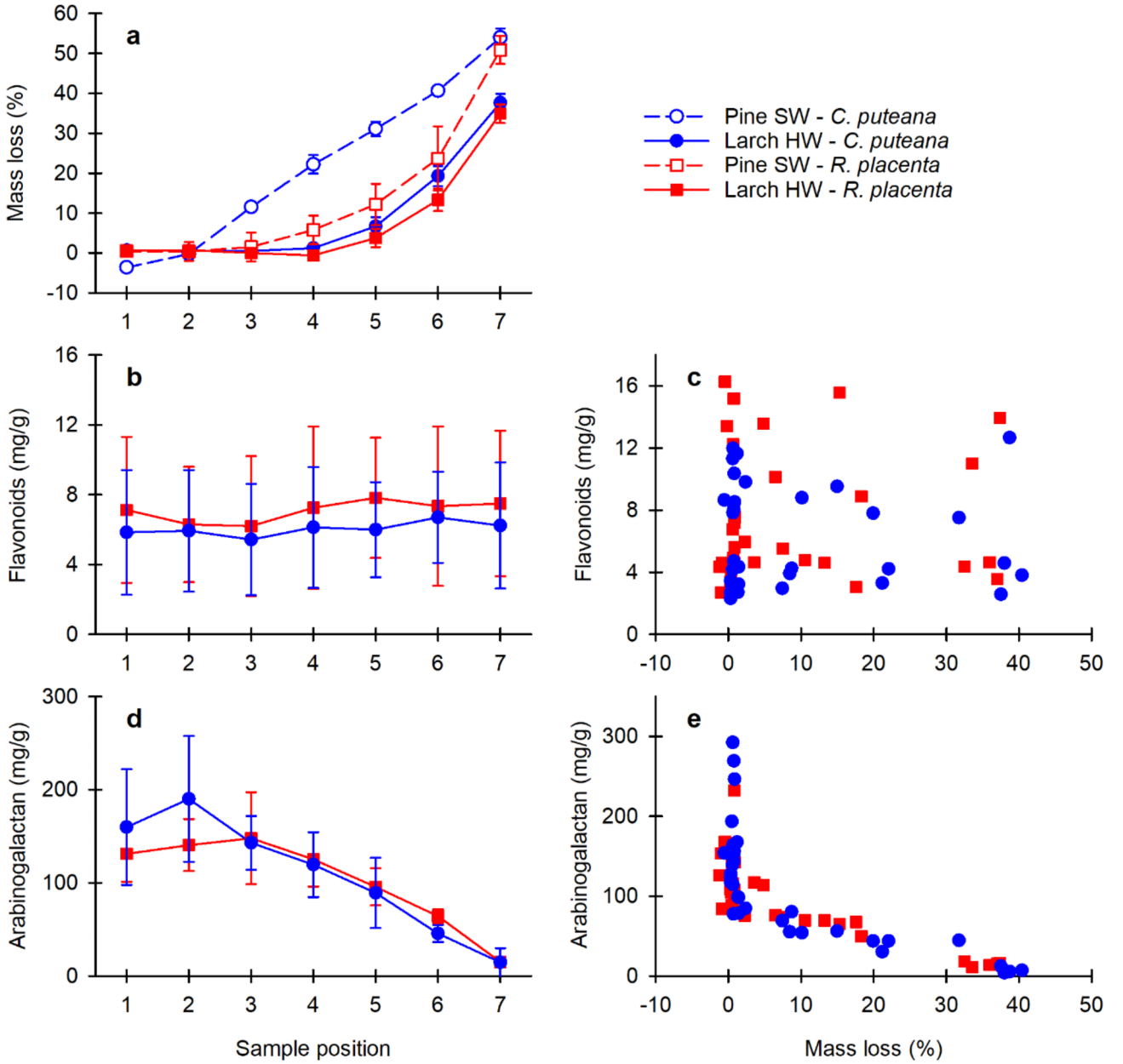


**Fig. 1** Mass loss and extractive content after decay test 1. Average mass loss of pine sapwood and larch heartwood as a function of sample position (**a**), average flavonoid content (**b**) and arabinogalactan (AG) content (**d**) as a function of sample position, and flavonoid content (**c**) and AG content (**e**) of individual samples as a function of mass loss. Error bars in a, b and d are ± standard deviation ($N = 5$). Sample position 1 is the topmost block and position 7 the bottommost block in the stacked-sample setup (see Supplementary Figure S1). Extractive contents are given on a decayed mass basis

mass loss from −4% at position 1 (top) to 54% at position 7 (bottom). The average mass loss of larch heartwood remained low until position 5 and then increased rapidly, reaching 38% at position 7. *R. placenta* caused a non-linear increase in mass loss from 0% at position 1 to 51% at position 7 in pine sapwood. The mass losses of larch heartwood followed a similar nonlinear pattern and reached 35% at position 7.

GC-MS analysis of the decay test samples (see Supplementary Figure S2) showed that the organic solvent-soluble extractives of larch heartwood consisted primarily of flavonoids, most notably taxifolin, in agreement with previous results (Füchtner et al. 2020). The average flavonoid contents of the decayed samples are shown in Fig. 1b as a function of sample position, while the flavonoid contents of the individual samples are shown in Fig. 1c as a function of

mass loss. Resin acids and fatty acids were present in minor amounts, and their contents are given in Supplementary Figure S3. Despite attempts to minimise variations in extractive content by taking samples from the same annual ring position in the log, wide variation was observed in extractive content. However, the data clearly showed that there was no change in flavonoid content as a function of sample position (Fig. 1b) or mass loss (Fig. 1c), which means that neither fungus caused preferential degradation of flavonoids. Fatty acid content showed no change as a function of sample position or mass loss, while resin acid content showed an increase (Supplementary Figure S3). The lack of flavonoid degradation differentiates larch heartwood from Scots pine heartwood, which showed a rapid decrease in heartwood stilbene content in the early stages of *R. placenta* decay (Belt et al. 2022). The differences in the fate of flavonoids and stilbenes may be due to differences in their chemistry and mode of action. Pine stilbenes protect wood by antifungal action (Hart and Shrimpton 1979) and appear to be actively degraded by the fungus (Belt et al. 2022), while flavonoids such as taxifolin are thought to protect wood primarily by antioxidant action (Füchtner et al. 2020) and might not be actively degraded due to their (presumed) lower toxicity.

The average AG contents of the decayed samples are shown in Fig. 1d as a function of sample position and the AG contents of the individual samples in Fig. 1e as a function of mass loss. Unlike flavonoid content, AG content clearly decreased as a function of sample position and mass loss. Although there was substantial variation in initial AG content, samples with > 2% mass loss showed a clear linear decrease in AG content with increasing decay. In the early stages of decay (sample positions 1–5, mass loss < 10%) the observed mass losses may be primarily due to the degradation of AG, while in more advanced decay (sample positions 6–7, mass loss > 10%) the degradation of cell wall polymers becomes evident as the measured mass losses match or exceed the initial AG content of the samples (average AG content at position 1 was 164 mg/g for samples exposed to *C. puteana* and 136 mg/g for samples exposed to *R. placenta*). The role of AG in the decay resistance of larch heartwood is unclear, given that it is a polysaccharide that presents an easily accessible source of sugars to the fungi. Previous studies have found a significant (Gierlinger et al. 2004) or insignificant (Venäläinen et al. 2006) negative correlation between mass loss due to decay and AG content, which suggests that AG may play a role in decay resistance. The results of this study do not offer insight into the role of AG in decay resistance, but they show that the polymer is degraded by fungi over the course of decay.

To confirm the results obtained from decay test 1, decay test 2 was established using increment core samples from five different trees. The average mass losses of the samples at different timepoints due to *C. puteana* and *R. placenta* are given in Fig. 2a. The mass losses of the heartwood samples due to *C. puteana* increased linearly with time, while mass losses due to *R. placenta* increased rapidly initially and then plateaued. The average flavonoid and AG contents of the samples after decay are given in Fig. 2b and d as a function of time, while the flavonoid and AG contents as a percentage of the undecayed control value are shown in Fig. 2c and e as a function of mass loss. Resin acid and fatty acid contents are given in Supplementary Figure S4. Although there was wide variation in the flavonoid and AG content of the samples, the results agreed with the results obtained from decay test 1. Flavonoid content showed no change as a function of time (Fig. 2b) or mass loss due to either fungus (Fig. 2c), while AG content decreased over time due to *C. puteana* (Fig. 2d) and as a function of mass loss due to both fungi (Fig. 2e). Fatty acids content showed a slightly decreasing trend but high variation, while resin acid content showed no change (Supplementary Figure S4).

## 4 Conclusion

The two decay tests showed that neither fungus caused the preferential degradation of flavonoids under the employed test conditions, while both fungi caused a clear gradual decrease in AG content in both tests. The relationship between extractive degradation and mode of action requires further investigation, as does the role of AG in larch decay resistance.

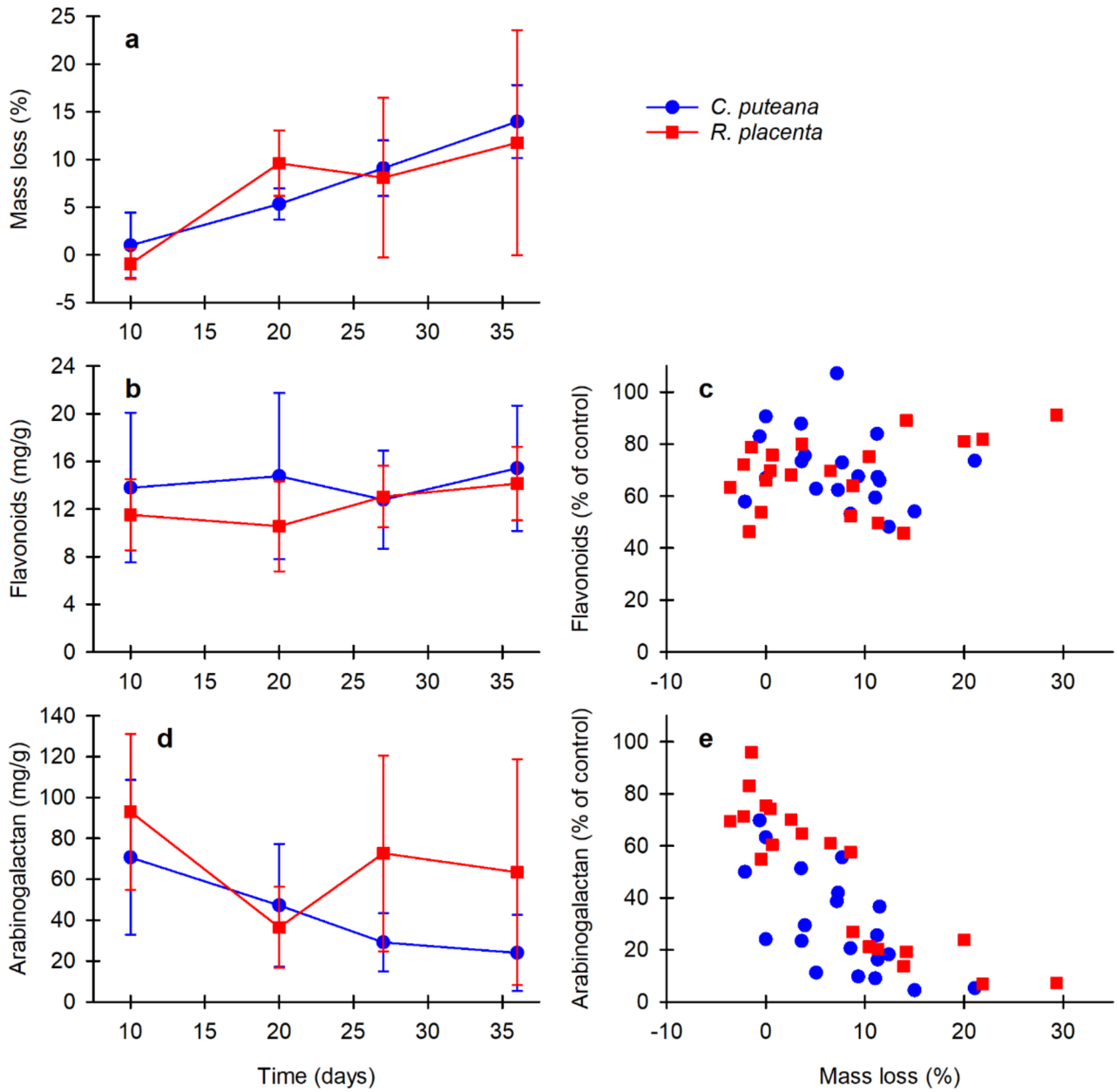


**Fig. 2** Mass loss and extractive content after decay test 2. Average mass loss of larch heartwood as a function of time (**a**), average flavonoid content (**b**) and arabinogalactan content (**d**) as a function of time, and flavonoid content (**c**) and arabinogalactan content (**e**) of individual samples as a percentage of the undecayed control value as a function of mass loss. Error bars in a, b and d are ± standard deviation ($N = 5$). Extractive contents are given on a decayed mass basis

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00107-024-02146-3.

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**Data availability** The datasets generated and analysed during the current study are available in the Zenodo repository, https://doi.org/10.5281/zenodo.10781274.

## Declarations

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.



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