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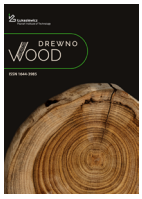

Fungal Assemblages Associated with the Ambrosia Beetle *Xyleborus Monographus* (Fabricius) on Oak Wood: Potential Role of the Insect in Spreading Blue-Stain and Wood-Decaying Fungi

Izabela Anna Bąk*
Aleksander Dziuk
Katarzyna Sikora
Miłosz Tkaczyk

Department of Forest Protection, Forest Research Institute, Sękocin Stary, Poland

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The ambrosia beetle *Xyleborus monographus* (Fabricius) inhabits oak wood (*Quercus* sp.). These insects carry fungal inoculum in specialised structures called mycangia. It is assumed that they may transport fungi potentially responsible for blue stain or wood decay. The composition and diversity of fungi isolated from the mycangia and bodies of X. monographus beetles were examined at two locations in Poland: the Krotoszyn FD (Forest District) and the Nowa Sól FD. The fungal colonies obtained were identified based on genetic sequences and morphology. In total, 143 isolates were obtained, representing 20 taxa from 16 fungal genera. The most frequently isolated genus was *Penicillium* (95% of samples), while *Talaromyces*, *Mucor*, *Yamadazyma*, and *Trichoderma* were found in 20–30% of the individuals examined. Fungi potentially associated with blue stain and wood decay were also detected: *Graphium basitruncatum*, *Sporothrix pseudoabietina*, *Clonostachys rosea*, *Fusarium solani*, and *Diaporthe* sp. These fungi are known components of the wood microbiome, capable of causing discolouration and degradation of sapwood, which may lead to economic losses in oak timber. Results suggest that *X. monographus* may potentially act as a carrier of saprotrophic and blue-stain–associated fungi. However, the actual role of *X. monographus* in the active transmission and colonisation of oak wood by these fungi requires experimental confirmation. The data provide a basis for further research on the role of ambrosia beetles in the dissemination of staining and wood-decaying fungi in hardwoods, as well as for developing methods to reduce economic losses caused by wood discolouration and degradation.

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Introduction

Oak wood is one of the most valuable forest resources in Central Europe due to its high durability, aesthetic qualities, and wide range of applications in furniture making, joinery, and construction. However, the quality of this material can be significantly reduced by discolouration and blue stain ("blue stain" or "sap stain"), as well as by the early stages of decay, which affect its market and functional value. Blue stain in wood results from fungal colonisation of the sapwood, leading to characteristic dark or bluish discolourations. Although these changes often do not immediately alter the mechanical properties of the wood, they substantially decrease its aesthetic and commercial value (Humar et al., 2008). Moreover, when discolouration

* Corresponding author: i.bak@ibles.waw.pl

is accompanied by the development of decay fungi or the formation of insect galleries, the strength and durability of the wood may also deteriorate (Dhiman et al., 2017).

In the case of oak, the distinction between heartwood and sapwood is of particular economic importance. Oak heartwood is preferred in high-grade applications (e.g., veneer, furniture, cooperage), whereas sapwood is often classified as lower quality material due to its lighter colour, lower natural durability, and higher susceptibility to fungal colonisation (Muñoz et al. 2014). In certain grading systems, the presence of sapwood in structural or decorative oak products may already reduce the commercial class of timber, even in the absence of visible blue stain. Therefore, fungal discolouration of sapwood further aggravates the economic loss by affecting material that is already more vulnerable and less durable.

A key factor contributing to such wood damage is the activity of xylophagous and ambrosia insects, which not only mechanically damage the wood by boring galleries but may also transmit fungi responsible for blue stain and wood decay. Ambrosia beetles, such as species of the genus *Xyleborus*, possess highly specialised structures – mycangia – that enable the transport of fungal symbionts between generations and substrates (Batra 1967; Jankowiak et al. 2019; Joseph et al. 2025). This symbiosis is mutualistic: the fungi play a crucial role in the insect's life cycle, serving as their food source and facilitating colonisation of new wood fragments (Mayers et al., 2015; Hulcr & Stelinski, 2017). In turn, the insects provide the fungi with dispersal opportunities and access to fresh, carbohydrate-rich tissues (Carrillo et al. 2014). The presence of these insects in wood can therefore indicate not only mechanical or physiological damage to the tree, but also an increased risk of microbiological degradation caused by the transport of blue-stain and decay fungi (Grousset et al., 2020).

Numerous studies have shown that improper timber management – such as delayed processing, poor storage, or lack of sorting of material showing signs of insect activity – accelerates the development of blue stain and results in value loss. For instance, industrial reports indicate that stained wood is often heavily discounted or even rejected, even though some forms of blue stain do not reduce mechanical strength. However, due to aesthetic and market requirements, such material is classified as lower grade (Komut, 2020). The market value of stained wood can drop by several dozen percent compared to unstained material, leading to significant economic consequences for forest owners and the wood industry.

In the case of oak wood, which has a high unit value, any signs of discolouration or degradation can lead to a substantial price reduction and exclusion from high-quality applications. Therefore, it is important not only to identify the presence of wood-inhabiting insects but also to characterise the microbiological load they may carry. In this context, the species *Xyleborus monographus*, which infests oaks and may serve as a vector for fungi responsible for blue stain and wood degradation, deserves particular attention (Rodríguez-Becerra et al. 2024).

Considering these factors, the aim of this study is to determine the spectrum of fungi transmitted by *X. monographus* on oak wood, with special emphasis on taxa potentially responsible for blue stain and wood decay, and to assess the relationship between the presence of these fungi, their collection sites, and the body parts of the insect. This knowledge may have direct implications for evaluating the quality of timber infested by this beetle.

Materials and methods

1. Research material

The research material comprised *X. monographus* beetles collected from two study sites. Wood infested by these insects was obtained from the Krotoszyn Forest District, compartment 172g (WGS84: 51.7281 N, 17.5763 E) on 27 March 2025, and from the Nowa Sól Forest District, various subcompartments within compartment 304 (WGS84: 51.6913 N, 15.6787 E) on 23 April 2025. These sites were selected because both districts reported recent occurrences of *Xyleborus monographus* infestations in oak stands and represent comparable yet geographically separated oak-dominated forest complexes. The inclusion of two spatially distinct locations allowed for preliminary assessment of potential variation in fungal assemblages associated with the beetle under different local forest conditions. Only females were included in the study. Although both sexes may occur in infested wood, females are responsible for dispersal, host colonisation, and the transmission of fungal symbionts, whereas males are flightless and typically remain within the galleries. Therefore, females constitute the ecologically relevant sex in the context of fungal vectoring and were selected for analysis (Szujecki, 1995; Michalski & Mazur, 1999).

The collected wood sections (*Q. robur* L.) were 60 cm long and 40–45 cm in diameter (measured at mid-length). The material originated from recently felled trees showing visible symptoms of ambrosia beetle infestation, including entry holes and initial gallery formation. The wood consisted of stem sections with intact bark and sapwood, without advanced signs of decay at the time of sampling. After cutting, the sections were stored outdoors under shaded conditions for a short period (not exceeding several days) before being transported to

the laboratory at the Forest Research Institute. The first adult beetles began to emerge approximately two weeks after transport. During the emergence period, a total of 44 adult beetles were obtained from the collected wood fragments. As expected for this species, nearly all individuals were females, and only sporadic males (n = 2) were observed. Due to their extremely low abundance and their minor role in fungal transmission (males are flightless and remain within galleries), only females (n = 20) were included in further analyses.

For analysis, 10 mycangia from *X. monographus* individuals were used from each location (Krotoszyn and Nowa Sól). Additionally, for five individuals from each site, the remaining body parts were also analysed.

The collected beetles were stored at –2°C until analysis.

2. Mycangia isolation

Before isolation, *X. monographus* beetles underwent external surface sterilisation to remove environmental contaminants. Each insect was rinsed individually in distilled water for 1 minute, immersed in 70% ethanol for 10 seconds, and again rinsed in distilled water for 1 minute.

Isolation of heads containing preoral mycangia (Joseph et al. 2025) was carried out under sterile conditions using entomological pins and a scalpel. All tools were disinfected after each use in 70% ethanol and over a flame.

The head and the remaining body parts from each beetle were placed in separate sterile Eppendorf tubes containing 300 µl of distilled water. After 30 minutes at room temperature, the material was crushed using a sterile pestle, and the volume of each sample was adjusted to 1 ml with distilled water. Samples were stored at 4°C for at least 12 hours.

3. Fungal culturing

Fungal culturing was performed on a 1:1 mixture of MEA (Malt Extract Agar) and WA (Water Agar) media supplemented with streptomycin. A 200 µl suspension from the mycangia and body samples was applied to two Petri dishes per sample. The suspension was evenly spread across the surface using a sterile spreader.

Plates were incubated at 24°C. After two, four, and seven days, emerging fungal colonies were subcultured onto new MEA:WA plates. Additionally, on the seventh day after inoculation, the number of colony-forming units (CFUs) was counted, and the presence of fungal colonies was recorded.

After 1–2 weeks of growth, the subcultured colonies were grouped into morphotypes based on macroscopic characteristics, growth rate, and microscopic observations.

4. Molecular analysis

Polymerase Chain Reaction (PCR) was performed using the Phire® Plant Direct PCR Kit (Thermo Fisher Scientific). Each reaction (20 µl total volume) contained: 1× Phire® Plant PCR Buffer, 0.4 µl Hot Start II DNA polymerase, 0.5 µM ITS1 and ITS4 primers (White et al. 1990), and 0.5 µl of DNA template. The method allowed amplification of DNA directly from fragments of fungal mycelium suspended in the Dilution Buffer provided with the kit, without prior DNA extraction.

Amplification was conducted in a Veriti 96 Thermal Cycler (Life Technologies™, USA) according to the following protocol: initial denaturation at 98°C for 5 min; 40 amplification cycles consisting of denaturation at 98°C for 5 s, primer annealing at 58°C for 5 s, and elongation at 72°C for 40 s; followed by a final elongation step at 72°C for 1 min.

PCR products were separated on a 1% agarose gel stained with Nancy-520 (Sigma-Aldrich, Germany). PCR products were purified using the Clean-up kit (A&A Biotechnology, Gdynia, Poland), and their concentration was measured spectrophotometrically using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). Sequencing was carried out on a 3500 Genetic Analyzer (Genomed, Poland). Sequence quality was assessed and trimmed using Chromas 2.6.6 (Technelysium Pty Ltd). Final sequences were compared with the NCBI (National Library of Medicine, NIH) database using the BLAST tool to assign isolates to the lowest possible taxonomic level. Species-level identification was defined based on the maximum (>99%) sequence similarity level.

5. Statistical analysis

Statistical analyses were conducted using R software. Normality of the data distribution was assessed with the Shapiro-Wilk test. For normally distributed data, Student's t-test was applied, while for non-normal distributions, the Mann-Whitney test was used. The number of colony-forming units (CFUs) was compared between locations, sample types (mycangia vs body), and their interactions.

Results and discussion

1. Presence of fungal material in *Xyleborus monographus* beetles

A total of twenty female *X. monographus* individuals from two locations were analysed: Krotoszyn FD (KJ) and Nowa Sól FD (NS). Fungal colonies appeared on all Petri dishes inoculated from both mycangia and other

body parts, indicating a widespread presence of fungal material in the examined beetles.

The number of colony-forming units (CFUs) varied depending on the beetles' origin and the body part analysed. For body samples, counts ranged from 59 to 213 CFUs, while for mycangia, from 15 to 121 CFUs. In one individual from Krotoszyn, the number of colonies obtained from the mycangium reached 344 CFUs; however, this value was considered an outlier and excluded from further statistical analyses. The results are presented in Table 1.

The mean number of fungal colonies in the mycangia of beetles from the Krotoszyn FD was significantly higher than in individuals from Nowa Sól (Student's t-test, $p = 0.0277$). Likewise, the CFU count obtained from body samples was higher than from mycangia, both for the Krotoszyn FD ($p = 0.0415$) and Nowa Sól ($p = 0.0070$). A paired Student's t-test confirmed a significantly greater number of fungal colonies in samples derived from beetle bodies compared to mycangia ($p = 0.0012$). This indicates that most fungi associated with *X. monographus* occur on the external surfaces or within the body, although mycangia also serve as a source of fungal material. The higher CFU counts obtained from body samples compared to mycangia suggest that a substantial proportion of the fungal assemblage may be externally acquired rather than specifically maintained symbionts.

Results for individual beetles are summarised in Table 2. The number of fungal colonies varied among individuals, ranging from 15 to 532 CFUs, and the number of detected taxa per individual ranged from 1 to 7. Greater

Table 1. List of analyzed *X. monographus* beetles with sampling location (KJ – Krotoszyn, NS – Nowa Sól), total number of colony-forming units (CFUs), CFUs for individual tagmata, number of detected taxa, and their corresponding list

Beetle	Location	Total CFUs	Head CFUs	Body CFUs	No. of taxa	Taxa
KJ-1	KJ	251	38	213	4	*Penicillium* spp., *Talaromyces* ruber, *Talaromyces* spp., *Yamadazyma* sp.
KJ-2	KJ	82	23	59	6	*Chalara* sp., *Microdochium nivale*, *Penicillium* spp., *Sporothrix pseudoabietina*, *Talaromyces* ruber, *Trichoderma* spp.
KJ-3	KJ	215	77	138	5	*Diaporthe* sp., *Penicillium* spp., *Talaromyces ruber*, *Talaromyces* spp., *Yamadazyma* sp.
KJ-4	KJ	532	344	188	6	*Chalara* sp., *Clonostachys rosea*, *Mucor* spp., *Penicillium* spp., *Talaromyces ruber*, *Talaromyces variabilis*
KJ-5	KJ	174	38	136	7	*Chalara* sp., *Graphium basitruncatum*, *Mucor* spp., *Penicillium* spp., *Talaromyces ruber*, *Trichoderma* spp., *Yamadazyma* sp.
KJ-6	KJ	121	121		2	*Penicillium* spp., *Talaromyces* spp.
KJ-7	KJ	87	87		3	*Penicillium* spp., *Talaromyces* spp., *Talaromyces variabilis*
KJ-8	KJ	74	74		3	*Mucor* spp., *Penicillium* spp., *Talaromyces ruber*
KJ-9	KJ	102	102		2	*Beauveria bassiana*, *Penicillium* spp.
KJ-10	KJ	76	76		2	*Beauveria pseudobassiana*, *Penicillium* spp.
NS-1	NS	81	21	60	4	*Fusarium solani*, *Mucor* spp., *Penicillium* spp., *Sporothrix* sp.
NS-2	NS	190	37	153	2	*Penicillium* spp., *Trichoderma* spp.
NS-3	NS	176	39	137	3	*Mucor* spp., *Penicillium* spp., *Yamadazyma* sp.
NS-4	NS	116	22	94	2	*Penicillium* spp., *Sporothrix* sp.
NS-5	NS	202	92	110	3	*Alternaria infectoria*, *Penicillium* spp., *Trichoderma* spp.
NS-6	NS	64	64		2	*Cladosporium* spp., *Penicillium* spp.
NS-7	NS	15	15		2	*Aureobasidium pullulans*, *Penicillium* spp.
NS-8	NS	17	17		1	*Penicillium* spp.
NS-9	NS	15	15		1	*Trichoderma* spp.
NS-10	NS	57	57		1	*Penicillium* spp.

Table 2. Frequency (%) of occurrence of the identified fungal taxa. Sample size: total $n = 20$; Krotoszyn (KJ), Nowa Sól (NS): $n = 10$; head KJ, head NS: $n = 10$; body KJ, body NS: $n = 5$

Taxa	All	Krotoszyn FD (KJ)	Nowa Sól FD (NS)	Head (KJ)	Head (NS)	Body (KJ)	Body (NS)
Penicillium spp.	95	100	90	80	100	100	90
Talaromyces ruber	30	60	0	100	0	50	0
Mucor spp.	25	30	20	40	20	10	10
Trichoderma spp.	25	20	30	40	40	0	10
Talaromyces spp.	20	40	0	20	0	30	0
Yamadazyma sp.	20	30	10	20	20	20	0
Chalara sp.	15	30	0	0	0	30	0
Sporothrix sp.	10	0	20	10	0	0	10
Talaromyces variabilis	10	20	0	20	0	10	0
Alternaria infectoria	5	0	10	0	0	0	10
Aureobasidium pullulans	5	0	10	0	0	0	10
Beauveria bassiana	5	10	0	0	0	10	0
Beauveria pseudobassiana	5	10	0	0	0	10	0
Cladosporium spp.	5	0	10	0	0	0	10
Clonostachys rosea	5	0	10	20	0	0	0
Diaporthe sp.	5	10	0	0	0	10	0
Fusarium solani	5	10	0	0	20	0	0
Graphium basitruncatum	5	0	10	20	0	0	0
Microdochium nivale	5	10	0	0	0	10	0
Sporothrix pseudoabietina	5	10	0	0	0	10	0

taxonomic diversity was observed in samples from Krotoszyn, where an average of 4.3 ± 1.8 taxa per individual was obtained, compared to 2.3 ± 1.1 taxa in samples from Nowa Sól.

2. Molecular analyses and identification of fungal taxa

A total of 143 fungal isolates were obtained, of which 116 were identified to the genus or species level. For 27 isolates, taxonomic affiliation could not be determined due to the absence of pure cultures or unsuccessful amplification of the ITS region under the applied PCR conditions.

Sequence analyses revealed 20 distinct taxa belonging to 16 fungal genera (Table 2). Among these, 11 taxa were identified to the species level. The most frequently isolated genus was *Penicillium*, present in 95% of all examined individuals. These fungi occurred in both study locations and were detected in both mycangia and body samples.

The second most common taxon was *Talaromyces ruber*, found in 30% of individuals, exclusively in samples from Krotoszyn. Fungi from the genera *Mucor* and *Trichoderma* were recorded in 25% of samples, while *Yamadazyma* sp. and *Talaromyces* spp. were detected in 20%. Other fungi such as *Chalara* sp., *Graphium basitruncatum*, *Clonostachys rosea*, *Sporothrix pseudoabietina*, *Fusarium solani*, and *Alternaria infectoria* occurred sporadically (≤10%), typically in specific body parts or individual beetles only.

The identified taxa represented several ecological groups:

– Saprotrophic and opportunistic fungi – *Penicillium* spp., *Talaromyces* spp., *Mucor* spp., *Trichoderma* spp., *Cladosporium* spp., *Aureobasidium pullulans*;

- Entomopathogenic fungi – *Beauveria bassiana*, *Beauveria pseudobassiana*, *Fusarium solani*;
- Fungi potentially associated with wood decay or blue stain – *Graphium basitruncatum*, *Sporothrix pseudoabietina*, *Clonostachys rosea*, *Fusarium solani*, *Diaporthe* sp.

The occurrence of the latter taxa, including species belonging to the *Graphium*/*Sporothrix* complex, suggests that *X. monographus* may play a role in transmitting fungi associated with blue stain and decay processes in oak wood.

3. Variation in fungal composition between locations and body parts

The structure of the mycobiota differed between *X. monographus* populations from Krotoszyn FD (KJ) and Nowa Sól FD (NS). Samples from Krotoszyn exhibited greater taxonomic richness (14 taxa), whereas samples from Nowa Sól contained 10 taxa. The higher taxonomic richness observed in Krotoszyn may indicate more advanced microbial succession or differences in wood moisture and stand conditions. Alternatively, it may reflect stochastic variation due to limited sample size. In Krotoszyn, the genera *Penicillium*, *Talaromyces*, *Mucor*, and *Trichoderma* were dominant, while in Nowa Sól, the mycobiota was mainly represented by *Penicillium* and *Trichoderma*, with a minor contribution from other saprotrophic fungi.

In mycangia samples, taxa typically associated with blue stain, such as *Graphium basitruncatum* and *Sporothrix pseudoabietina*, were more frequently isolated, whereas saprotrophic and opportunistic fungi (*Penicillium*, *Talaromyces*, *Mucor*) dominated in body samples. This suggests that mycangia may serve as storage sites for specific symbionts or wood pathogens, while the insect's body surface reflects contact with fungi present in the environment. Table 2 provides a detailed overview of the frequency (%) of individual taxa by location and analysed body part.

Our study demonstrates that *X. monographus* carries a rich and diverse assemblage of fungi, including saprotrophic and opportunistic species as well as taxa associated with blue stain and wood decay. The presence of fungal colonies in all analysed samples (mycangia and body parts) confirms that this ambrosia beetle functions as a significant vector of wood-associated microflora. This is consistent with previous observations that ambrosia beetles transport a variety of fungi within their mycangia, on their body surfaces, and in galleries (Schedl, 1964; Gebhardt et al., 2004; Hulcr & Stelinski, 2017; Rassati et al., 2019). In oak timber, sapwood constitutes a relatively narrow outer zone compared to heartwood. However, despite its limited width, it plays a critical role in grading and market valuation. In many quality classifications, heartwood-dominated logs are preferred for high-value products, whereas visible sapwood may result in downgrading depending on the intended end use. Moreover, sapwood is biologically more susceptible to colonisation by staining and decay fungi. The impact of blue stain is therefore particularly relevant in oak, as it affects primarily the sapwood zone, which may already be considered a defect in premium assortments. In lower-grade or industrial assortments, sapwood is accepted; however, fungal staining may still reduce value by affecting visual quality or processing properties (McCarthy et al., 2012).

4. Association of detected taxa with blue stain and wood decay

Unlike the "typical" blue stain in conifers, blue stain in oaks and other hardwoods can be caused by more diverse fungal assemblages. Among the fungi isolated from beetles, both common saprotrophs (*Penicillium*, *Talaromyces*, *Mucor*, *Trichoderma*) and species belonging to ophiostomatoid or related clades (*Graphium*, *Sporothrix*, *Diaporthe*, *Fusarium*) were identified (Romón et al., 2014; Rassati et al., 2019; Trollip et al., 2021; Osborn et al., 2022). In this study, the most frequently isolated genus was *Penicillium* (95% of samples), which should be interpreted with caution. Species of this genus are ubiquitous environmental saprotrophs and are known for their rapid growth on nutrient-rich media such as MEA. Therefore, their dominance in culture-based studies may reflect competitive advantages under laboratory conditions rather than ecological dominance within the beetle mycobiome. It is also possible that some isolates originated from environmental contamination or secondary colonisation during wood storage before beetle emergence. Consequently, the high prevalence of *Penicillium* does not necessarily indicate a specific or functional association with *X. monographus*. Culture-independent methods would be necessary to more accurately assess its relative abundance in situ. Taxa potentially involved in blue stain or wood decay included *Graphium basitruncatum*, *Sporothrix pseudoabietina*, *Fusarium solani*, and *Clonostachys rosea*.

These groups are associated with wood discolouration and/or decay. *Ophiostomatoid* taxa (and close relatives, such as species from the *Graphium*/*Sporothrix* complex) are classical agents of blue stain and are widely documented as associates of bark beetles and ambrosia insect partners (Wang et al., 2019; Trollip et al., 2021).

It should be emphasised, however, that not every species detected in culture is necessarily a "pathogen" of wood. Many species, particularly *Penicillium*, *Talaromyces*, and *Mucor*, are common saprotrophs in wood and the environment. They primarily cause superficial

discolouration and rapid colonisation of wood following injury or death, and their impact on mechanical properties may be limited compared to aggressive decay fungi. Blue stain (sap stain) often reduces the aesthetic value and usability of timber, whereas full wood decay leads to much greater volumetric and mechanical losses (Findlay, 1959; Komut, 2020; Musah et al., 2022). It should be clearly stated that the mere isolation of fungal taxa from beetles does not confirm their ability to colonise oak wood under natural conditions, nor does it demonstrate active transmission by the insect. Some isolates may represent incidental environmental contaminants. Experimental inoculation trials and transmission studies are required to determine whether *X. monographus* functions as an effective biological vector rather than a passive carrier.

5. Ecological significance and vectoring by *X. monographus*

The mechanism of fungal transmission by ambrosia beetles is well documented: mycangia and the insect's body surface serve as carriers for symbionts and other associated microbes. Beetle-fungus associations are often flexible; the insect can transport both its resident nutritional symbionts and other environmental fungi, including potential wood pathogens (Gebhardt et al., 2004; Mayers et al., 2022; Menocal et al., 2023). For *X. monographus*, previous reports have documented its associations with various fungi in oak habitats (e.g., populations in Portugal, Germany, and other regions), which aligns with our observations of ophiostomatoid and related taxa on these beetles (Bellahirech et al., 2021). Compared with studies conducted in southern Europe, where ophiostomatoid fungi were more frequently reported as primary associates of *X. monographus*, our material showed a stronger representation of opportunistic saprotrophs. This may reflect climatic differences or differences in sampling strategy (culture-based isolation vs. direct wood sampling).

6. Economic implications – impact of blue stain and decay on oak timber value

From a timber market perspective, two aspects are particularly important: (1) aesthetics and intended use (furniture, decorative materials, or lower-value industrial applications) and (2) mechanical properties and durability (structural safety, suitability for drying and impregnation).

Various reports and reviews indicate that economic losses related to blue stain and fungal contamination can be significant and depend on the time elapsed between felling and processing, storage conditions, and demand for high-quality timber (Musah et al., 2022). In practice, rapid felling, processing, and drying reduce the risk of quality loss; conversely, delays and storage under moist conditions favour the development of staining and decay fungi (Das, 2005).

In the literature on blue stain, representatives of the *Ophiostomatales/ophiostomatoids* (e.g., *Ophiostoma*, *Graphium*, *Sporothrix*) are most frequently reported, but some species from other clades (e.g., *Fusarium*, *Ceratocystis under* certain conditions) are also associated with significant reductions in wood quality (Wang et al., 2019; Trollip et al., 2021; Osborn et al., 2022). The detection of blue-stain–associated taxa (*Graphium*, *Sporothrix*) and decay-associated taxa (*Fusarium*, *Diaporthe*, *Clonostachys*) in *X. monographus* suggests that the presence of this beetle in oak wood may justify a more thorough assessment of raw material quality before sale.

In practice, the occurrence of numerous saprotrophic colonies combined with the presence of ophiostomatoids may warrant a price reduction, especially for timber intended for applications where colour and finish are critical (furniture, veneer) (Ross, 2014). In contrast, in species lacking clearly differentiated heartwood (e.g., diffuse-porous hardwoods with uniform colour) or in species where sapwood is commercially acceptable (e.g., birch, beech, or many softwoods), the economic consequences of blue stain may differ. In such cases, aesthetic impact and structural degradation play a more decisive role than the mere presence of sapwood. Therefore, the practical importance of fungal transmission by ambrosia beetles extends beyond oak and may concern a broader range of hardwood and softwood species (Arisandi, 2021).

The high frequency of *Penicillium* is consistent with findings from studies of wood and insect galleries, where *Penicillium* and related saprotrophs rapidly colonise available substrates and may affect aesthetics and drying processes (Komut, 2020). The presence of specific fungal taxa on beetles should be considered a cue for detailed evaluation of the wood (e.g., microscopic or colony-based analyses of wood from the beetle's habitat) before placing it on the market (Menocal et al., 2023).

Study limitations and future research directions

This study should be regarded as a preliminary (pilot) investigation. The relatively small sample size (20 individuals in total, with detailed body analyses conducted on five individuals per location) limits the statistical power of the analyses and the generalizability of the results. Consequently, comparisons between locations and body parts should be interpreted with caution. Although ITS-based isolations and identifications

provide important information on the spectrum of fungi associated with *X. monographus*, they do not conclusively determine pathogenicity to oak or the ability of a given isolate to cause blue stain or accelerated wood decay. Therefore, we recommend the following future research directions: (1) Expanded mycobiota surveys – investigate a larger number of *X. monographus* individuals and populations to determine the prevalence of fungi associated with wood decay or blue stain, and broaden the scope to include other ambrosia beetle species infesting oak (e.g., *Platypus cylindrus*), enabling comparative analyses of their fungal communities and vectoring potential; (2) Pathogenicity tests and controlled inoculations – conduct in situ and in vitro experiments on oak wood to assess which isolates induce blue stain and/or decay; (3) Quantitative impact measurements – evaluate the effects of individual taxa on wood, including changes in mechanical strength, mass loss, and reduction in aesthetic value; (4) Extended temporal and spatial sampling – investigate the dynamics of fungal transmission and the persistence of colonisation over time and across locations; (5) Multigene markers and metagenomics – employ comprehensive molecular approaches to fully characterise the microbiome of beetles and wood, potentially revealing previously undetected symbionts or pathogenic species (Rassati et al., 2019; Osborn et al., 2022; Menocal et al., 2023).

Conclusions

1. *X. monographus* carries a diverse range of fungi, from common saprotrophs to taxa associated with blue stain and wood decay.
2. The detection of species such as *Graphium*, *Sporothrix*, *Fusarium*, and *Diaporthe* in the examined beetles indicates a possible association with fungi known to cause blue stain or wood decay. However, the present study does not demonstrate active transmission, successful colonization of oak wood, or pathogenicity of these isolates. Therefore, the potential impact of *X. monographus* on timber quality should be regarded as hypothetical and requires confirmation through controlled inoculation experiments.
3. From a forest management and timber industry perspective, the detection of these characteristic taxa on beetles should prompt stricter quality control of harvested wood and rapid preventive measures to minimise economic losses caused by downgrading or repurposing timber for lower-value uses.

Conflict of interest

The author(s) declare(s) that there is no conflict of interest concerning the publication of this article.

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