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"PASTE TEST" – A METHOD FOR EVALUATING THE FUNGAL INHIBITION POTENTIAL OF GROUND WOOD SAMPLES
"TEST LESNE PASTE" – METODA ZA PREVERJANJE GLIVNE INHIBICIJE VZORCEV ZMLETEGA LESA

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ABSTRACT
As part of the »ULTRA – University of Ljubljana for a Sustainable Society« project, a new test method was introduced into the curriculum to provide insight into key properties that affect wood durability. This study aimed to adapt and verify the "paste test", which uses wood dust in nutrient media to assess the inhibitory effect of wood components on fungal growth. The method was adapted to match the available laboratory equipment and simplified for educational use by students at the Department of Wood Science and Technology (Biotechnical Faculty, University of Ljubljana, Slovenia). The study involved testing fungal growth on nutrient media supplemented with spruce wood dust, with the goal of evaluating different grinding methods, nutrient media, and data analysis. Potato dextrose agar nutrient media was confirmed as a suitable alternative to malt extract agar. The wood dust fraction size had no significant effect on fungal inhibition, enabling the use of coarser, less time-saving grinding methods. Image analysis and logistic growth modelling were conducted using freely available software (ImageJ and R-Commander), which proved to be effective alternatives to proprietary tools. Visualization of growth using logistic growth rates offered a clearer comparative analysis, despite some loss of dynamic growth information.

Keywords: inhibition, wood chemistry, nutrient media, white and brown rot fungi, logistic growth rate

IZVLEČEK
V okviru projekta »ULTRA – Univerza v Ljubljani za trajnostno družbo« smo želeli v učni program uvesti nov način testiranja, da bi nam omogočil vpogled v izbrane lastnosti, ki vplivajo na odpornost lesa. V ta namen smo preizkusili in optimizirali »Test lesne paste«, pri katerem se za oceno zaviralnega učinka lesnih komponent na rast gliv v hranilno gojišče doda lesni prah. Postopek je bilo treba prilagoditi razpoložljivi laboratorijski opremi in za izvedbo v okviru študijskega procesa na Oddelku za lesarstvo (Biotehniška fakulteta, Univerza v Ljubljani). S testiranjem preraščanja gliv na hranilnih gojiščih z dodanim smrekovim prahom smo ocenjevali uporabo različnih metod mletja in hranilnih gojišč ter možnosti analize podatkov. Ugotovili smo, da je hranilno gojišče s krompirjevim dekstroznim agarjem primerna alternativa gojišču iz sladnega agarja. Izkazalo se je, da velikost frakcije lesnega prahu nima bistvenega vpliva na zaviranje rasti gliv, zato je za test možno uporabiti tudi manj fine frakcije, ki jih dobimo z manj zamudnimi postopki mletja. Analizo slik in modeliranje logističnih krivulj rasti smo opravili z uporabo prosto dostopne programske opreme (ImageJ in R-Commander), ki je učinkovita alternativa plačljivim orodjem. Čeprav pri vizualizaciji hitrosti rasti z uporabo logističnih stopenj rasti izgubimo del informacije o dinamiki rasti, se je ta način izkazal za bolj preglednega v primerjavi s prikazom rastnih krivulj.

Ključne besede: inhibicija, kemija lesa, hranilno gojišče, glive bele in rjave trohnobe, logistična stopnja rasti

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1 INTRODUCTION
1 UVOD

To reduce human impact on the environment, the European Green Deal promotes the increased use of natural, renewable materials, including wood. The use of wood biomass should be prioritized due to its high economic and environmental added value. As a result, the use of wood is encouraged in increasingly demanding applications, especially in construction, where the material is exposed to outdoor conditions and thus requires high durability (EN 335, 2013). The durability of wood can be improved through specific wood preservation methods. Traditionally, biocides containing heavy metals and mineral oils, such as copper-based wood preservatives and creosote oil, were used to protect wood. These treatments considerably extended the service life of wood but were later found to be harmful to human health and the environment (Schultz et al., 2007; Humar, 2011). Consequently, the use of some active ingredients is now restricted (Regulation (EU) No 528/2012, 2012). In recent years, several alternative protection methods have emerged, including wood modification. However, these methods do not achieve the same level of effectiveness as traditional wood preservatives (Zelinka et al., 2022). Therefore, there is continued interest in developing new protection methods or using existing materials more thoughtfully, particularly those that offer sufficient durability for specific environments. A sound understanding of the properties of the wood species available on the market is essential in this context.

The durability of wood species depends on various material characteristics, including chemical composition, anatomical structure, and moisture dynamics (De Ligne et al., 2021). Among the main chemical components of wood, the composition and proportion of lignin significantly contribute to wood durability. For instance, conifers are more resistant to white rot fungi than deciduous trees due to their higher content of guaiacyl lignin, which is very compact and difficult to oxidize (Martín and López, 2023). Conversely, cellulose and hemicelluloses, along with the presence of nutrients (such as starch) in the wood, serve as the main nutrient source for decomposers (Evans et al., 2022). Extractives, which are species-specific, also play a crucial role in wood durability. These compounds form during the wood maturation process, and, although they make up only a relatively small proportion of the wood, they significantly influence its properties and potential applications (Kirker et al., 2013). Some extractives possess antifungal properties, which may be linked to mechanisms such as metal and free radical scavenging activity, direct interaction with enzymes, and disruption of membrane integrity and ion homeostasis (Fernández-Costas et al., 2017; Valette et al., 2017).

There are several options for testing the antifungal effects of individual material characteristics that affect durability. The inhibitory effect or fungicidal potential of various active substances (biocides, extractive substances) can be assessed through screening tests carried out directly on nutrient media, followed by tests on wood samples or in field trials. Screening tests are typically conducted in Petri dishes with solid nutrient media. The active substance can be mixed directly into the media, inserted into holes, or applied to filter paper placed around the edge of the Petri dish (Vek, 2015; Vek et al., 2020a; Vovchuk et al., 2024). The effect of extractives on wood durability can also be tested by comparing leached and unleached wood samples (Kirker et al., 2013), or by using extractive-impregnated samples (Vek et al., 2020b). A relatively new method known as the "paste test" has also been identified in the literature, revealing the influence of wood chemistry, including nutrients and antifungal components, on a wood species' resistance to fungi. As described by De Ligne et al. (2021), the "paste test" involves adding wood dust to the nutrient medium. This setup removes the influence of wood structure and moisture dynamics. In one example, De Ligne et al. (2021) demonstrated that fungi grew similarly on extracted sapwood from various species, indicating the levelling effect of this test design.

The aim of this study was to assess the feasibility of the "paste test" as part of the educational process at the Department of Wood Science and Technology (Biotechnical Faculty, University of Ljubljana, Slovenia). The research activities were carried out as part of the "ULTRA – University of Ljubljana for a Sustainable Society" project, a pilot initiative that promotes the integration of competencies crucial for the green and digital transition into the renewal of professional study programmes. Since some equipment described in the literature (De Ligne et al., 2021) is not available in the laboratories of the Department of Wood Science and Technology (Biotechnical Faculty, University of Ljubljana, Slovenia), and certain procedures are too time-consuming for educational use, this study focused on adapting and verifying a simplified version of the "paste test" for laboratory practice. As part of the study, fungal growth was tested on nutrient media supplemented with spruce wood dust to evaluate different nutrient media, milling procedures, and data analysis approaches. The adapted methods were validated using three wood species as examples.

2 MATERIALS AND METHODS

2 MATERIALI IN METODE

2.1 Materials

2.1 Materiali

Fresh wood from three species was used in the tests: Scots pine (*Pinus sylvestris*), black locust (*Robinia pseudoacacia*) heartwood, and spruce (*Picea abies*) sapwood, which served as the control. The wood was sourced from a sawmill (LESCOM, d.o.o., Medvode, Slovenia). Prior to the experiments, the wood was dried to an absolutely dry state. Two commercially available nutrient media mixtures were used: Malt Extract Agar (MEA; DIFCO™, Becton, Dickinson and Company, USA) and Potato Dextrose Agar (PDA; DIFCO™, Becton, Dickinson and Company, USA). The fungal cultures *Gloeophyllum trabeum* (ZIM L018) and *Trametes versicolor* (ZIM L056) were obtained from the fungal culture collection of the Department of Wood Science and Technology (Biotechnical Faculty, University of Ljubljana, Slovenia).

2.2 Nutrient media

2.2 Hranilno gojišče

De Ligne et al. (2021) used MEA as the nutrient medium in the "paste test». Since fungal tests at the Department of Wood Science and Technology (Biotechnical Faculty, University of Ljubljana, Slovenia) are typically performed on PDA, this study examined whether the "paste test" could also be conducted using PDA. Because the fungal isolates from the department's culture collection are adapted to grow on PDA, they were inoculated several times in succession on MEA before testing to acclimate them to the new nutrient source. One gram of spruce dust, ground as prescribed by De Ligne et al. (2021), was added to 17 ml of nutrient medium in Petri dishes. The medium mixed with wood dust was sterilized by autoclaving at 121 °C and 150 kPa for 20 minutes. After the nutrient medium mixed with wood dust had cooled and solidified, each Petri dish was inoculated with the fungal isolate (d = 0.7cm). The growth of *Gloeophyllum trabeum* (brown rot) and *Trametes versicolor* (white rot) was evaluated. For each nutrient medium and fungus, three replicates of Petri dishes with added spruce dust (0.12 mm) and one replicate of the control medium without added dust were prepared.

2.3 Sample grinding
2.3 Mletje vzorcev

According to De Ligne et al. (2021), grinding is performed in two stages: initial grinding with a cutting mill (SM 2000; Retsch GmbH, Haan, Germany) using a 0.25 mm sieve, followed by finer grinding with a centrifugal mill (ZM 200; Retsch GmbH, Haan, Germany) using a 0.1 mm sieve. Both mills are available in the laboratories at the Department of Wood Science and Technology (Biotechnical Faculty, University of Ljubljana, Slovenia). However, grinding with the centrifugal mill (ZM 200; Retsch GmbH, Haan, Germany) quickly leads to overheating, requiring the use of dry ice, which causes additional dust due to rapid evaporation. This method is also very time-consuming. To enable the use of the method in a teaching laboratory setting, the grinding process was adapted and simplified, including the use of pre-ground samples. Several grinding procedures were tested to determine whether less fine fractions could be used effectively (Fig. 1). In procedure A, grinding was conducted using a process nearly identical to that described by De Ligne et al. (2021), first applying a 1 mm sieve in the cutting mill and then a 0.12 mm sieve in the centrifugal mill. In procedure B, grinding was performed with a cutting mill (0.25 mm sieve). The samples were previously ground using a 1 mm sieve or consisted of tiny pieces obtained using a chisel (Fig. 2b, c). Procedure C involved further grinding the samples with an IKA mill (undefined particle size) after processing on a cutting mill (1 mm sieve). The resulting wood fractions were mixed with the selected nutrient medium (described in Section 2.1) at a ratio of 1 g wood to 17 ml of medium to form a paste. Fungal isolates were then introduced, and incubation conditions followed the protocol outlined in Section 2.1.

2.4 Monitoring of fungal growth
2.4 Spremljanje rasti gliv

Petri dishes were incubated for 10 days at 25 °C and 80% relative humidity. Mycelial growth was monitored by capturing images using a flatbed scanner once a day (Mustek A3 2400S, Mustek Systems Inc., Taiwan). Image analysis was performed with Fiji (Schindelin et al., 2012), which is based on ImageJ (Schneider et al., 2012). The mycelial surface area was measured using

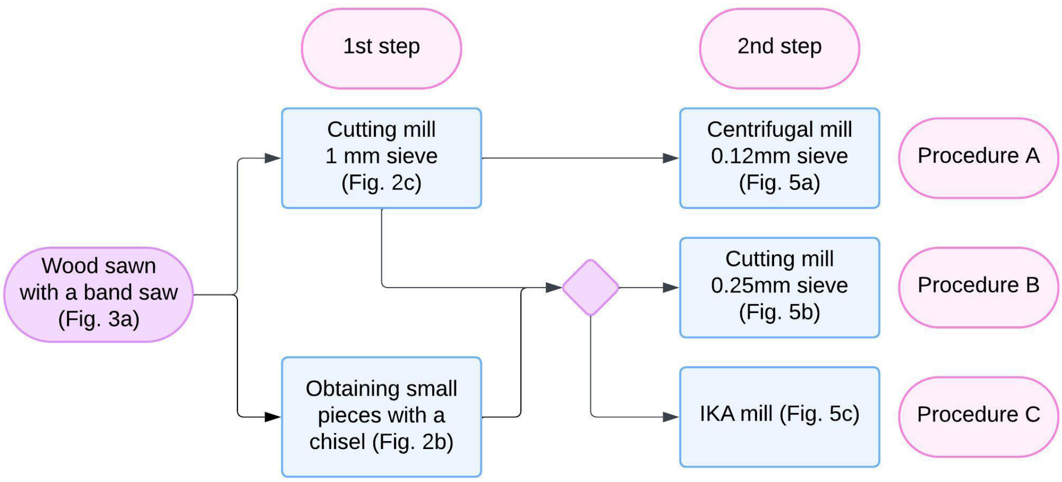

Fig. 1: Flowchart of grinding procedures.

Slika 1: Shema postopkov mletja

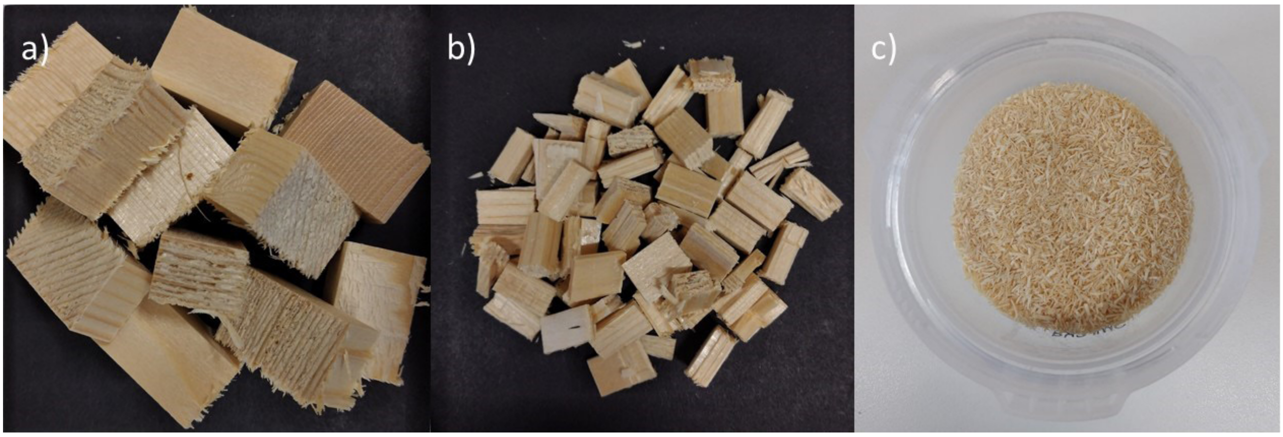

Fig. 2: Fractions suitable for grinding in different mills: a) samples prepared with a band saw, with dimensions of approximately 1.0 x 1.5 x 2.0 cm3, suitable for the first stage of grinding; b) samples sawn and later split using a chisel and hammer; and c) fraction obtained after grinding with a cutting mill (thickness and width less than 1 mm).

Slika 2: Frakcije, primerne za mletje na različnih mlinih; a) vzorci pripravljeni s tračno žago, dimenzij približno (1,0 x 1,5 x 2,0) cm3, primerni za prvo stopnjo mletja, b) vzorci, ki so bili razžagani in kasneje razklani s pomočjo dleta in kladiva, in c) frakcija po mletju z rezalnim mlinom (debelina in širina sta manjši od 1 mm)

the Wand tool in Fiji. Before measurement, the image background was subtracted, and the images were segmented using adaptive thresholding (Fig. 3). Each image was then manually reviewed. In cases where images were unclear, for example, due to shadows or condensation, the mycelial surface area was determined manually using the Oval tool.

R-Commander (Fox, 2005) was used to fit logistic growth curves (Fig. 4) per Petri dish, from which the logistic growth rate (C) per replicate was derived (Cheng, 2014):

$$f(x) = \frac{A}{1 + e^{-(B+Cx)}} \tag{1}$$

where A represents the curve's asymptote and C is the logistic growth rate or steepness of the curve (Fig. 4).

2.5 Validation of the customised test on three wood species

2.5 Validacija prilagojenega testa na treh lesnih vrstah

The "paste test" was used to evaluate the fungal inhibition potential of black locust (*Robinia pseudoacacia*), Scots pine (*Pinus sylvestris*), and Norway spruce (*Picea abies*). Black locust is considered a highly durable wood species due to its high content of extractives (Vek et al., 2019), while Scots pine and Norway spruce are moderately to less durable (EN, 2016). The experiment followed the same preparation procedure as described in the previous chapters. All adjustments summarized in Table 1 were used.

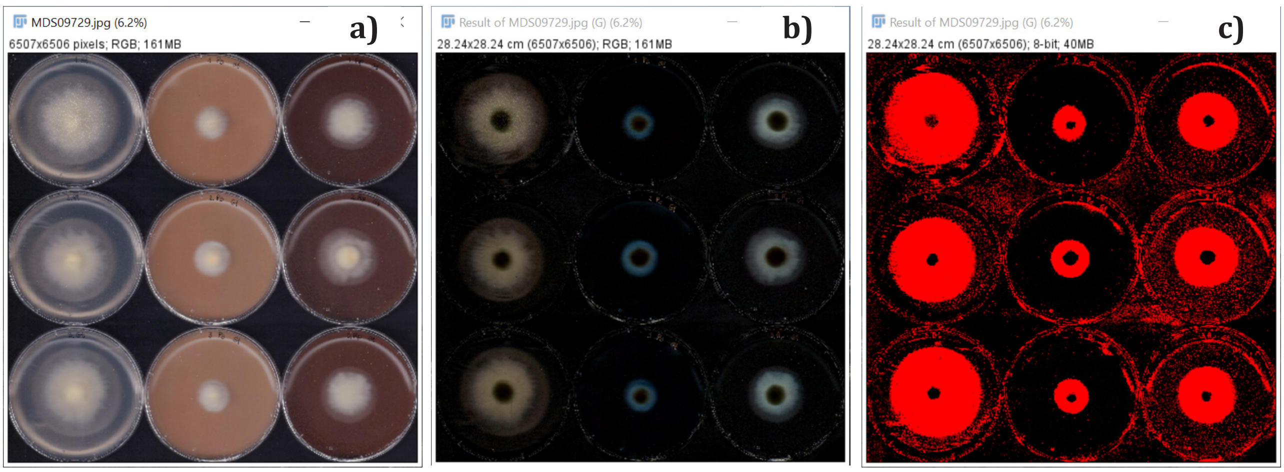

Fig. 3: Image processing workflow for measuring the mycelial surface area: a) raw image, b) image after background subtraction and c) image segmented by adaptive thresholding.

Slika 3: Postopek obdelave slike za meritve površine micelija: a) neobdelana slika, b) slika z odštetim ozadjem, in c) segmentirana slika s prilagojenim pragom

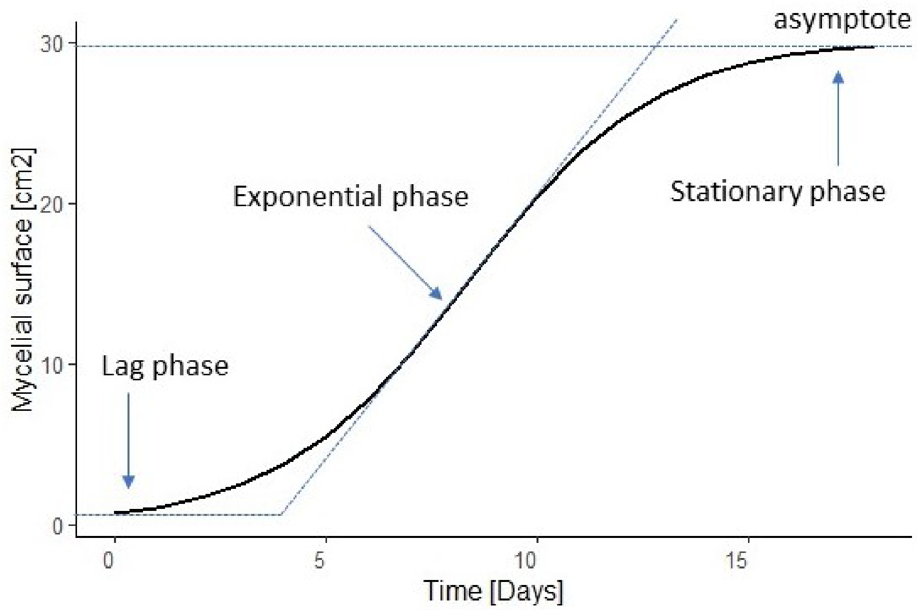

Fig. 4: Logistic growth curve showing typical growth phases.

Slika 4: Logistična krivulja rasti z značilnimi fazami rasti

3 RESULTS AND DISCUSSION

3 REZULTATI IN RAZPRAVA

To perform the "paste test" during laboratory practice in our facilities, the procedure described by De Ligne et al. (2021) had to be slightly adapted (Table 1). Some adaptations were due to differences in available laboratory equipment, while others required testing for suitability. Since our laboratory does not have access to gamma irradiation sterilization equipment, sterilization was conducted using autoclaving. The fungal strains used in our study were those most commonly employed in other wood durability tests. Growth conditions in our growth chambers were not specifically modified for this test, which resulted in slight differences from those reported by De Ligne et al. (2021). We concluded that capturing images once a day, excluding weekends, was sufficient for monitoring fungal growth. Due to the lower resolution of our flatbed scanner (Mustek A3 2400S) compared to the one used in the original study, adaptations to the image analysis procedure were required, as described in Section 2.3. Instead of the licensed software MATLAB, the freely available programs ImageJ (Fiji) and R-Commander were used for image analysis and growth curve modelling.

3.1 Nutrient media

3.1 Hranilno gojišče

Even after repeated inoculations, fungal growth on malt agar medium remained sparse and less visible, which posed challenges for capturing and further analysing images (Fig. 5). The inhibitory effect of

Table 1: Test execution procedures, necessary equipment cited in the literature (De Ligne et al., 2021), and adaptations used in this study.

Preglednica 1: Postopki izvedbe poizkusa oz. potrebna oprema, navedeni v literaturi (De Ligne et al., 2021) in njihove prilagoditve

Procedure	Literature (De Ligne et al., 2021)	Adaptations
Grinding procedure	Procedure A	Procedure C
Sterilisation	25–50 kGy Gama irradiation	Autoclaving
Nutrient medium	2% malt, 2% agar	Potato dextrose agar
Fungi	*C. puteana* *T. versicolor*	*G. trabeum* *T. versicolor*
Number of repetitions	6	3
Growing conditions	22 °C in 70% RH	25 °C in 85% RH
Growth monitoring	10 days	10 or 14 days
Image acquisition	Twice daily	Once daily (except weekends)
	Flatbed scanner (Epson perfection V750 Pro)	Flatbed scanner (Mustek A3 2400S)
Image processing	MATLAB	Image J (Fiji)
Logistic growth rate modelling	MATLAB	R-Commander

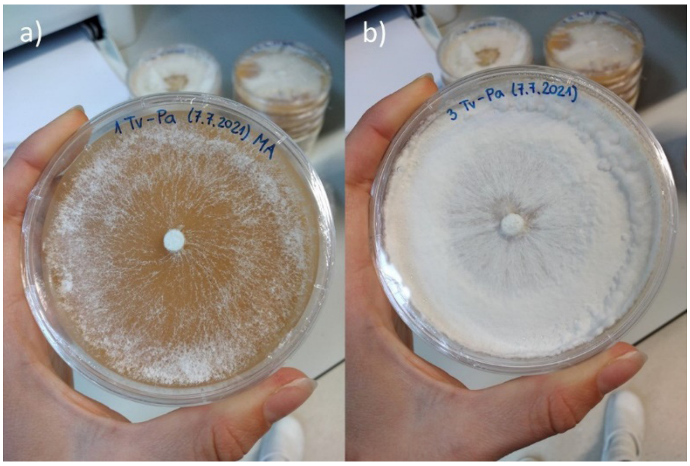

Fig. 5: Growth density of *T. versicolor* on nutrient media: a) malt extract agar, b) potato dextrose agar.

Slika 5: Različna gostota rasti *T. versicolor* na hranilnem gojišču iz a) sladnega agarja ter b) krompirjevega dekstroznega agarja

wood dust on fungal growth was relatively consistent across both media. The variation in fungal growth between the isolates in individual Petri dishes was lower on the PDA medium compared to MEA (Fig. 6). Based on these results, PDA was determined to be the more suitable option in our conditions. Our scanner has a significantly lower optical resolution (2400 dpi) than the one used in the referenced study (6400 dpi), making the contrast between the fungal mycelium and the medium even more crucial for subsequent image processing and analysis. Higher contrast can be achieved if the fungus grows more densely, more fully covering the surface of the medium.

3.2 Sample grinding

3.2 Mletje vzorcev

The mycelial growth test on different substrates was conducted using nutrient media containing wood dust ground to various particle sizes. Figure 7 shows the wood fractions produced by three different griding procedures. In procedure A, the material was finely ground using a centrifugal mill with a 0.12 mm sieve, resulting in a defined particle size and a homogeneous appearance (Fig. 7a). Although effective, this method is time-consuming and requires dry ice to prevent overheating, making it impractical for use in routine laboratory practice. Therefore, pre-ground material

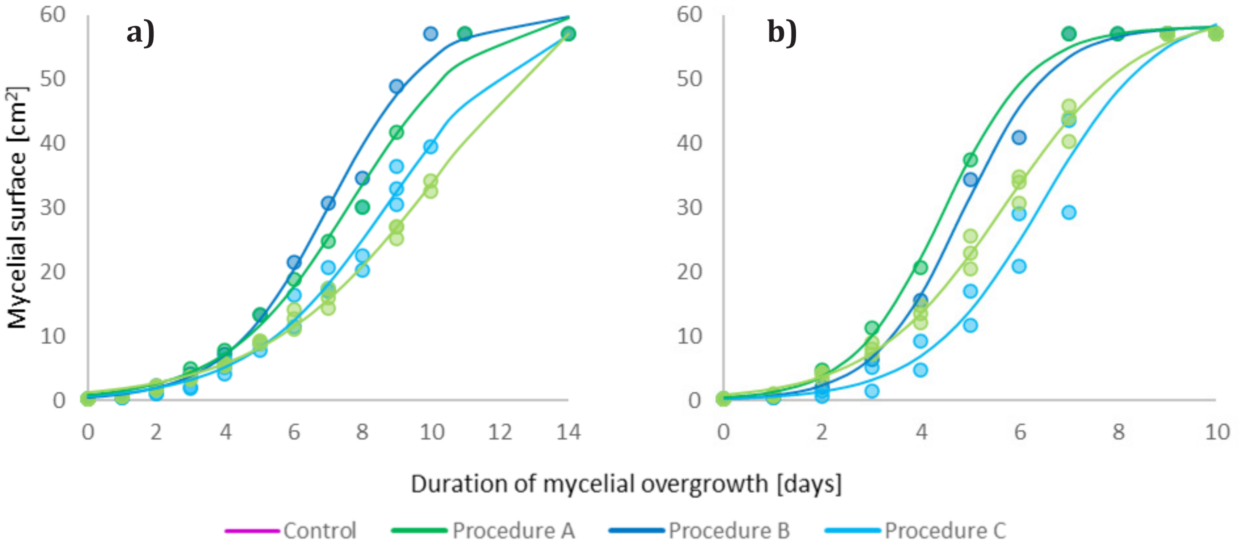

Fig. 6: Growth curves of a) *Gloeophyllum trabeum* and b) *Trametes versicolor* on malt extract agar (MEA C), malt extract agar with added spruce wood (MEA S), potato dextrose agar (PDA C), and potato dextrose agar with spruce wood (PDA S). Points represent actual measurements, while the logistic curves are calculated from the mean values.

Slika 6: Krivulje preraščanja a) *Gloeophyllum trabeum* in b) *Trametes versicolor* na sladnem agarju (MEA C), sladnem agarju z dodano smrekovino (MEA S), krompirjevem dekstroznem agarju (PDA C) in krompirjevem dekstroznem agarju s smrekovino (PDA S). Točke ponazarjajo realne meritve, logistične krivulje so izračunane iz povprečja meritev.

Fig. 7: Samples after the second grinding stage: a) Procedure A – centrifugal mill (0.12 mm sieve), b) Procedure B – cutting mill (0.25 mm sieve), and c) Procedure C – IKA grinder (undefined size).

Slika 7: Vzorci po drugi fazi mletja: a) Postopek A: pomleti s centrifugalnim mlinom (0,12 mm sito), b) Postopek B: z rezalnim mlinom (0,25 mm sito) in c) Postopek C: mlinčkom IKA (nedefinirana velikost)

or alternative grinding methods must be considered. In procedure B, the material was ground using a cutting mill with a 0.25 mm sieve. The resulting powder also had a defined particle size (< 0.25 mm), but small chips were visible in the powder (Fig. 7b). Procedure C involved grinding the samples with an IKA mill, producing material that appeared identical to that from Procedure B. However, the particle size was not clearly defined due to nature of the grinding method (Fig. 7c).

As shown in Figure 8, none of the fungi tested demonstrated significant differences in growth rate based on the fraction of wood dust mixed with the nutrient medium. *Gleophyllum trabeum* required 10 days to fully colonise the nutrient medium, while *Trametes versicolor* did so in only 7 days. However, for more precise analyses, it would be advisable to follow the procedure described in the literature (Procedure A), as the presence of chips in the fractions obtained via Procedures B and C may introduce structural variables that cannot be completely excluded. Nevertheless, both alternative procedures (B and C) are suitable for laboratory practice and basic analysis. Procedure C is preferable, as griding with the IKA mill is somewhat faster and requires less cleaning than the cutting mill with a 0.25 mm sieve. Regardless of the nutrient medium used, it was observed that the *Gloeophyllum trabeum* mycelium became sparser toward the edge of the Petri dish, irrespective of the wood powder used. Therefore, in addition to image analysis, visual assessment should also be considered when assessing growth.

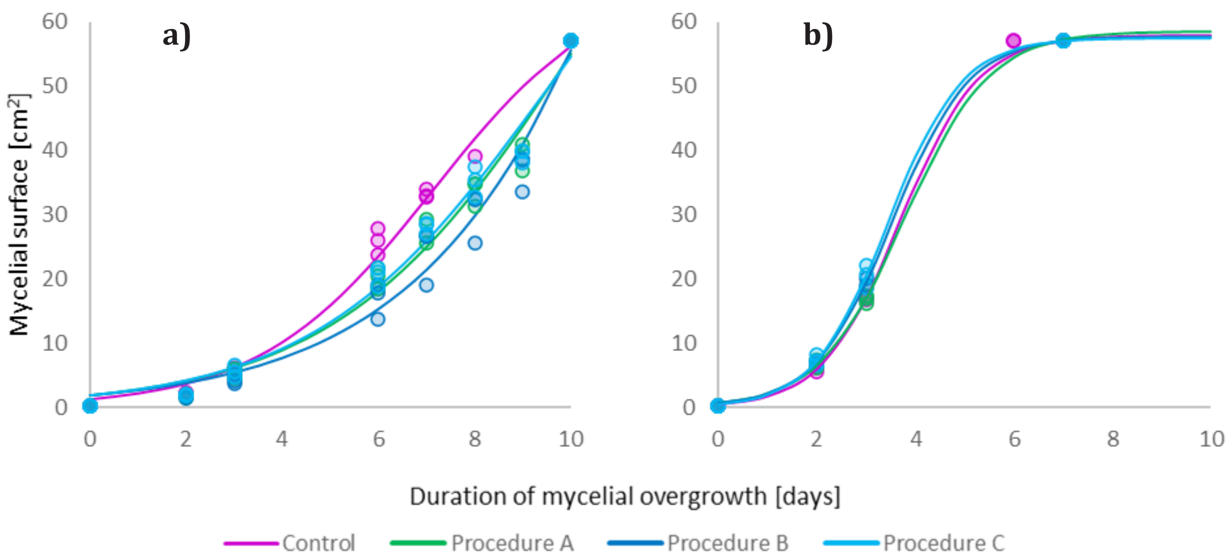

Fig. 8: Growth curves of a) *Gloeophyllum trabeum* and b) *Trametes versicolor* on PDA nutrient medium (Control) and nutrient media supplemented with the various fractions shown in Figure 7. Points represent actual measurements, while the logistic curves are calculated from the mean values.

Slika 8: Krivulje preraščanja a) *Gloeophyllum trabeum* in b) *Trametes versicolor* na čistem hranilnem gojišču (Control) in hranilnih gojiščih, ki so jim bile dodane različno pomlete frakcije, predstavljene na sliki 7. Točke ponazarjajo realne meritve, logistične krivulje so izračunane iz povprečja meritev.

2.3 Monitoring of the fungal growth and validation of the customised test on three wood species

2.3 Spremljanje rasti gliv in validacija prilagojenega testa na treh lesnih vrstah

All tested wood species inhibited the growth of the fungi to a certain degree. Since both coniferous wood species showed weaker inhibition of white rot fungus (*Trametes versicolor*) than brown rot fungus (*Gloeophyllum trabeum*), it can be concluded that lignin composition (Martín and López, 2023) does not significantly affect fungal inhibition in this test. Due to the collapsed cellular structure of the wood dust in this experiment, nutritional components are more readily accessible to fungi, and the non-enzymatic degradation strategy that naturally favours brown rot fungi in wood decomposition is not expressed (Brischke and Meyer-Veltrup, 2016; Thybring et al., 2018). Therefore, the inhibitory effect of the wood species is more pronounced in *Gloeophyllum trabeum* compared to *Trametes versicolor*. Based on these findings and those previously reported by De Ligne et al. (2021), it is assumed that the inhibition potential of the wood species observed in this test mainly results from the antifungal effect of the extractive substances. The extractive content in the studied wood species was determined in previous studies conducted on parallel samples of the same materials applied in the current "paste test", as summarised in Table 2.

The strongest inhibitory effect was observed in Scots pine samples and the weakest in Norway spruce samples (Figs. 9 and 10). The weak inhibitory effect of spruce was expected and is consistent with its low resistance to fungal decay, as defined in the standard (EN, 2016). Compared with other wood species, Norway spruce contains significantly lower amounts of extractives. Furthermore, no index extractive could be detected in Norway spruce. Scots pine, in contrast, contains 2–3% hydrophilic extractives and 1.6% lipophilic extractives, with the stilbenes pinosylvin and pinosylvin monomethyl ether predominating (Keržič et al., 2023). Vek et al. (2020c) demonstrated that pinosylvins extracted from pine knotwood inhibit the growth of both white and brown rot fungi. Black locust contains an even higher proportion of hydrophilic extractives, approximately 5% (Humar et al., 2024). Black locust is primarily characterized by the flavonoids dihydrorobinetin and robinetin, which have antifungal effects (Sergent et al., 2014). Despite this higher proportion of extractives, black locust exhibited a less pronounced inhibitory effect on *Gloeophyllum trabeum* in the "paste test" and comparable inhibition on *Trametes versicolor* compared to Scots pine. These results indicate that the inhibitory effect of wood species is primarily influenced by the composition of the extractives rather than their quantity. Furthermore, the inhibitory effect of the wood's chemical components is not the only factor influencing its durability against fungal decay. In the case of black locust, in addition to the extractives, its excellent wood durability is also supported by favourable moisture behaviour (Humar et al., 2024). Moreover, its durability is closely related to the proportion of earlywood vessels containing tyloses (Brischke et al., 2024).

The approach employed by De Ligne et al. (2021) was also tested to illustrate the results of the "paste test" (Fig. 10). In this approach, growth was summarised with the coefficient C (Equation 1, Fig. 4), which represents the logistic growth rate or the slope of the curve. This coefficient best describes the exponential growth phase, although it does not account for the lag or stationary phases, which are lost in this form of data presentation. The inhibitory effect of substances on fungal growth can be reflected in a prolonged lag phase, meaning slower initial fungal growth, as the fungus must still acclimatise to the substrate (Lekou-

Table 2: Proportions [%] of extractive substances soluble in different solvents and index extractives in absolutely dry wood of the studied wood species, summarised according to Keržič et al. (2023, 2024).

Preglednica 2: Deleži [%] ekstraktivnih snovi, topnih v različnih topilih in indeksnih ekstraktivov v absolutno suhem lesu preučevanih lesnih vrst. Povzeto po Keržič et al. (2023, 2024).

Procedure	Norway spruce (*Picea abies*)	Scots pine (*Pinus sylvestris*)		Black locust (*Robinia pseudoacacia*)	
Hexane-soluble extractives	0.30	1.61		0.05	
Acetone-soluble extractives	0.73	2.00		3.32	
Ethanol-soluble extractives	1.05	2.76		8.17	
Methanol-soluble extractives	1.41	3.08		9.84	
Water-soluble extractives	2.05	2.15		8.86	
Index extractives	-	Pinosylvin	0.17	Dihydrorobinetin	0.74
		Pinosylvin monomethyl ether	0.12	Robinetin	0.58

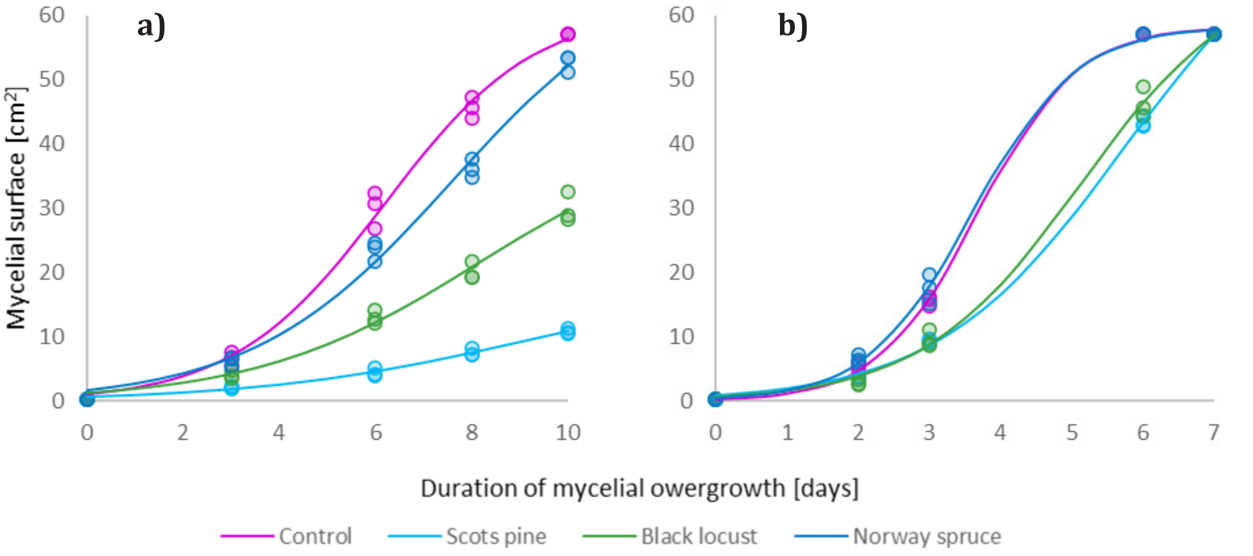

Fig. 9: Growth curves of a) *Gloeophyllum trabeum* and b) *Trametes versicolor* on nutrient media supplemented with wood dust from different wood species. Points represent actual measurements, while the logistic curves are calculated from the mean values.

Slika 9: Krivulje preraščanja a) *Gloeophyllum trabeum* in b) *Trametes versicolor* na hranilnih gojiščih, ki jim je bil dodan prah različnih lesnih vrst. Točke ponazarjajo realne meritve, logistične krivulje so izračunane iz povprečja meritev.

nougou et al., 2008). Therefore, results expressed solely by relative growth rates must be interpreted with caution and should be reserved for exceptional cases where a large number of comparisons are being made, and where presenting the results as logistic growth curves (Fig. 9) would be too visually complex.

4 CONCLUSIONS

4 ZAKLJUČKI

In the present study, several important adaptations of the "paste test", originally described by De Ligne et al. (2021), were successfully tested to facilitate its application in laboratory work and teaching at the Department of Wood Science and Technology (Biotechnical Faculty, University of Ljubljana, Slovenia). In particular, the test was adapted for use with potato dextrose agar instead of malt extract agar. It was also demonstrated that the size of the wood fraction has no significant influence on the inhibitory effect of wood components on the growth of the fungal species studied. Grinding methods that produce less fine fractions but are also less time-consuming can therefore be used

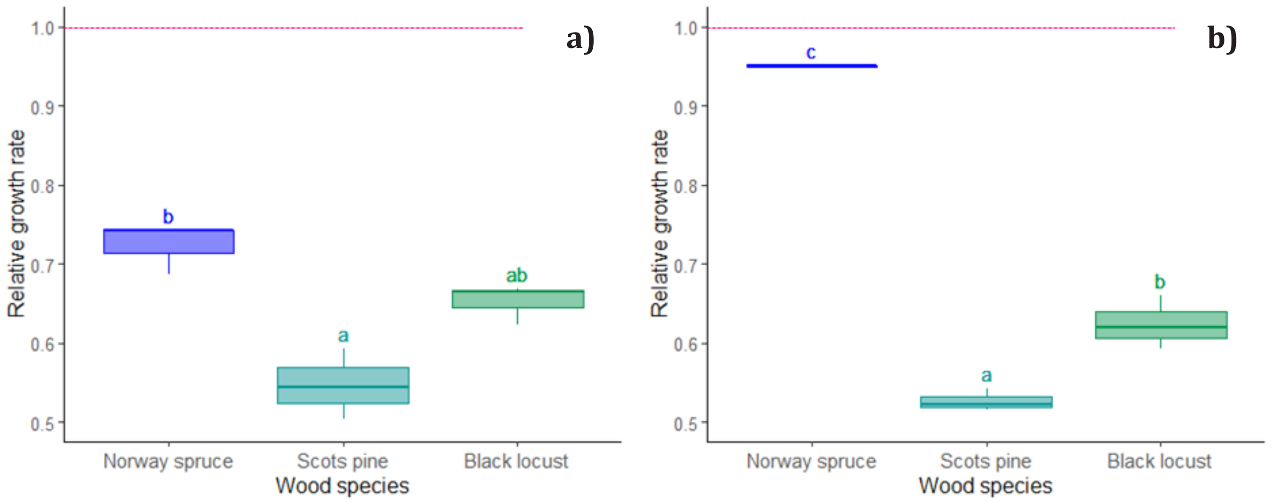

Fig. 10: Relative growth rates of a) *Gloeophyllum trabeum* and b) *Trametes versicolor* on nutrient media supplemented with wood dust from different wood species. The dashed line shows the median relative growth rate on control medium without added wood dust.

Slika 10: Relativna stopnja rasti a) *Gloeophyllum trabeum* in b) *Trametes versicolor* na gojiščih, ki jim je bil dodan lesni prah različnih lesnih vrst. Črtkana črta prikazuje mediano relativne stopnje rasti na kontrolnem gojišču brez dodanega lesnega prahu.

for basic analysis. The use of freely available computer programs for data processing was also investigated. Two approaches to graphical data visualization were considered: plotting growth curves, which provide insight into growth dynamics over the full observation period, and plotting logistic growth rates, which reflect only the speed of the exponential growth phase. While the latter method omits some information, it offers a clearer and more concise way to present a large number of comparisons.

5 SUMMARY

5 POVZETEK

Dobro poznavanje lastnosti lesa različnih lesnih vrst, ki so nam na voljo na našem tržišču, je ključnega pomena za razvoj novih postopkov zaščite oz. čim bolj premišljeno uporabo že obstoječih materialov. V ta namen smo preverili možnost uvedbe »Testa lesne paste« v učni program. Raziskovalne aktivnosti smo opravljali v okviru projekta »ULTRA – Univerza v Ljubljani za trajnostno družbo«, ki celostno obravnava integracijo kompetenc, ključnih za zeleni in digitalni prehod, v strokovne študijske programe. Pri »Testu lesne paste« se v hranilno gojišče doda fino mlet lesni prah, nanj se cepi glivno kulturo in nato spremlja rast gliv. Tako lahko raziščemo vpliv kemije lesa na odpornost lesne vrste na glive. Na Oddelku za lesarstvo (Biotehniška fakulteta, Univerza v Ljubljani, Slovenija) nimamo popolnoma enake opreme oz. določene postopke navadno opravljamo na nekoliko drugačen način kot je navedeno v literaturi, v kateri smo zasledili »Test lesne paste«. Da bi test lahko opravljali pri študentskih vajah v naših laboratorijih, smo si zastavili cilj, da poizkus naveden v literaturi nekoliko prilagodimo.

Nekatere prilagoditve se nanašajo zgolj na uporabo druge laboratorijske opreme, medtem ko je bilo treba nekatere prilagoditve preizkusiti, če so ustrezne. Ker nimamo opreme za sterilizacijo z gama žarki, smo sterilizacijo opravili z avtoklaviranjem. Uporabili smo glivi *Gloeophyllum trabeum* in *Trametes versicolor*, ki ju najpogosteje uporabljamo tudi pri drugih testiranjih odpornosti lesa na glive. Rastnih pogojev, ki so splošno nastavljeni v naših rastnih komorah, nismo spreminjali posebej za izvedbo tega preizkusa, zato so se nekoliko razlikovali od tistih, navedenih v literaturi. Sklenili smo, da je za namen opravljanja vaj za spremljanje rasti gliv zadostno izvajati zajemanje slik enkrat dnevno, pri čemer so vikendi izvzeti. Za zajem slik smo uporabljali skener Mustek A3 2400S, ki ima slabšo ločljivost kot skener, uporabljen v literaturi, zato smo morali prilagoditi nadaljnje postopke analize slike. Za analizo slik in modeliranje krivulje rasti gliv smo namesto plačljivega Matlab-a uporabili prosto dostopna programa ImageJ (Fiji) in R-Commander.

Preizkusili smo, kako izolati iz zbirke Oddelka za lesarstvo, ki so navadno gojeni na krompirjevo glukoznem agarju (PDA) preraščajo gojišče iz sladnega agarja. Obenem smo preverili tudi, ali se hitrost rasti razlikuje med različnima gojiščema oz. ali je možno namesto sladnega agarja uporabiti kar PDA-gojišče, ki ga navadno uporabljamo. Čeprav smo pred preizkusom glive večkrat zaporedoma precepili na sladno agarno gojišče, da so se lahko nekoliko navadile na nov vir hranil, je bila rast izolatov na sladnem agarju zaradi manj gostega prepleta hif slabše razvidna, kar bi lahko pomenilo težavo pri zajemu slik in nadaljnji analizi. Zaviralni učinek lesnega prahu na preraščanje gliv je bil relativno enak za obe gojišči. Odkloni med posameznimi petrijevkami so bili manjši na PDA-gojišču. Na podlagi rezultatov smo se odločili, da je uporaba PDA-gojišča v našem primeru najbolj primerna. Naš optični bralnik ima namreč veliko slabšo optično ločljivost od tistega, uporabljenega v izbranem članku, zato je kontrast med glivnim micelijem in gojiščem še toliko bolj pomemben za nadaljnjo obdelavo in analizo slik.

Ugotovili smo, da je mletje precej zahteven oz. predvsem zamuden postopek. Mletje v skladu z literaturo poteka v dveh stopnjah: 1. mletje na rezalnem mlinu Retsch SM 2000 z 0,25 mm sitom; 2. mletje na centrifugalnem mlinu Retsch ZM 200 z 0,12 mm sitom. Pri mletju na centrifugalnem mlinu je hitro prihajalo do pregrevanja, zato smo uporabili suhi led, ki pa je zaradi hitrega izhlapevanja povzročal dodatno prašenje. Za izvedbo tega eksperimenta pri vajah bi bilo tako treba imeti vnaprej pomlete vzorce oz. prilagoditi oz. poenostaviti postopek mletja. Poleg postopka, opravljenega v literaturi, smo preizkusili še dve opciji ter preverili, ali je možno uporabiti tudi manj fine frakcije. Pri obeh alternativnih postopkih mletja smo dobili manj fino pomlet material, kot je naveden v literaturi, vendar pri obeh testiranih glivah ni bilo opaziti večjih razlik v hitrosti preraščanja glede na uporabljeno frakcijo lesnega prahu, primešanega gojišču. Tako smo sklenili, da je za osnovno analizo in izvedbo pri študentskih vajah možno uporabiti tudi načine mletja, pri katerih sicer dobimo manj fine frakcije, vendar so manj časovno zamudni. Kljub temu bi bilo za natančnejše raziskave primerneje uporabiti enak postopek, kot je naveden v literaturi, saj glede na prisotnost iveri v frakcijah, pridobljenih z alternativnima postopkoma mletja, ni možno popolnoma izključiti vpliva strukture materiala.

»Test lesne paste« smo validirali na primeru robinije, bora in smreke. Robinija velja za zelo odporno lesno vrsto, medtem ko bor in smreka veljata za zmerno oz. slabše odporni lesni vrsti. Vse lesne vrste so do določene mere zavirale rast testiranih gliv. Največji inhibitoren učinek so imeli vzorci bora ter najmanjšega smreka. Slab inhibitoren učinek smrekovine je bil pričakovan in v skladu z njeno slabo odpornostjo na glivni razkroj. Na drugi strani je bila inhibicija rasti obeh gliv pri boru najbolj očitna, čeprav le-ta velja za zmerno odporno vrsto. Glede na dejstvo, da je bil inhibitoren učinek lesa robinije, kljub večji vsebnosti ekstraktivov, manjši ali primerljiv učinku bora, smo zaključili, da ima vrsta ekstraktivov večji vpliv na odpornost lesa kot njihova količina. Poleg tega smo sklepali, da inhibitoren učinek kemijskih komponent lesa ni edini dejavnik, ki vpliva na odpornost lesa proti glivnemu razkroju. Tako npr. pri robiniji poleg ekstraktivov k odlični odpornosti lesa pomembno prispeva tudi njena ugodna odpornost proti navlaževanju. Poleg tega je odpornost lesa robinije tesno povezana tudi z deležem otiljenih trahej v ranem lesu.

Ker smo ugotovili, da prikaz rezultatov z logističnimi krivuljami preraščanja micelija pri večjem številu primerjav postane nepregleden, smo za predstavitev rezultatov uporabili še koeficient C, ki ponazarja logistično stopnjo rasti oz. strmino krivulje v eksponentni fazi rasti. Ta koeficient najbolje opiše eksponentno fazo rasti, informacije o preostalih fazah pa pri tem prikazu rezultatov izgubimo. Čeprav pri slednjem načinu prikaza podatkov izgubimo del informacije, je le-ta bolj pregleden in s tem primernejši za prikaz večjega števila primerjav.

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REFERENCES
VIRI

Brischke C., Meyer-Veltrup L. 2016. Modelling timber decay caused by brown rot fungi. Materials and Structures, 49: 3281–3291. https://doi.org/10.1617/s11527-015-0719-y

Brischke C., Stolze H., Koddenberg T., Vek V., Caesar C.M.C., Steffen B., Taylor A.M., Humar M. 2024. Origin-specific differences in the durability of black locust (*Robinia pseudoacacia*) wood against wood-destroying basidiomycetes. Wood Science and Technology, 58: 1427–1449. https://doi.org/10.1007/s00226-024-01571-0

Cheng B. 2014. Modeling logistic growth data in R. Marine global change ecology, https://bscheng.com/2014/05/07/modeling-logistic-growth-data-in-r/ (March 8, 2024)

De Ligne L., Van den Bulcke J., Baetens J., De Baets B., Wang G., De Windt I., Beeckman H., Van Acker J. 2021. Unravelling the natural durability of wood: revealing the impact of decay-influencing characteristics other than fungicidal components. Holzforschung, 75, 4: 368–378. https://doi.org/10.1515/hf-2020-0109

EN 335: Durability of wood and wood-based products - Use classes: definitions, application to solid wood and wood-based products. 2013. https://standards.iteh.ai/catalog/standards/cen/e5d368b1-2232-47e2-8349-ee85cb6c895b/en-335-2013?srsltid=AfmBOoo364bRzMAOvwFcI5YI-sop3S4qjihKdrGryifTdsoOLMEY4fm7 (April 2, 2025).

EN 350: Durability of wood and wood-based products. Testing and classification of the durability to biological agents of wood and wood-based materials. 2016. https://standards.iteh.ai/catalog/standards/cen/b02d18a7-87ce-4a20-84c7-c0de641a2780/en-350-2016?srsltid=AfmBOorZxW_DKJLeDzVUsssm_St-kG_bHUpgWRa3DPpGi3XsFQWugCm (April 2, 2025).

Evans, P.D., Matsunaga, H., Preston, A.F., Kewish C.M. 2022. Wood protection for carbon sequestration - a review of existing approaches and future directions. Current Forestry Reports, 8: 181–198. https://doi.org/10.1007/s40725-022-00166-x

Fernández-Costas C., Palanti S., Charpentier J.P., Ángeles Sanromán M., Moldes D. 2017. A sustainable treatment for wood preservation: Enzymatic grafting of wood extractives. Sustainable Chemistry & Engineering, 5: 7557–7567. http://dx.doi.org/10.1021/acssuschemeng.7b00714

Fox, J. 2005. The R Commander: a basic statistics graphical user interface to R. Journal of Statistical Software, 14, 9: 1–42. https://doi.org/10.18637/jss.v014.i09

Humar M. 2011. Wood preservation after implementation of the biocidal products directive. IN: Wood is Good - EU Preaccession Challenges of the Sector. Grbac I. (ed.). Zagreb, Croatia, CABI Journals: 51–57. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20113406438 (November 19, 2024)

Humar M., Malik J., Levanič J., Kržišnik D., Keržič E. Lesar B. 2024. The durability and mechanical properties of 40-year-old black locust poles. In: Proceedings IRG Annual Meeting. Tennessee Scientific Conference on Wood Protection Knoxville.

Keržič E., Humar M., Oven P., Vek V. 2023. Development of extraction methodology for identification of extractive-compounds indexing natural durability of selected wood species. Wood Material Science & Engineering, 18, 6: 1940–1950. https://doi.org/10.1080/17480272.2023.2207529

Keržič E., Vek V., Oven P., Humar M. 2024. Changes in wood durability due to leaching of biologically active substances (extractives) resulting from weathering. Case Studies in Construction materials, 21. https://doi.org/10.1016/j.cscm.2024.e03921

Kirker G.T., Blodgett A.B., Arango R.A., Lebow P.K., Clausen C.A. 2013. The role of extractives in naturally durable wood species. International Biodeterioration & Biodegradation, 82: 53–58. http://dx.doi.org/10.1016/j.ibiod.2013.03.007

Lekounougou S., Jacquot J.P., Gerardin P., Gelhaye E. 2008. Effects of propiconazole on extra-cellular enzymes involved in nutrient mobilization during *Trametes versicolor* wood colonization. Wood Science and Technology, 42: 169–177. https://doi.org/10.1007/s00226-007-0158-1

Martín, J. A., López, R. 2023. Biological deterioration and natural durability of wood in Europe. Forests, 14, 2, 283. https://doi.org/10.3390/f14020283

Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. 2012. Official Journal of the European Union, 2009/0076/COD. http://data.europa.eu/eli/reg/2012/528/oj (April 2, 2025).

Schindelin J., Arganda-Carreras I., Frise E., Kaynig V., Longair M., Pietzsch T., Preibisch S., Rueden C., Saalfeld S., Schmid B., Tinevez J.Y., White D.J., Hartenstein V., Eliceiri K., Tomancak P., Cardona A. 2012. Fiji: An open-source platform for biological-image analysis. Nature Methods, 9, 7: 676–682. https://doi.org/10.1038/nmeth.2019

Schneider C.A., Rasband W.S., Eliceiri K.W. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9, 7: 671–675. https://doi.org/10.1038/nmeth.2089

Schultz T.P., Nicholas D.D., Preston A.F. 2007. A brief review of the past, present and future of wood preservation. Pest Management Science, 63: 784–788. https://doi.org/10.1002/ps.1386.

Sergent, T., Kohnen, S., Jourez, B. C. Beauve, Schneider Y.-J., Vincke C. 2014. Characterization of black locust (*Robinia pseudoacacia* L.) heartwood extractives: identification of resveratrol and piceatannol. Wood Science and Technology 48: 1005–1017. https://doi.org/10.1007/s00226-014-0656-x

Thybring E.E., Kymäläinen M., Rautkari L. 2018. Moisture in modified wood and its relevance for fungal decay. iForest, 11: 418–422. https://doi.org/10.3832/ifor2406-011

Valette N., Perrot T., Sormani R., Gelhaye E., Morel-Rouhier M. 2017. Antifungal activities of wood extractives. Fungal biology reviews, 31: 113–123. http://dx.doi.org/10.1016/j.fbr.2017.01.002

Vek V. 2015. Uporaba ekstraktivov bora za zaščito lesa: končno poročilo o delu na operaciji. Ljubljana, Biotehniška fakulteta, Oddelek za lesarstvo.

Vek V., Vivod B., Poljansek I., Oven P. 2019. Vsebnost ekstraktivov v skorji in lesu robinije (*Robinia pseudoacacia* L.) = Content of extractives in bark and wood of black locust (*Robinia pseudoacacia* L.). Acta Silvae et Ligni, 119: 13–25. https://doi.org/10.20315/ASetL.119.2

Vek V., Poljanšek I., Humar M., Willför S., Oven P. 2020a. In vitro inhibition of extractives from knotwood of Scots pine (*Pinus sylvestris*) and black pine (*Pinus nigra*) on growth of *Schizophyllum commune*, *Trametes versicolor*, *Gloeophyllum trabeum* and *Fibroporia vaillantii*. Wood Science and Technology, 54: 1645–1662. https://doi.org/10.1007/s00226-020-01229-7

Vek V., Balzano A., Poljanšek I., Humar M., Oven P. 2020b. Improving fungal decay resistance of less durable sapwood by impregnation with Scots pine knotwood and black locust heartwood hydrophilic extractives with antifungal or antioxidant properties. Forests, 11, 9: 1–23. https://doi.org/10.3390/F11091024

Vek V., Poljanšek I., Humar M., Willför S., Oven P. 2020c. In vitro inhibition of extractives from knotwood of Scots pine (*Pinus sylvestris*) and black pine (*Pinus nigra*) on growth of *Schizophyllum commune*, *Trametes versicolor*, *Gloeophyllum trabeum* and *Fibroporia vaillantii*. Wood Science and Technology, 54, 6: 1645–1662. https://doi.org/10.1007/s00226-020-01229-7

Vovchuk C.S., González Garello T., Careaga V.P., Fazio A.T. 2024. Promising antifungal activity of *Cedrela fissilis* wood extractives as natural biocides against Xylophagous fungi for wood artwork of cultural heritage. Coatings, 14, 237. https://doi.org/10.3390/coatings14020237

Zelinka S.L., Altgen M., Emmerich L., Guigo N., Keplinger T., Kymäläinen M., Thybring E.E., Thygesen L.G. 2022. Review of wood modification and wood functionalization technologies. Forests, 13, 7: 1–46. https://doi.org/10.3390/f13071004