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Decay capacity and degradation patterns of *Xylaria hypoxylon* on different wood species

Ehsan Bari[1], Geoffrey Daniel[2], Adya Singh[3], Jeffrey Morrell[4]

[1] Department of Wood Sciences and Engineering, Technical and Vocational University (TVU), Tehran, Iran. E-mail: bari_lenzites@yahoo.com

[2] Department of Forest Biomaterials and Technology/Wood Science, Swedish University of Agricultural Sciences, Box 7008, Uppsala, Sweden. E-mail: geoffrey.daniel@slu.se

[3] Scion, Private Bag 3020, Rotorua, New Zealand. E-mail: adyasingh@hotmail.com

[4] National Centre for Timber Durability and Design Life, University of the Sunshine Coast, Brisbane. Australia. E-mail: jmorrell@usc.edu.au

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IRG SECRETARIAT
Box 5604
SE-114 86 Stockholm
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[1] Department of Wood Sciences and Engineering, Technical and Vocational University (TVU), Tehran, Iran. E-mail: bari_lenzites@yahoo.com

[2] Department of Forest Biomaterials and Technology/Wood Science, Swedish University of Agricultural Sciences, Box 7008, Uppsala, Sweden. E-mail: geoffrey.daniel@slu.se

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[4] National Centre for Timber Durability and Design Life, University of the Sunshine Coast, Brisbane. Australia. E-mail: jmorrell@usc.edu.au

Abstract

A host of physical and environmental factors may influence fungal decay including the wood substrate, temperature, moisture, oxygen, light, pH, and nitrogen. Understanding the effects of these factors on fungal decay is important for the effective utilization of wood decay fungi in biotechnological processes and for understanding the role of these organisms in global carbon cycling. The ascomycete Xylaria hypoxylon causes white rot of hardwoods, but remains relatively under-characterized. In this investigation, the decay capability of this fungus was studied using beech, hornbeam, oak and pine. Although Xylaria species are considered as causing white rot decay, Type II soft rot erosion was observed on hornbeam, Type I soft rot cavities were noted on beech, simultaneous rot was found on oak and selective rot on pine. Results indicated that both wood species and cell wall chemistry affected morphological decay patterns illustrating the relationships between fungal enzymatic capacity and wood cell wall structure/chemistry.

Keywords: White rot, *Xylaria hypoxylon*, soft rot, wood species, cell wall chemistry, fungal decay strategies

1. INTRODUCTION

Fungi are one of the indispensable components of the forest ecosystem and play an important role in salvaging carbon stored in the form of different complex organic materials (Fazio *et al.* 2010, Sanghvi *et al.* 2013). Wood degradation is an important part of the carbon recycling process under natural conditions, but it also causes extensive economic losses by reducing the quality of timber, furniture, ancient sculptures, and many other wood products. The process of wood degradation is categorized into three major classes based on removal of wood cell wall components and morphological appearance, viz., white rot, brown rot, and soft rot (Blanchette *et al.* 1990, Liese 1970, Eaton and Hale 1993, Worrall *et al.* 1997, Daniel 2003, Schwarze 2007, Koyani *et al.* 2010, Hickman *et al.* 2011). These categories are increasingly viewed as artificial, but they serve as guides to relative decay capabilities of fungi.

While basidiomycetes are viewed as the most important wood degraders in most terrestrial environments, some Ascomycetes such as *Xylaria hypoxylon* cause white rot decay in hardwoods (Nilsson *et al.* 1989, Schmidt 2006). *Xylaria* species have been reported to cause both soft rot and white rot decay (Nilsson *et al.* 1989, Worrall *et al.* 1997, Anagnost 1998). Under certain

conditions, these fungi may switch from white- to soft rot decay (Bari *et al.* 2017a), which can be influenced by moisture, temperature and pH. Substrate characteristics may also influence fungal behavior. Species of the genus *Xylaria* usually grow on rotten wood, but can also be found in soils or on various substrates such as fallen leaves, herbaceous stems, dung, grasses, seeds or fruits and wood (Rogers 1986). Some *Xylaria* species have also been reported to be endophytes, illustrating the wide array of potential roles for this genus.

Three species of *Xylaria* have been reported from Iran prior 2009 (Ershad 2009), and a more recent taxonomic and phylogenetic study of the genus in the Northern provinces of Iran has led to eight new records for Iranian mycobiota, including one *Xylaria longissima* sp. nov (Hashemi 2015). However, much of the information available on members of this genus relates to medical and ecological applications, and the destructive behavior of this fungus on wood remains poorly characterized. Thus, the aim of the current study was to determine the biological, chemical, and anatomical characteristics of *X. hypoxylon* on four wood species (beech, oak, pine, and hornbeam.

2. MATERIALS AND METHODS

2.1 Source of *Xylaria hypoxylon* isolate

Xylaria hypoxylon (MF682345) was collected from the Guilan and Mazandaran Provinces of Iran. Type specimens were deposited in the fungal collection of the Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Iran (GUM) and the Fungal Reference Collection of the Ministry of Jihad-e Agriculture (IRAN) located at the Iranian Research Institute of Plant Protection, Tehran. Duplicates of studied fungal materials are kept in the personal herbaria of S. A. Hashemi at Shahed University, Iran.

2.2 Wood samples preparation

Sapwood lumber was cut from beech (*Fagus orientalis* Lipsky), oak (*Quercus castaneifolia* C.A.M.), hornbeam (*Carpinus betulus* L.) and pine (*Pinus brutia* var. *eldarica*) trees (about 70-120 year old trees) at breast height. The boards were air-dried for two months then samples were cut (30×10×5 mm) for decay tests. Samples were initially conditioned to constant weight at 25°C and 45% RH, before being oven dried at 103 °C and weighed. The blocks were then sterilized by heating for 20 minutes at 121 °C.

2.3 Decay capabilities

Decay capabilities of the test fungus were determined following procedures described in Standard EN-113 as modified by Bravery (1978). Briefly, the test fungus was inoculated onto Petri dishes containing 1.5 % malt extract agar and incubated until the fungus covered the agar surface. The sterile test blocks were then placed on glass rods on the agar surface. Forty blocks were evaluated per wood species. The plates were sealed to minimize the risk of contamination and incubated at 22±2°C and 65±5% RH. Ten replicate specimens of each wood species were removed at 30, 60, 90 and 120 days of incubation. Mycelia were removed from the block surfaces and the blocks were weighed, oven-dried at 103 °C and weighed again to determine the mass loss (ML) and moisture content (MC) as per the procedures described in EN-113 (1997).

2.4 FT-IR spectroscopy

The effect of fungal decay on wood chemistry was investigated using FT-IR spectroscopy. Sound and fungal exposed samples of each wood species were ground to pass a 20-mesh screen, then mixed with potassium bromide and pressed into a pellet that was examined using a Shimadzu 8400s FT-IR Spectrometer equipped with a DLATGS detector. All samples were examined at a spectral resolution of 4 cm^{-1} with 30 scans per sample. Background scans were also carried out

using a blank collector. A rubber band method was used for each spectra with baseline correction. The band for CO_2 was removed to make a suitable baseline correction (Mohebby 2003).

2.5 Light microscopy

For anatomical analysis, three sapwood blocks (8×8×5 mm) per wood species were prepared from the degraded wood specimens and sections (10-15 µm) cut on a sliding microtome (GSL-1 microtome, WSL). Ten consecutive sections were prepared from each block, oriented in cross, radial and tangential directions. Due to severe decomposition, some wood blocks needed to be embedded in polyethylene glycol 1500 for one day (Sigma-Aldrich). Sections were stained in a 1:1 (vol/vol) mixture of 0.5 % safranin and 0.3 % astra blue (Gartner and Schweingruber 2013, Bari *et al.* 2015). The stained samples were washed in distilled water for 1-3 min, followed by dehydration in an alcohol series. After rinsing in xylol for 1-2 min, sections were mounted in Mountalan glue, and examined by light microscopy using an Olympus E-210 microscope and photographed with an Olympus E-450 camera (Tokyo, Japan). Non-decayed samples were processed at the end of the incubation period in the same way.

3. RESULTS AND DISCUSSION

3.1 Wood decay test and fungal metabolism

Mass losses for the four species were relatively modest, ranging from 5.14 to 11.54 % at the end of fungal exposure. The highest- and lowest mass losses occurred in oak (11.54%) and pine (5.14%), respectively, after 120 days incubation (Figure 1). Mass losses were also observed for hornbeam (9.66%) and beech (6.41%). Nilsson *et al.* (1989) found average mass losses produced by *X. hypoxylon* in beech and pine were 40.6% and 3.3%, respectively, after 4 months incubation. Eriksson *et al.* (1990) showed that *X. hypoxylon* caused mass losses of 18.6% and 3.9% ML in birch and pine, respectively, after 12 weeks incubation. The mass losses in the current tests were somewhat lower suggesting some variations in isolate decay capabilities.

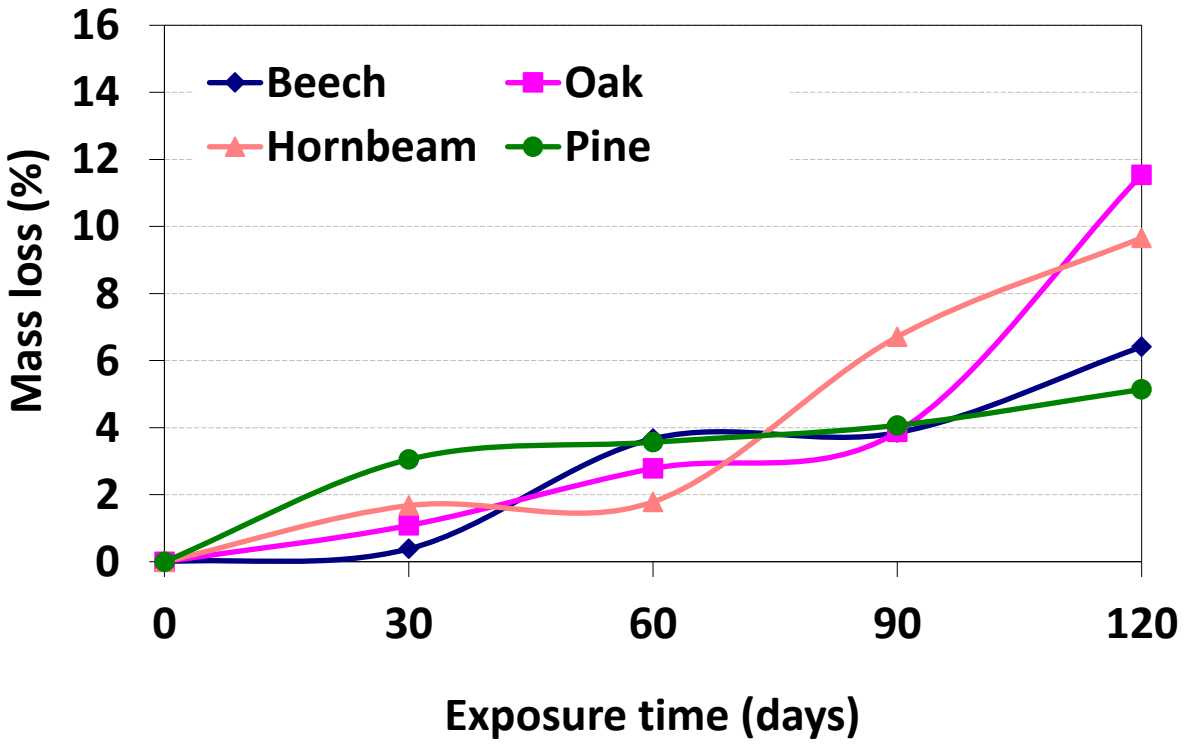

Figure 1: Average mass losses for four wood species exposed to *Xylaria hypoxylon* over 120 days in an EN-113 decay test.

Merrill *et al.* (1964) found that several different Xylariaceae species caused weight losses of 12 to 25 % in different hardwood species. These studies indicated that *Xylaria* species, while capable of decaying wood of various species, are far less aggressive than more typical wood decay fungi such as *Trametes versicolor* or *Coniophora puteana* that would be expected to produce weight losses in the 20 to 30% range under similar conditions. One factor that can affect mass loss is wood moisture content. Many fungi tend to be most aggressive as moisture contents increase from 30 to 60 to 80 %, and then decline at higher moisture levels, most likely due to oxygen limitations (Zabel and Morrell 2020). Moisture contents of the test blocks were all over 40 % after 30 days of incubation and then varied slightly with further exposure. The pine and oak blocks were the wettest, ranging from 80 to 100 % at the end of the test. Beech and hornbeam blocks had the lowest moisture levels at the end of the test (~40 %), while the moisture contents of beech blocks steadily increased from ~40 to 60 % over the 120-day incubation period. The results suggest that moisture contents were well within the range expected for fungal attack, although it is unclear whether these ranges were optimal for the current *Xylaria* species. Standard decay test methods are typically developed using fungi that are easily cultured and conditions that closely match the requirements for growth. It is important to note that *Xylaria* species are not typically evaluated using this method and that there may be more suitable methods for assessing their decay capabilities.

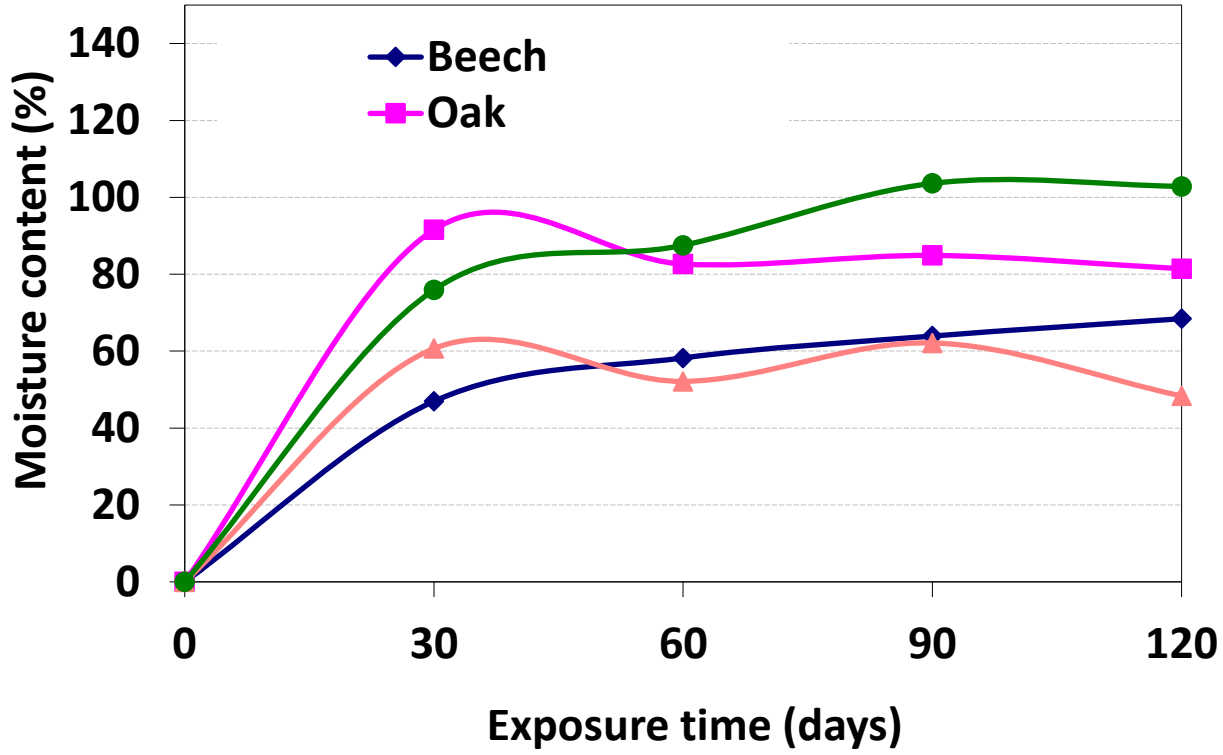

Figure 2: Moisture contents of four wood species exposed to *Xylaria hypoxylon* over 120 days in an EN-113 decay test.

3.2 Changes in the chemistry of decayed wood

FT-IR analysis of sound and decayed samples showed relatively few differences in the spectra (Figures 3 & 4). A slight decline in the peak at 1155 cm^{-1} was noted for oak samples exposed to the fungus, corresponding to the C-O-C bonds for cellulose and hemicellulose (asymmetric stretching in cellulose I and cellulose II), but there were only relatively minor differences in spectra for the decayed wood (Pandey and Pitman 2003, Mohebby 2005, Schwanninger *et al.* 2004, Bari *et al.* 2018). The results are consistent with the premise of white rot fungi that degrade all three wood polymers more or less uniformly, leaving relatively sound residual material. The limited

weight losses likely also limit the ability to detect changes. A slight reduction in the peak at 1138 cm^{-1} was seen for pine that is related to aromatic C–H in-plane deformation; typical for G units, where G is condensed and etherified (Pandey and Pitman 2003, Schwanninger *et al.* 2004). Notable decreases in the peaks at 1500 cm^{-1} and 1589 cm^{-1} were obtained for oak and correspond to C=C stretching of the aromatic ring (G) as well as C=C stretching of the aromatic ring (S) (Pandey and Pitman 2003, Schwanninger *et al.* 2004).

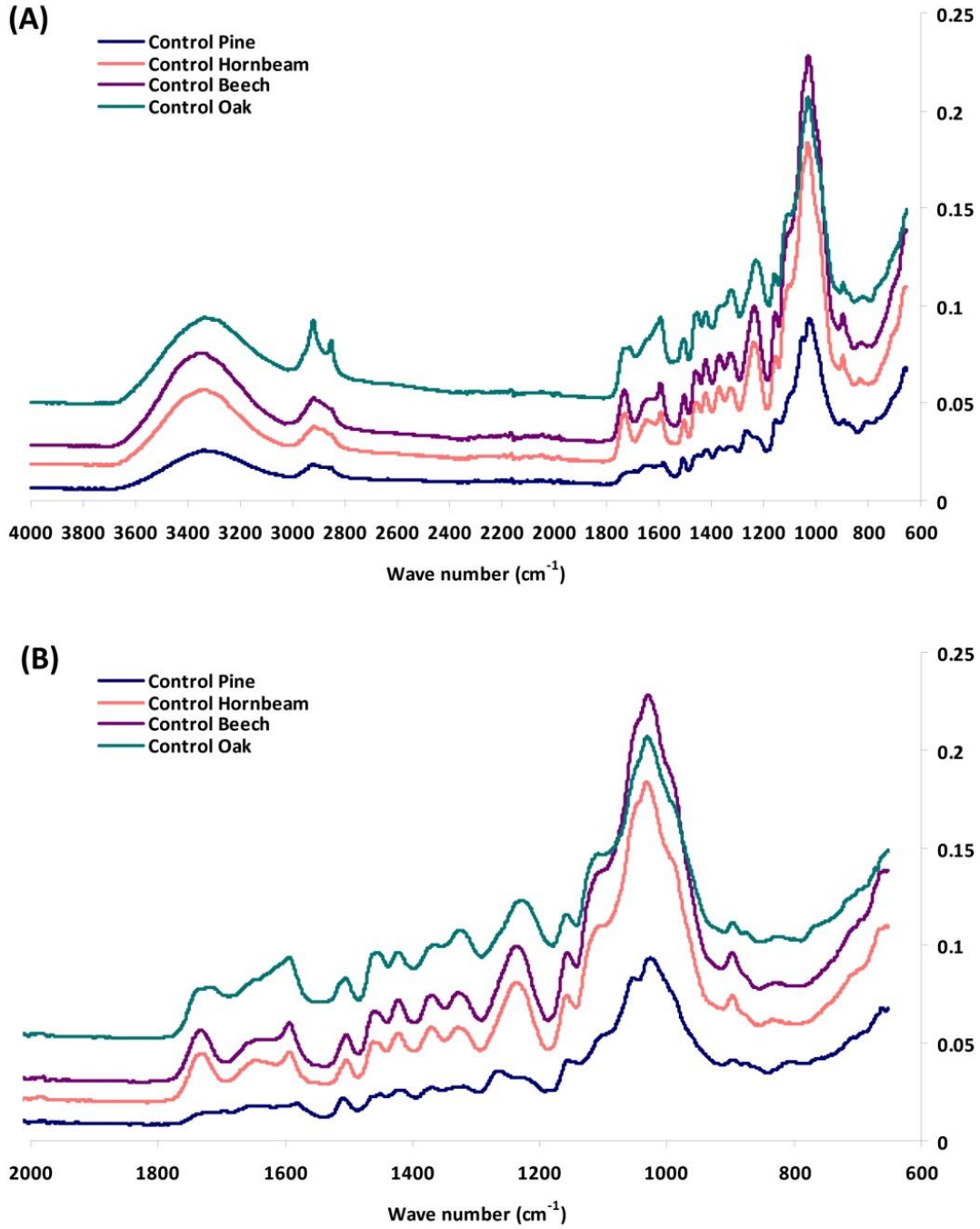

Figure 3: Total FT-IR spectra (A) and the fingerprint region of (B) control wood samples from four wood species.

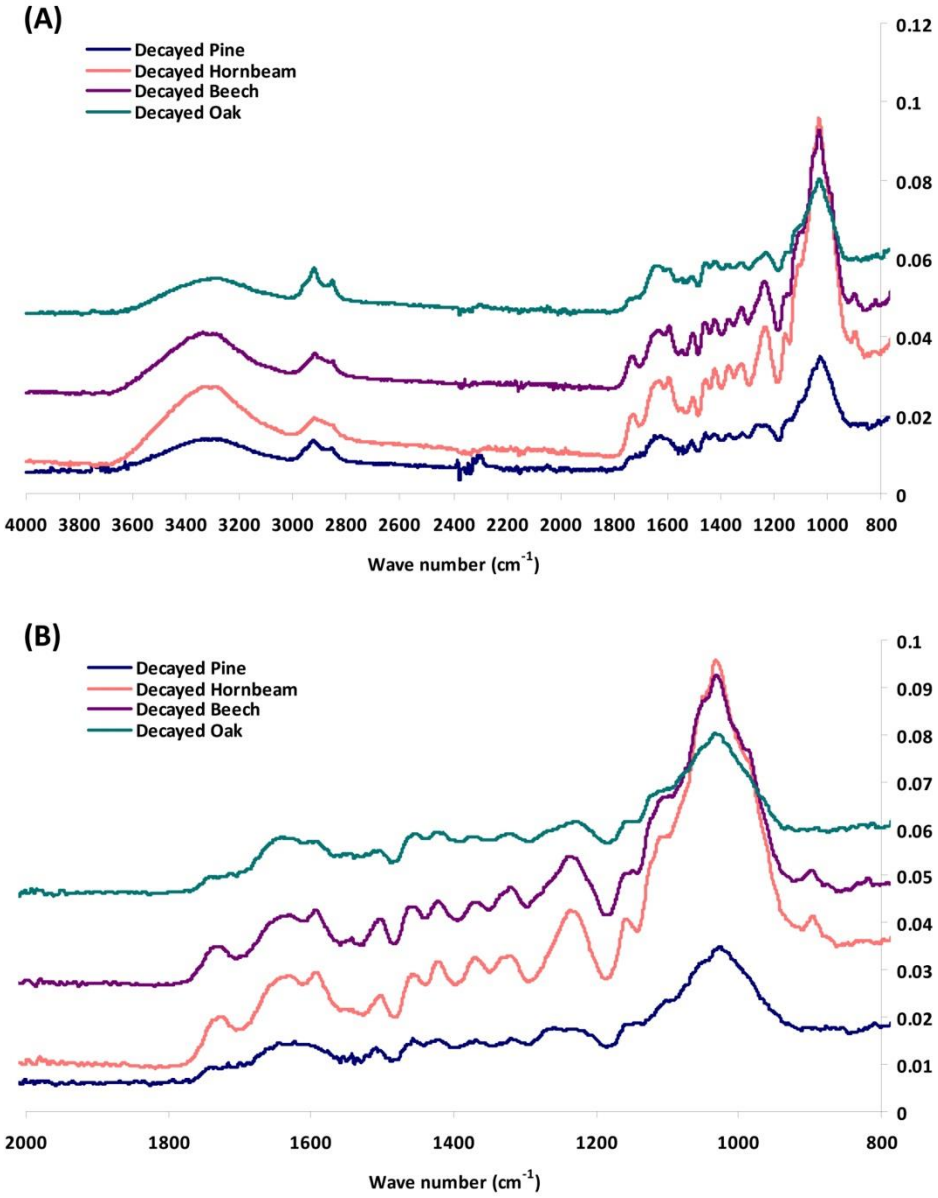

Figure 4: Total FT-IR spectra (A) and the fingerprint region (B) from wood of four species exposed to *Xylaria hypoxylon* for 4 months in an EN-113 decay test.

3.3 Anatomical investigations

Microscopic analysis of thin sections cut from the decayed wood samples are shown in Figures 5 and 6. The decay pattern produced by the fungus varied with wood species. The test fungus caused Type I soft rot in beech wood as shown by the presence of bore holes on fiber lumen surfaces (Fig. 5A), diamond-shaped cavities in the cell walls (Fig. 5B) and small bore holes developing from ray cells (Fig. 5C). The decay pattern in hornbeam was suggestive of Type II soft rot, as indicated by erosion of the lumen cell walls (Fig. 5D, E). Bore holes were produced by fungal hyphae in vessels and fibers (Fig. 5E) and decay caused separation in the cellular structure of ray cells (Fig. 5F), possibly due to lignin attack. *X. hypoxylon* caused a different morphological decay pattern in oak wood samples producing white rot of the cell wall layers in fibers (Fig. 6A), bore holes (Fig. 6B) and severe degradation in the ray parenchyma (Fig. 6C). Growth of hyphae and enlargement of pits and cell wall bore holes were observed in radial sections (Fig. 5B).

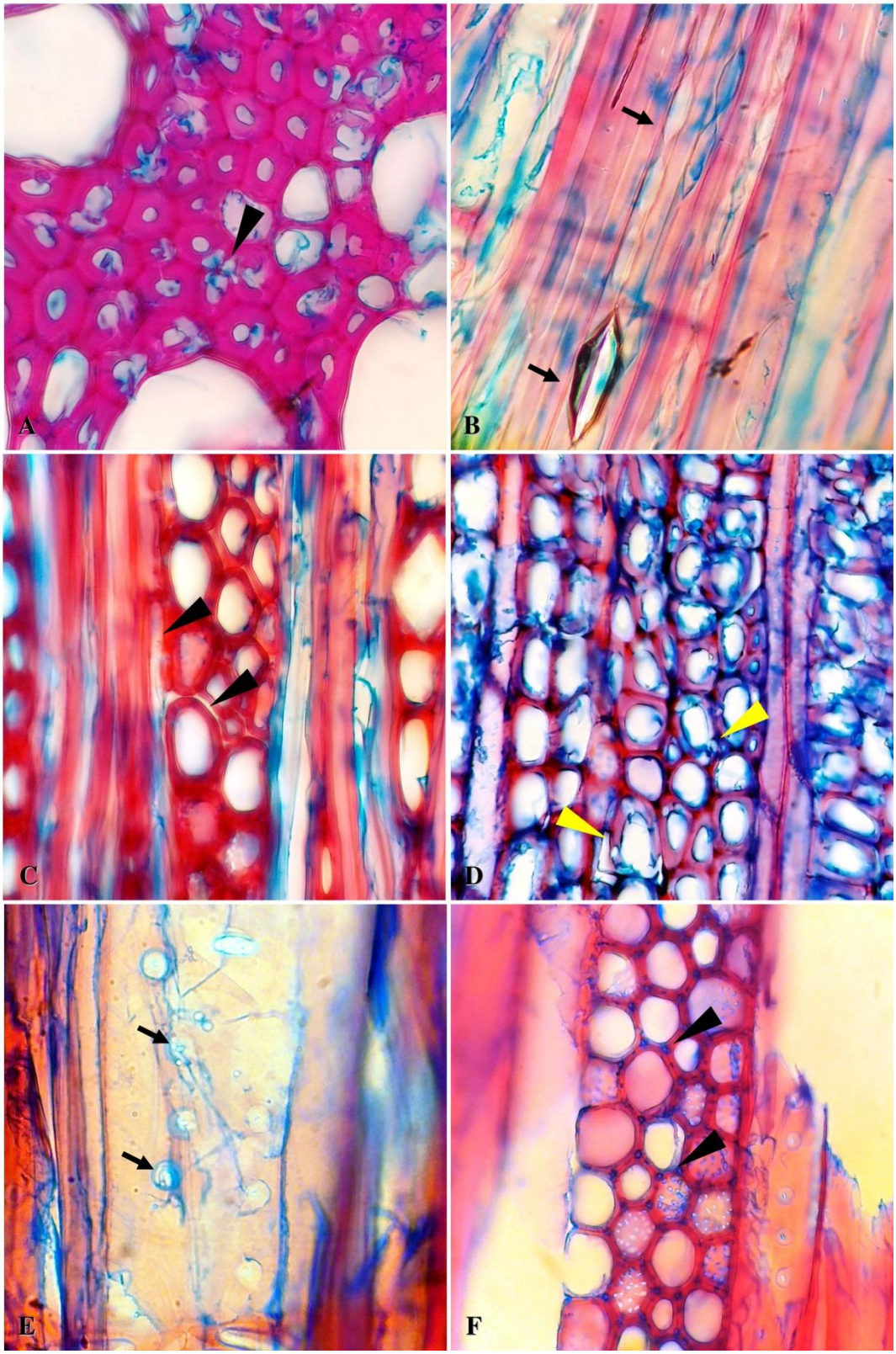

Figure 5: Micrographs of thin sections cut from beech (A-C) and hornbeam (D-F) wood following exposure to *Xylaria hypoxylon* for 120 days in an EN-113 decay test.

- Beech wood: formation of diamond-shaped cavities (*arrows*) as well as decay of lignin between ray parenchyma and adjacent cells (*arrowheads*) were obtained in cross, radial, and tangential sections, respectively.
- Hornbeam wood: erosion of cell walls (*arrowheads*) and production of bore holes in vessel elements (*arrows*), coupled with formation of bore holes in the ray parenchyma (*arrowheads*) as observed in cross, radial, and tangential sections, respectively.

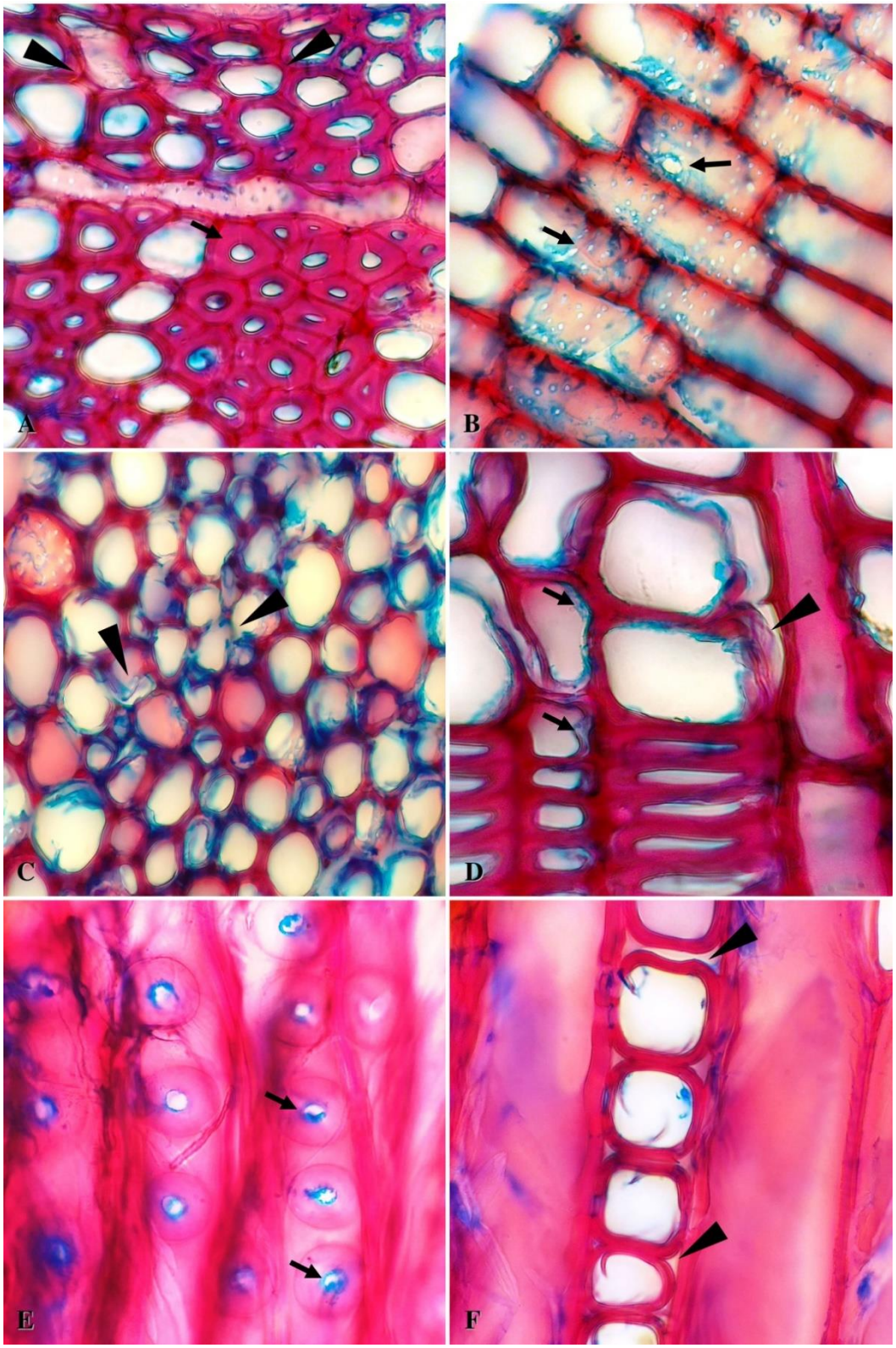

Figure 6: Micrographs of thin sections cut from oak (A-C) and pine (D-F) exposed to *Xylaria hypoxylon* for 120 days in an EN-113 decay test.

- Oak wood: degradation of cell wall layers (*arrowheads*), intact fibers (*arrows*) and production of bore holes (*arrows*) as well as severe decay of ray parenchyma (*arrowhead*) were observed in cross, radial, and tangential sections, respectively.
- Pine wood: separation (*arrowhead*) and degradation of the S3 cell wall layer due to lignin degradation (*arrow*) and degradation of pit membranes by hyphal penetration (*arrow*) as well as lignin removal between ray parenchyma and adjacent cells (*arrowheads*) were observed in cross, radial, and tangential sections, respectively.

A number of species of *Xylariaceae* and *Diatrypaceae* have been reported as causing white rot in wood (Campbell and Wiertelak 1935, Hinds 1981, Eriksson *et al.* 1990). Nilsson *et al.* (1989) showed that *X. hypoxylon* caused both Type I and Type II soft rot decay in birch as well as in pine wood samples after 4 months incubation. Interestingly, Koyani *et al.* (2017), commenting on the destructive behavior of *X. hypoxylon*, found the fungus was capable of causing both soft- and white rot decay in three hardwood species. In our work, the test fungus caused both soft- and white-rot decay in the four different wood species examined. These behaviors suggest that the fungus produces enzymes/agents capable of attacking all three wood polymers but the patterns of release or the arrangement of polymers in the wood cell walls affects the decay pattern. This is also a characteristic of some white-rot fungi (Eslyn and Nakasone 1984, Bari *et al.* 2017a, b). The ability to degrade lignin in wood is a typical feature of white rot fungi. However, significant losses of lignin have also been reported for wood attacked by typical soft-rot fungi (Levi and Preston 1965, Eslyn *et al.* 1975). The variation in decay patterns with different wood species is likely due to the chemical components in the cell walls and their accessibility for fungal decay. Differences in lignin type and level also likely affect differences in decay of gymnosperms and angiosperms by white rot fungi (Highley 1982, Faix *et al.* 1985, Daniel 2014). Typical gymnosperm lignins are composed almost entirely of guaiacyl propane units (G) (Obst and Landucci 1986). In contrast, most angiosperm lignin contains both guaiacyl propane and syringyl propane units.

4. CONCLUSIONS

Xylaria hypoxylon caused relatively low weight losses on the four wood species tested, but produced different decay patterns on each species. These results illustrate that interactions between fungal enzymes and the wood cell chemistry/morphology play an important role in the resulting condition of the wood.

5. ACKNOWLEDGMENTS

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