Original article

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Unraveling the natural durability of wood: revealing the impact of decay-influencing characteristics other than fungicidal components

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Abstract: The effect of fungicidal components in wood has been known for ages, yet there is no method to assess the impact of such components on the durability of a wood species, as compared to other material characteristics that influence decay. In this paper, the importance of fungicidal effects on the natural durability of 10 wood species is assessed in relation to other decay-influencing factors with a new test, the so-called 'paste test'. By comparing results from this test with the 'mini-block test', on both heartwood and leached sapwood, insight is gained into the significance of fungicidal components on the one hand and other material characteristics on the other hand. The durability of species such as *Prunus avium* was attributed mainly to fungicidal components. For species such as *Pterocarpus soyauxii*, durability seemed to be an effect of both fungicidal components and moisture-regulating components, while the latter seemed to be of main importance in regulating the decay of *Aucoumea klaineana* and *Entandrophragma cylindricum*. Wood-anatomical features, such as the parenchyma content (in case of brown rot fungi) and the vessel-fiber ratio, possibly affect degradation as well. This work shows that fungicidal components are not always of major importance for the durability of a wood species. The authors hereby emphasize the importance of moisture-regulating components and wood anatomy on the durability of wood.

Keywords: fungicidal components; heartwood; moisture-regulating components; natural durability; wood anatomy.

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1 Introduction

Environmental and economic concerns are stimulating the development of construction materials made from renewable resources (Bourmaud et al. 2018; Churkina et al. 2020). Wood, engineered wood products and high performance plant fiber composites are gaining importance (Bechthold and Weaver 2017; Bourmaud et al. 2018; Wimmers 2017). Many of these materials are biodegradable, an excellent quality at the end of a material's service life as it solves waste issues, but a less desirable feature during use. The most crucial factors for fungal degradation to occur are material moisture content (MC), temperature and durability of the material (Meyer-Veltrup et al. 2017; Zabel and Morrell 2012). When optimal fungal growth conditions occur, the durability of the material will therefore be a key element determining the degree of degradation.

With the advent of new renewable materials and modification technologies, there is an increasing need for more insight into how different material characteristics, such as the material's chemistry, structure and moisture dynamics, influence durability. The current standards for testing (treated) wood give an adequate indication of durability, but were never designed to provide fundamental insight in the aforementioned characteristics. Additionally, they are not intended to assess certain engineered wood products and wood modification technologies (Candelier et al. 2016; Kutnik et al. 2014; Ormondroyd et al. 2015; Ringman et al. 2014). Unraveling the role of different material characteristics on the overall durability

of a material will guide us towards more insightful design and use of renewable construction materials.

When it comes to wood chemistry and durability, research has been focused on wood cell wall polymers and the extraction and evaluation of possibly fungicidal wood components. Wood cell wall polymers (cellulose, hemicellulose and lignin) are the main components of wood and it is well known that cellulose and hemicellulose are essential nutrients for fungal growth (Cragg et al. 2015; Zabel and Morrell 2012). Lignin, however, acts as a barrier against decay. Brown- and white-rot fungi have developed different mechanisms to circumvent this barrier. While brown-rot fungi can tap into cellulose and hemicellulose as a nutrient source by slightly modifying the lignin, white-rot fungi have the ability to degrade lignin (Floudas et al. 2012; Schmidt 2006). For the latter fungi, the type of lignin directly affects the decay rate. In softwoods for instance, guaiacyl is the main lignin type, typically resulting in a slower decay rate of white-rot fungi on softwoods compared to hardwoods (Highley 1982; Li et al. 2015; Schmidt 2006; Schwarze 2007).

Similarly, the extraction and evaluation of possibly fungicidal wood components (fatty acids, organic acids, terpenes, tannins, and other benzenoid compounds) have been studied extensively. The most common method for evaluating the toxicity of such extracted components is the assessment of the effect of an extractive on the development of pure fungal cultures growing in or on a nutrient solution or agar (Hart 1989; Tchinda et al. 2018). The effect of a specific fungicidal component can also be assessed by impregnating a wood sample with such a component and evaluating its decay resistance. However, when artificially impregnating wood with an extractive, the interaction between extractive and wood differs from extractives naturally present in the heartwood. Furthermore, a uniform concentration is hard to achieve. The effect of extractives on the overall durability of wood can also be tested by comparing decay between extracted and non-extracted samples. It is important to note, however, that often only a weak correlation can be found between a wood's durability and the concentration of extractives (Scalbert 1992), except in the case of certain highly fungicidal extractives such as tropolones in Cupressaceae (Hart 1989). This confirms that, for most wood species, other components or material characteristics besides fungicidal extractives contribute to the overall natural durability. Chemical components that have been overlooked, for instance, are moisture-regulating components, even though these are considered to play a role in the natural durability of certain heartwood species (De Angelis et al. 2018; Harju et al. 2002; Song et al. 2014).

While literature is available on possibly fungicidal components in wood, there is no method to assess their importance in the durability of a wood species or bio-based building material. This paper proposes the 'paste test' in combination with the 'mini-block' test (Bravery 1978) to assess the importance of fungicidal components on the natural durability of 10 wood species. In the 'paste test', wood is pulverized to a fine powder, limiting the influence of wood anatomy on fungal growth, and mixed with water and agar, to minimize the role of moisture-regulating components. The 'mini-block' test (Bravery 1978) is used to test the durability of solid wood blocks, for which other material characteristics besides fungicidal components are expected to play a role as well. Both sapwood and heartwood samples of the 10 wood species are subjected to the 'paste' and 'mini-block' tests. Since sapwood generally does not contain fungicidal components (Hart 1989; Hillis 2012), sapwood samples are included to confirm the validity of the 'paste test' and to reveal the role of material characteristics besides fungicidal components. This study aims to prove that besides fungicidal components, other material characteristics play a crucial role in durability. For those wood species with a durability not related to any fungicidal effect, moisture-regulating components, lignin type and wood anatomy proved to be key in the durability.

2 Materials and methods

2.1 Wood specimens

Mini-blocks of 30 × 10 × 5 (axial) mm^3 were prepared from 10 wood species, covering the full range of natural durability. Table 1 gives an overview of the 10 wood species, their durability class (CEN EN 350 standard 2016), absorption and desorption class (Van Acker et al. 2014), and a selection of wood-anatomical features described in literature: vessel/fiber ratio, tracheid proportion and parenchyma content (Wagenführ and Scheiber 1974). The absorption and desorption classes described in Van Acker et al. (2014) are determined on the floating test, in which wood samples (5 × 5 × 2.5 cm^3) are laid afloat a water surface for 144 h of absorption and left to dry for 144 h of desorption in a climate chamber (20 °C and 65% RH) (CEN/TS 16818 standard 2018). To confirm whether the paste test adequately eliminates the influence of structure and moisture-regulating components on fungal growth, wood samples with clear differences in structure and hygroscopic components, but with similar nutritional values and fungicidal components, need to be selected. The heartwood of the selected wood species (Table 1) contains various fungicidal and moisture-regulating components. Removing these from heartwood is an arduous process requiring a sequence of severe leaching methods. Still, a complete removal of all fungicidal components from the heartwood cannot be guaranteed (Pettersen 1984). Therefore, sapwood, generally containing no fungicidal components (Hart 1989; Hillis 2012), was used as well. Leaching is, however, still necessary in

Table 1: Durability (EN 350 standard 2015), absorption and desorption class (Van Acker et al. 2014), vessel/fibre ratio, tracheid proportion and parenchyma content (Wagenführ and Scheiber 1974) of selected wood species.

Wood species	Durability of heartwood against basidiomycetes in lab conditions	Absorption/Desorption class heartwood	Average vessel/fibre ratio (%)	Tracheid proportion (%)	Parenchyma content (%)
Hardwoods					
Pterocarpus soyauxii Taub	DC 1	4/2	16.3	–	23.0
Robinia pseudoacacia L.	DC 1–2	2/4	25.9	–	6.0
Castanea sativa Mill.	DC 1	5/4	46.5	–	Insignificant
Distemonanthus benthamianus Baill.	x	2/3	37.8	–	14.0–23.0
Entandrophragma cylindricum Sprague	DC 3–4	4/5	39.3	–	10.5–30.5
Prunus avium L.	x	5/4	76.6	–	Insignificant
Aucoumea klaineana Pierre	DC 4–5	3/3	28.8	–	0.0–3.0
Fagus sylvatica L.	DC 4–5	6/7	99.8	–	3.5–7.0
Softwoods					
Picea abies (L.) Karst	DC 4–5	5/5	–	93.1	–
Pinus sylvestris L.	DC 2–5	5/5	–	95.3	0.0–5.8

The durability class of a wood species in EN 350 is assigned according to the Basidiomycete test that resulted in the highest median mass loss, which could be a white or brown rot fungus (CEN EN 350 standard 2016). The absorption class ranges from 1–8, with 1 indicating the wood sample has absorbed less than 750 g water per m^2 of sample surface and 8 indicating the sample has absorbed more than 5000 g water per m^2 of sample surface after 144 h of absorption. The desorption class ranges from 1–8, with 1 indicating that less than 250 g water per m^2 of sample surface remained after 144 h of desorption and 8 indicating that more than 2000 g water per m^2 of sample surface remained after 144 h of desorption.

order to better mimic the heartwood, as leaching removes nutrients such as starch. Thus, from each species, 40 heartwood and 40 sapwood specimens were collected for the mini-block tests and 20 heartwood and 20 sapwood specimens for the paste test, with the exception of *Robinia pseudoacacia* for which no adequate sapwood specimens could be obtained, due to the small amount of sapwood present on the available *R. pseudoacacia* wood sources. The sapwood specimens were leached according to the EN 84 standard (1997). The heartwood specimens can contain both fungicidal substances as well as moisture-regulating components, while sapwood specimens were leached and assumed free of fungicidal components, yet moisture-regulating components might still have been present.

2.2 Fungal species

Trametes versicolor (strain MUCL 11665) and *Coniophora puteana* (strain MUCL 11662) were used, since both fungi have evolved differently and have developed different mechanisms of degradation (Floudas et al. 2012; Goodell and Jellison 1990; Mester et al. 2004; Schmidt 2006). It is also obligatory to test both fungi for standard testing of (natural) durability against Basidiomycetes (CEN/TS 15083–1 standard 2006; EN 113 standard 1996; CEN EN 350 standard 2016).

2.3 Mini-block test

All specimens were conditioned in a climate chamber with 20 ± 2 °C and 65 ± 5% relative humidity. The mini-block test specimens were weighed (m_1) to the nearest 0.001 g and sterilized using 25–50 kGy Gamma irradiation (Synergy Health, Etten-Leur, The Netherlands). Ten additional heartwood and sapwood specimens were weighed ($m_{1,ref}$) per species, then oven-dried at 103 °C ± 2 °C for 24 h and weighed ($m_{0,ref}$) once more to the nearest 0.001 g to determine an average reference moisture content (MC [–]) per species:

$$\mathrm{MC} = \frac{m_{1,ref} - m_{0,ref}}{m_{0,ref}}. \quad (1)$$

This average moisture content was then used to determine the dry weight of the mini-block specimens (m_0 [g]):

$$m_0 = \frac{m_1}{\mathrm{MC} + 1}. \quad (2)$$

Wood specimens were exposed to actively growing, pure cultures of *C. puteana* and *T. versicolor* (20 heartwood and 20 sapwood specimens each). Both fungi were cultivated on Petri dishes (90 mm diameter, 16 mm deep) containing 20 ml of 3% malt/2% agar medium (Thermo Fisher Diagnostics B.V., Landsmeer, The Netherlands). The mini-blocks were placed on sterilized metal meshes, with three mini-blocks in each Petri dish. The Petri dishes were incubated for 10 weeks at 22 °C and 70% relative humidity. Subsequently, the mini-blocks were cleaned, weighed, oven-dried and weighed again (m_1 [g]) to determine the loss in dry mass (ML) due to fungal degradation:

$$\mathrm{ML} = \frac{m_0 - m_1}{m_0}. \quad (3)$$

The durability of the samples was rated between 1 (Very durable) and 5 (Not durable) based on the percentage of mass loss due to fungal degradation, as described in standard TS 15083-1 (CEN standard 2006). This rating scale is intended for larger block sizes (5 × 2.5 × 1.5 cm^3)

exposed for 16 weeks, but Deklerck et al. (2019) reported that there is adequate variation in mass loss for mini-blocks of various wood species, exposed for 8 and 12 weeks, to compare durability of the wood species. Additionally, mini-block durability ratings were similar as those described in standard EN 350 (CEN EN 350 standard 2016).

2.4 Paste test

The heartwood and leached sapwood specimens were grinded to a coarse powder with a cutting mill with mesh size 0.25 mm (SM 200, Retsch GmbH, Haan, Germany), and then milled to a fine powder (particle size <0.1 mm) with a centrifugal mill (ZM 200, Retsch GmbH, Haan, Germany). The powders were sterilized using 25–50 kGy Gamma irradiation (Synergy Health, Etten-Leur, The Netherlands). For each combination of wood species and fungal type, six heartwood and six sapwood pastes were prepared. The pastes consisted of 20 ml agar medium (2% malt, 2% agar) and 1.2 g wood powder per Petri dish. The pastes were inoculated with a fungal inoculum disc of 0.7 cm^2. Again, *C. puteana* and *T. versicolor* were used. Additionally, six Petri dishes containing only agar medium were inoculated as controls.

The Petri dishes were incubated for 10 days at 22 °C and 70% relative humidity. Images of the growing fungi were captured twice a day using a flatbed scanner (Epson perfection V750 Pro) on which two rows of three Petri dishes were mounted, inspired by Vidal-Diez de Ulzurrun et al. (2015). The mycelial area was calculated by means of an automated image analysis method developed in-house. First, the region of each Petri dish was extracted from the background by the circular Hough transform method (Hough 1962). Subsequently, the mycelial area in each Petri dish was segmented by combining a region growing algorithm, adaptive thresholding and mathematical morphological processing, all implemented in Matlab (MATLAB 2018). Adequate processing was manually verified for each image, and unsuccessfully analyzed images (19% for *C. puteana* and 53% for *T. versicolor*) were reprocessed with the Wand Tool in Fiji (Schindelin et al. 2012). Logistic growth curves were fitted per Petri dish (see Figure 1 for an example on *Pinus sylvestris* sapwood), from which the logistic growth rate (k) was derived per replicate:

$$f(x) = \frac{L}{1 + e^{-k(x-x_0)}}. \quad (4)$$

with L the curve's maximum value and k the logistic growth rate or steepness of the curve.

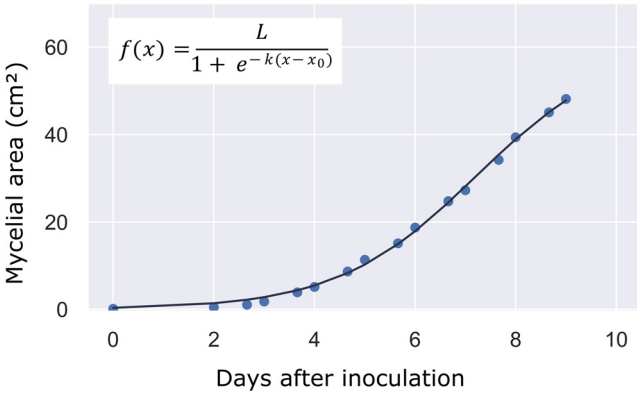

Figure 1: Logistic growth curve fitted to mycelial development of *Coniophora puteana* growing on a paste of *Pinus sylvestris* sapwood. L, the curve's maximum value; k, the logistic growth rate or steepness of the curve.

3 Results and discussion

3.1 Assessing fungicidal components with the paste test

In the paste test, the wood was ground to a fine powder with a particle size smaller than 0.1 mm. Therefore, the influence of the material structure on fungal growth should be limited. By mixing the wood powder with agar and water, water saturation of the wood powder was ensured, thereby also limiting the influence of water-regulating components. The paste test should thus give an indication of how the overall wood chemistry, consisting of the nutritional value and fungicidal components, influences the fungal susceptibility of a wood species.

First, an assessment was made to test whether the paste test adequately eliminates the influence of structure and moisture-regulating components. Fungal growth on the leached sapwood pastes is presented relative to the median growth rate of *C. puteana* and *T. versicolor* growing on a paste of pure malt agar without wood powder (5.2 ± 0.7 and 11.9 ± 1.9 cm^2 day^{-1}, respectively). *T. versicolor* grew at a similar rate on the different sapwood pastes compared to the pure malt agar pastes (Figure 2). Since the growth rate was similar on all leached sapwood pastes, this confirms that leaching sapwood removes any fungicidal component that has a growth inhibitory effect on *T. versicolor*. Furthermore, it also confirms that the paste test adequately eliminates the influence of structure and hydrophobic components on mycelial development of *T. versicolor* and thus allows to assess the influence of wood-fungicidal components. *C. puteana* grew faster on wood pastes than on the control pastes, with the exception of *Prunus avium* (Figure 2). The wood powder thus had a growth-promoting effect on *C. puteana*, indicating that *C. puteana* is cellulose-specific. While the growth rate of *C. puteana* was relatively low on *P. avium* and comparable to the growth rate on pure malt agar paste, it grew much denser (Figure 3b) on the sapwood of *P. avium* than on all other sapwood pastes, which had a mycelial density similar to the one displayed on the paste of *Pterocarpus soyauxii* sapwood (Figure 3a). The lower growth rate on *P. avium* sapwood was therefore most likely not the result of any fungicidal effect. Typical display of a growth inhibiting effect on the mycelial development of *C. puteana* can be seen for *P. soyauxii*

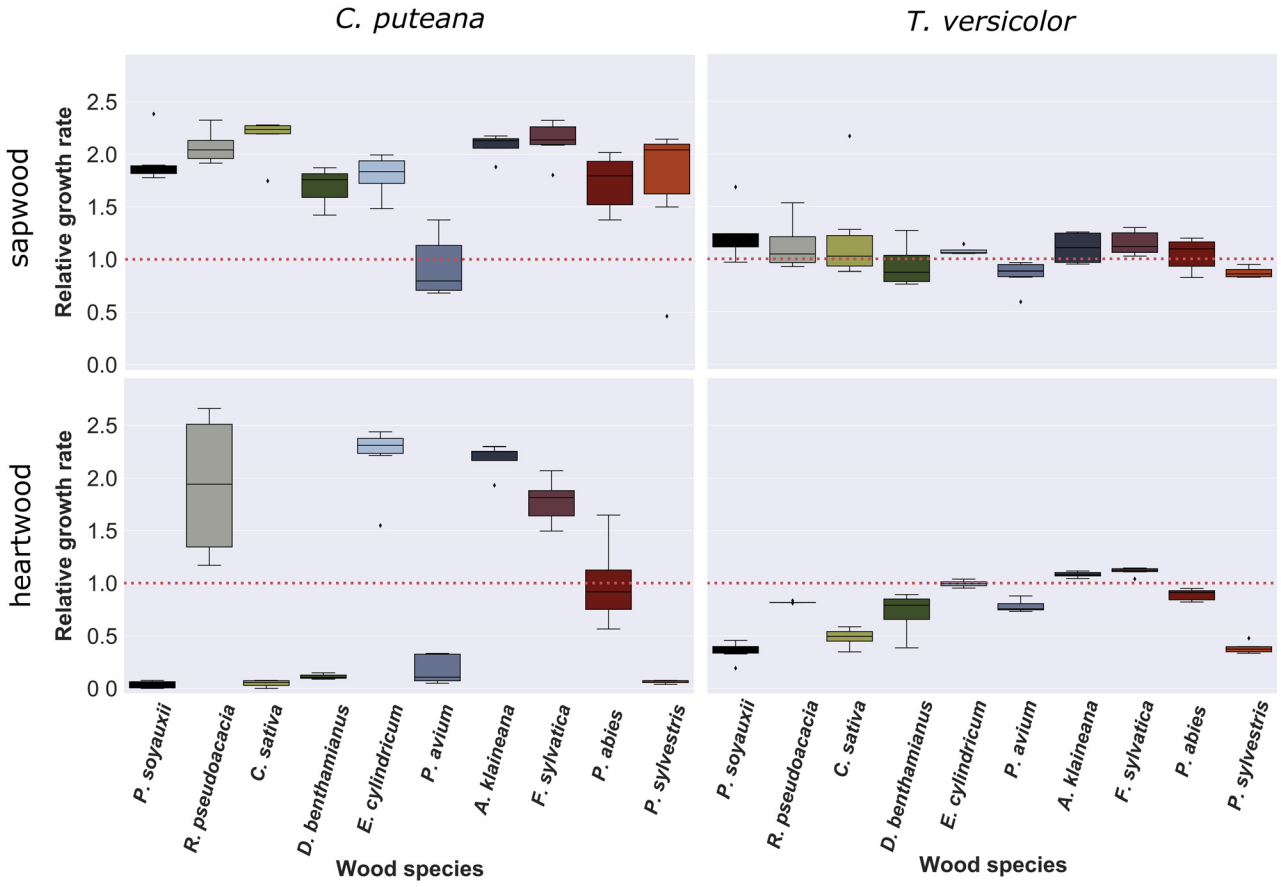

Figure 2: Paste test: relative growth rate of *Coniophora puteana* and *Trametes versicolor*, growing on sapwood and heartwood pastes of 10 wood species. The red dotted line corresponds to the median growth rate on malt agar medium without wood powder (5.25 cm^2 day^{-1} for *C. puteana* and 11.7 cm^2 day^{-1} for *T. versicolor*).

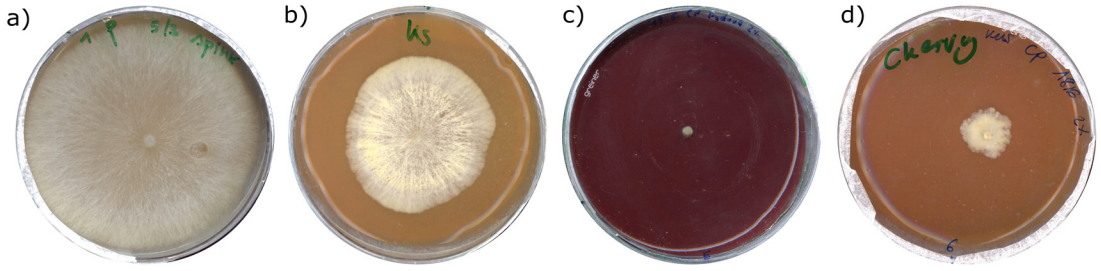

Figure 3: Mycelium of *Coniophora puteana* 9 days after inoculation on pastes of (a) leached *Pterocarpus soyauxii* sapwood, (b) leached *Prunus avium* sapwood, (c) *Pterocarpus soyauxii* heartwood and (d) *Prunus avium* heartwood.

heartwood (Figure 3c) and *P. avium* heartwood (Figure 3d). However, if this accumulation of mycelium were to occur abundantly in future experiments, it might be interesting to quantify this mycelium density in addition to mycelial area. In general, the paste test on leached sapwood specimens resulted in similar fungal growth behavior for the 10 wood species, indicating that grinding and moisture saturation adequately removes the influence of structure and moisture-regulating components on fungal growth.

Secondly, an assessment was made whether the paste test can be used to determine the presence of fungicidal components in wood. The results of the heartwood pastes (Figure 2) confirmed that the heartwood of *P. soyauxii*, *Castanea sativa*, *Distemonanthus benthamianus*, *P. avium* and *P. sylvestris* clearly contain fungicidal components that are growth inhibiting for *C. puteana*. While the growth rate of *C. puteana* approached zero, the one of *T. versicolor* was merely reduced.

The overall heartwood natural durability according to the mini-block test of the 10 wood species is shown in Figure 4. The results from the paste tests (Figure 2) and mini-block tests (Figure 4) were compared to determine if in addition to fungicidal components other wood characteristics, such as the wood-anatomical structure or hydrophobicity, have an impact on the overall natural durability of a wood species. Due to their size, the anatomical structure and water-regulating components were not expected to have a similar effect for mini-blocks as for wood in practice. Nevertheless, both factors seemed to have a considerable impact on durability, even in a mini-block test set-up as shown in Table 2. This table gives an overview of the heartwood durability of the 10 wood species and specific factors that have an impact on their resistance against *C. puteana* and *T. versicolor*.

3.2 Impact of moisture regulating components on natural durability

When analyzing each wood species, it became apparent that different wood species owe their durability to different factors. For instance, *P. soyauxii* clearly contained fungicidal components, as shown in the results from the heartwood paste test (Figure 2) and supported by previous studies on its extractives (Mounguengui et al. 2016). Growth of *C. puteana* on the *P. soyauxii* heartwood paste was limited, and the growth rate of *T. versicolor* was two times lower than on pure malt agar medium (2% MEA). However, the fungicidal components were not the only responsible factors for the high durability of *P. soyauxii*: sapwood mini-blocks, without any fungicidal components, showed resistance against degradation as well. *P. soyauxii* thus contains moisture-regulating components and/or has an anatomical structure that acted as a barrier to fungal growth. This additional protection was more effective against *C. puteana* (sapwood mini-block DC_m 1) than against *T. versicolor* (sapwood mini-block DC_m 3). Jankowska et al. (2018) indeed found a high percentage of moisture-regulating components in *P. soyauxii* heartwood, in the form of extractives soluble in ethanol-chloroform, which correspond to waxes, fats, resins and oils (ASTM standard 2013), and extractives soluble in cyclohexane, which probably correspond to oleoresins (Hillis 2012). *C. sativa* gave similar results as *P. soyauxii*, though less

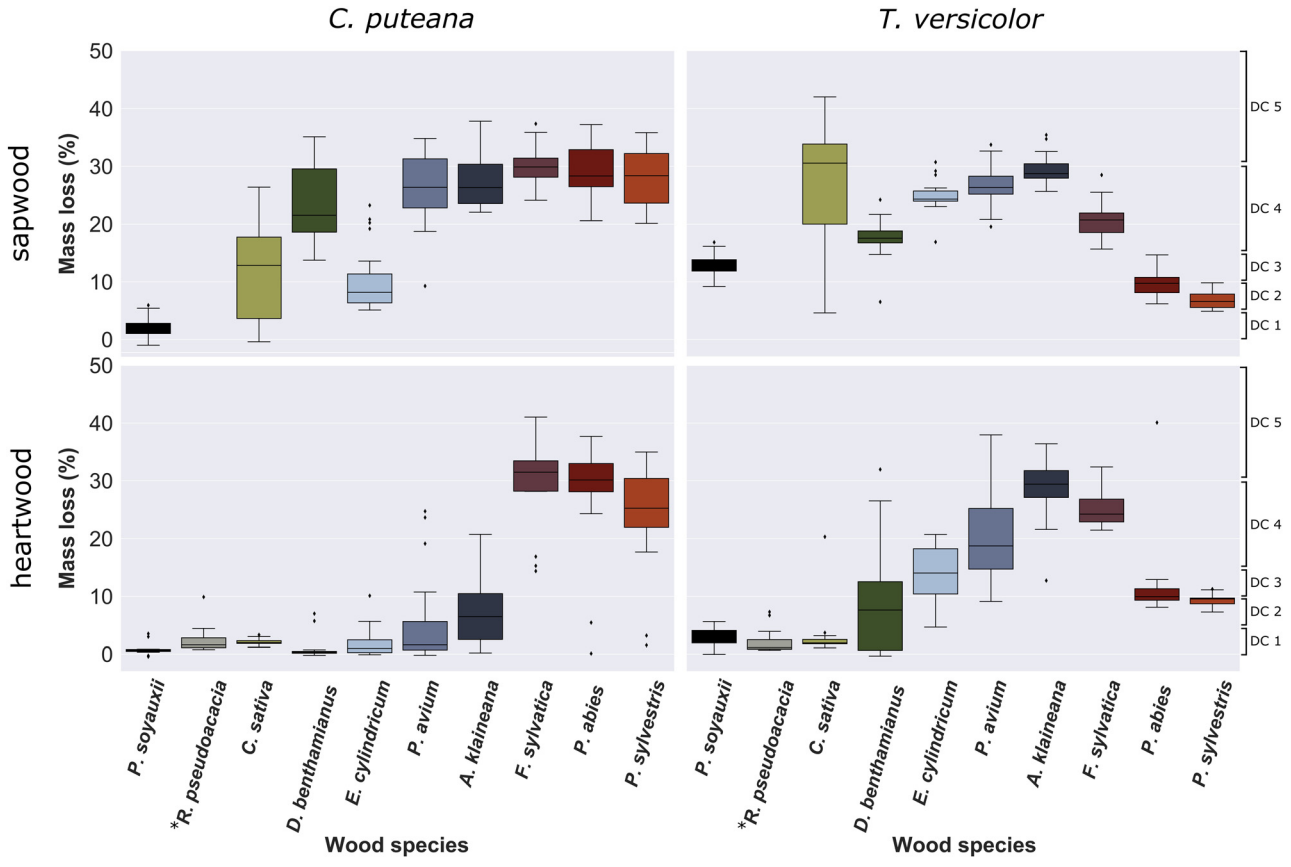

Figure 4: Mini-block test: mass loss after 10 weeks of fungal degradation by *Coniophora puteana* and *Trametes versicolor*, growing on sapwood and heartwood mini-blocks of 10 wood species. The axis on the right displays the durability classes as described by CEN/TS 15083-1. **Robinia pseudoacacia* was excluded from the sapwood mini-block test due to limited sapwood availability.

Table 2: Overview of factors contributing to the overall natural durability of the heartwood of 10 wood species against degradation by *Coniophora puteana* and *Trametes versicolor*, based on the paste and mini-block tests.

Wood species	Commercial name	*Coniophora puteana*				*Trametes versicolor*			
		DC_m HW	DC_m SW	F	WR/S	DC_m HW	DC_m SW	F	WR/S
Hardwoods									
Pterocarpus soyauxii Taub	African padauk	DC_m 1	DC_m 1	x	x	DC_m 1	DC_m 3	x	x
Robinia pseudoacacia L.	Black locust	DC_m 1	/	x	u	DC_m 1	/	x	x
Castanea sativa Mill.	Sweet chestnut	DC_m 1	DC_m 3	x	x	DC_m 1	DC_m 5	x	u
Distemonanthus benthamianus Baill.	Movingui	DC_m 1	DC_m 4	x	u	DC_m 2	DC_m 4	x	u
Entandrophragma cylindricum Sprague	Sapele	DC_m 1	DC_m 2		x	DC_m 3	DC_m 4		x
Prunus avium L.	European cherry	DC_m 1	DC_m 4	x	u	DC_m 4	DC_m 4	x	x
Aucoumea klaineana Pierre	Gaboon	DC_m 2	DC_m 4		x	DC_m 4	DC_m 4		
Fagus sylvatica L.	Beech	DC_m 5	DC_m 4			DC_m 4	DC_m 4		
Softwoods									
Picea abies (L.) Karst	Norway spruce	DC_m 5	DC_m 4			DC_m 2	DC_m 2		
Pinus sylvestris L.	Scots pine	DC_m 4	DC_m 4	x		DC_m 2	DC_m 2	x	

DC_m gives the durability class based on the median amount of mass loss (ML) after 10 weeks of degradation in a mini-block set-up, based on CEN/TS 15083-1 (DC_m1: ML <5%, DC_m2: 5% < ML ≤ 10%, DC_m3: 10% < ML ≤ 15%, DC_m4: 15% < ML ≤ 30%, DC_m5: 30% < ML) for heartwood (HW) and sapwood (SW). A potential effect of fungicidal components (F) and water regulating components and/or structure (WR/S) is indicated with an 'x'. In the WR/S columns the 'u' (unknown) is used when the influence of moisture-regulating components and/or structure can neither be proven nor rejected.

pronounced. In literature, the high durability of *C. sativa* heartwood is attributed to its high concentration of tannins (Eichhorn et al. 2017; Scalbert 1991).

The natural durability of *R. pseudoacacia* is attributed to phenolic compounds and the flavonoid robinetin (Dünisch et al. 2010; Hart 1989), while flavonoids in general have only a very low toxic effect on decay fungi (Hart 1989). The growth rate of *T. versicolor* on *R. pseudoacacia* heartwood paste was indeed only 0.8 times the growth rate on pure malt agar medium, indicating the presence of fungicidal components effective against *T. versicolor*. However, it did not contain components fungicidal for *C. puteana* (Figure 2). It is therefore plausible that moisture-regulating components and/or anatomical structure also have impact on the high durability of the heartwood of this wood species against both fungi. Indeed, *R. pseudoacacia* has excellent moisture properties, as it is classified in absorption class 2, indicating a low amount of water absorption during a floating test (Van Acker et al. 2014), although it performs less on desorption (class 4).

The high durability of *D. benthamianus* heartwood against *C. puteana* (DC_m 1) and *T. versicolor* (DC_m 2) seemed to be due to the presence of fungicidal components only (Figure 2), since sapwood mini-blocks of these species were not resistant (Figure 4). However, moisture-regulating components could be present in the heartwood and not in the sapwood, which have an additional effect on the overall durability. This was demonstrated by the heartwood resistance of *Aucoumea klaineana* (DC_m 2) against *C. puteana*. The *A. klaineana* heartwood paste had no fungicidal effect (Figure 2) and the sapwood mini-block test neither indicated an influence of moisture-regulating components nor of wood structure (Figure 4). This could point to the presence of moisture-regulating components in the heartwood, not present in leached sapwood mini-blocks, which is plausible since both *D. benthamianus* and *A. klaineana* belong to absorption and desorption classes 2/3 and 3/3 (Table 1) and contain high concentrations of sterols and terpenes (Mounguengui et al. 2016). These molecules were important in heartwood durability of *A. klaineana* against *C. puteana* (DC_m 2), but did not seem to affect the resistance against *T. versicolor* (DC_m 4). *Entandrophragma cylindricum*, like *A. klaineana*, was a distinct example of a wood species where moisture-regulating components and/or structure were of importance for the heartwood durability, given the absence of any fungicidal effect in the heartwood paste test, and the higher durability of the (leached) sapwood (Table 2). *E. cylindricum* indeed contains a relatively high concentration of extractives soluble in cyclohexane (Jankowska et al. 2018), which probably corresponds to oleoresins (Hillis 2012). Note that *E. cylindricum* neither belongs to a good absorption class, nor to a desorption class (Table 1). However, the floating test might give a distorted result in the case of *E. cylindricum*, as this species has interlocking grain patterns leading to increased absorption through the longitudinal face.

The high durability of *P. avium* heartwood (DC_m 1) against *C. puteana* seemed to be primarily due to the presence of fungicidal components (Figure 2), since sapwood mini-blocks of this wood species were degraded substantially by *C. puteana* (Figure 4). Nevertheless, moisture-regulating components in the heartwood, not present in the (leached) sapwood, could be of importance (see above). It should be noted, however, that in a previous study on *P. avium* extractives, Kebbi-Benkeder et al. (2015) found high concentrations of flavonoids, but did not mention fatty acids or resin acids. Also, *P. avium* belongs to absorption class 5 and desorption class 4 (Table 1). The growth rate of *T. versicolor* on *P. avium* heartwood paste was 0.8 times the growth rate on a pure malt extract paste. Then again, fungicidal components only had a minor effect on the heartwood resistance against *T. versicolor* (DC_m 4), which could be attributed to a stronger effect or higher accessibility of the fungicidal components in a paste set-up than in an actual mini-block test.

Fagus sylvatica did not seem to have much protection against fungal attack under laboratory conditions (Table 2), which is already well known (CEN EN 305 standard 2016). There was no indication of structure/moisture-regulating components having a major impact, and the heartwood paste test indicated no fungicidal effects. In fact, the heartwood mini-blocks seemed to be slightly more degraded by *C. puteana* than the leached sapwood, potentially due to a higher amount of nutrients in the heartwood compared to the leached sapwood.

Picea abies and *P. sylvestris* were not durable against brown-rot fungus *C. puteana*, while they showed a high resistance against *T. versicolor*. This confirms common knowledge, since white-rot fungi are known to degrade softwoods slower than hardwoods, due to the difference in the main lignin type (Highley 1982). It should be noted, however, that both species indicated fungicidal activity against *C. puteana* and *T. versicolor,* while the heartwood was not or only slightly more durable than the sapwood (Figure 4). This fungicidal activity was most prominent for *P. sylvestris*, with heartwood paste results similar to those of *P. soyauxii*, maybe due to stilbenes in *P. sylvestris* (Chiron et al. 2000). In practice, wood containing stilbenes often decomposes slowly. When tested on a nutrient agar substrate, stilbenes have been shown to be highly fungicidal (Hart 1989). On a woody substrate, however, the toxicity of stilbenes is reduced with 90 up to 99% (Hart 1989). Furthermore, even though conifers contain resins (Rissanen et al. 2019; Schmidt 2006), both wood species belong to absorption and desorption class 5 (Table 1). Obviously, the type of moisture-regulating component as well as the location in the wood structure can be of major importance. Furthermore, since *C. puteana* is a true softwood-degrading specialist, the fungus might have developed another way to circumvent this particular arrangement of moisture-regulating components.

3.3 Impact of wood anatomy on natural durability

Although the test set-up can be used to assess the importance of fungicidal effects versus other decay-influencing factors, it did not allow to distinguish between the impact of moisture-regulating components and structure separately. Also, anatomical structure and moisture dynamics are often related, especially in the over-hygroscopic range (Brischke and Alfredsen 2020; Fredriksson 2019). For instance, wood with a low density can take up more water, as it has a larger volume of voids. Also, the size of the pit openings in the wood cells affects the desorption rate of wood (Fredriksson 2019). The wood-anatomical structure also affects degradation and degradation patterns, as the ratio of different cell types influences the penetration and colonization ability of fungal hyphae, and determines whether the fungus can tap into the nutrient sources in the wood structure (Antwi-Boasiako and Atta-Obeng 2009; Bravery 1975; Daniel 2003; Schwarze 2007). When the paste and mini-block test indicated an influence of structure and/or moisture-regulating components, the latter concurred with literature on such components in the respective wood species and literature on moisture dynamics, such as the absorption and desorption classes described in Van Acker et al. (2014). Similarly, a connection between wood-anatomical features (Table 2) and durability might be found. The wood species of interest are those for which also the sapwood mini-blocks are durable against degradation, as was the case for *P. soyauxii*, *C. sativa* and *E. cylindricum* against *C. puteana* (Figure 4).

One of the wood-anatomical features that have been correlated to durability is the amount of vessels versus the amount of fibers in the wood, or the vessel-fiber ratio (Table 1). Antwi-Boasiako and Atta-Obeng (2009) attribute the high durability of the Ghanaian hardwood species *Milicia excelsa* (Welw.) C.C. Berg to its low vessel-fiber ratio (5–20%). This factor is therefore also a likely contributor to the high durability of *P. soyauxii*, which has an average vessel-fiber ratio of 16.3%. Another feature that possibly increases decay resistance is a high parenchyma content (Table 1), although only against brown-rot fungi. Schwarze (2007) postulates that this resistance is related to the cell wall morphology of parenchyma cells, possibly reflecting a low co-evolutionary adaptation of brown rot fungi to the

xylem of hardwood species. *E. cylindricum* and *P. soyauxii* both have high parenchyma contents, supporting this theory. However, similar parenchyma contents did not seem to lead to a similar resistance against *C. puteana* in the case of *D. benthamianus*.

3.4 Importance of testing both brown-rot and white-rot fungi

In general, unraveling the durability of heartwood mini-blocks against *T. versicolor* was less straightforward than for *C. puteana*, since the former was less affected by the fungicidal components in the paste set-up. Both fungi have evolved differently and have developed different mechanisms of degradation (Floudas et al. 2012; Goodell and Jellison 1990; Mester et al. 2004; Schmidt 2006). The results from this study emphasized the importance of testing with both fungi, since some wood-protection mechanisms resulted in a high resistance against one fungus, but not against the other. The lignin barrier, for instance, resulted in DC_m 2 for softwoods against *T. versicolor* and DC_m 4 and 5 against *C. puteana*. Furthermore, hydrophobicity and/or anatomy also affected the growth of both fungi differently. While *P. soyauxii* sapwood was classified as DC_m 1 against *C. puteana*, these wood characteristics had a lesser effect on *T. versicolor* (DC_m 3). A similar behavior was found for *E. cylindricum*, for which the sapwood was classified as DC_m 4 and the heartwood as DC_m 3 for *T. versicolor*, indicating an effect of moisture-regulating components and/or anatomical structure, but less important than in the case of *C. puteana*. Finally, the mode of action of fungicidal components affecting fungal growth was of importance as well. Since the extracellular enzymes of brown-rot and white-rot fungi differ in their mode of action and molecular size, many wood-fungicidal components are not able to affect growth of a broad spectrum of fungal species, but act fungus specific (Hart 1989), which was confirmed by the results in this paper. Not only had fungicidal components less impact on the mycelial development of *T. versicolor* than of *C. puteana*, there were also wood species for which the fungicidal components appeared to be more effective against *C. puteana*.

4 Conclusion

The approach presented in this paper is useful to assess the importance of fungicidal effects on the durability of wood and wood-based products, and to reveal the impact of other decay-influencing factors such as wood-anatomical features, moisture-regulating components and lignin type. It does not allow to distinguish between the impact of moisture-regulating components and wood anatomy. When the paste and mini-block tests hinted at an effect of moisture-regulating components on the durability, literature on such components and moisture dynamics in the respective wood species agreed with these findings. When it comes to wood-anatomical features, vessel-fiber ratio and parenchyma content were selected as factors possibly influencing decay, although many other wood-anatomical features could be of importance. The low vessel-fiber ratio of *P. soyauxii* likely contributed to its high durability. A high parenchyma content could possibly play a role in the durability of *P. soyauxii* and *E. cylindricum* sapwood and heartwood, although this was, for instance, not the case for *D. benthamianus* sapwood resistance against brown-rot fungus *C. puteana*. Overall, it is clear that different wood species owe their durability to a combination of properties. While some species combined fungicidal components with proper moisture control (*P. soyauxii*), other species were (moderately) durable due to their fungicidal components (*P. avium*), or relied partly (*R. pseudoacacia, D. benthamianus*, *C. sativa*) or mainly (*A. klaineana*, *E. cylindricum*) on moisture-regulating components. In the case of white rot, the presence of guaiacyl lignin also seemed to play a role (*P. abies*, *P. sylvestris*).

This paper shows that fungicidal components are not always of major importance for the durability of a wood species. The authors hereby emphasize the importance of moisture-regulating components and wood anatomy on the durability of wood. These factors are especially interesting when considering them for engineered wood products and bio-based insulation products, as there are many opportunities to optimize the structural design and to alter the material's moisture dynamics.

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