Comparison of Fungal Diversity on Woods with Different Fungal Susceptibility during Above-ground Exposure

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Sequential fungal changes that occur on three types of wood are described, namely naturally durable wood, preservative-treated wood, and radiata pine (considered as untreated wood) for above-ground use, during a four-year survey period. The surveys were conducted thrice at 1, 2, and 4 years of exposure. During the first and second years of exposure, both total fungal isolates and species diversity were low in each wood sample, with ascomycetes being the most prevalent. Additionally, the predominant species displayed a trivial impact on wood decay. However, after two years of exposure, the number of fungal communities increased sharply, accompanied by a considerable shift in the diversity of basidiomycetes, some of which established themselves as dominant species. The survey provides a snapshot of the initial stages of wood decay. Further, it reiterates that while fungal communities on wood are influenced by spores or hyphae in the surrounding air, colonization is further modulated by fungal susceptibility on the basis of durability. An expanded study is necessary for better comprehension of the processes involved in wood decay in their entirety.

DOI: 10.15376/biores.17.4.6740-6760

Keywords: Fungal diversity; Wood-inhabiting fungi; Wood deterioration; Tropical hardwoods; Preservative-treated woods; Susceptibility.

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INTRODUCTION

Achieving carbon neutrality is an urgent need worldwide. Towards this end, several visions and strategies have been proposed, including the usage of harvested wood products that have been recognized by the IPCC as a carbon storing material. Wood may be employed as a carbon neutralizing material by either long-term usage of large amounts for construction purposes, or as raw material for building interiors. Of these, the application of the former on a long-term basis is perceived as the key to achieving carbon neutrality. In accordance with this, there has been a gradual increase in the extent of wood utilization as a construction material for the structural components and exteriors of buildings. However, constant exposure to varied weather conditions causes changes in the internal moisture content of wood. Additionally, discoloration caused by exposure to the elements reduces its aesthetic value. Consequently, an analysis of discoloration causing fungi and other factors that may cause biological degradation is of paramount importance in order to prevent the same.

Fungi are well-established wood decomposers in natural ecosystems that play an important role in the carbon cycle (Freedman *et al*. 1996). Several studies on fungal diversity and succession in various wood species that aid comprehension of microbial wood decomposition are available (Fryar *et al*. 2004; Jönsson *et al*. 2008; Kebli *et al*. 2011). The composition of fungal populations has been shown to be influenced by several factors, including host species, wood size (Krah *et al.* 2018), and season (Olou *et al.* 2019). Additionally, depending on substrate availability, fungal succession in general has been observed to progress through soft rot, brown rot, and white-rot fungi sequentially (Jönsson *et al*. 2008; Fukasawa 2018). The authors previously reported the sequence of fungal succession during decay over a period of 54 months, along with differences in fungal diversity and species richness in two exposed wood species on the forest floor, namely *Pinus koraiensis* and *Pinus rigida* (Kim *et al*. 2009).

A proper comprehension of fungal diversity and succession in wood is essential to understand how fungi function in nature, in order to enable efficient usage of wood. This will help predict and prevent bio-deterioration that may be modulated by climatic factors, wood species, and moisture content of the surrounding areas. Thus far, most studies have primarily focused on wood that is susceptible to moisture-induced microbial degradation. Of these, the majority have concentrated on the diversity and succession of fungi on wood that is in contact with the ground, since it is affected by both ground and atmospheric moisture (Meier *et al*. 2010; Prewitt *et al*. 2014). However, wood that is used above ground is also susceptible to atmospheric moisture-induced degradation by aerial fungal spores or hyphae. Although this process is considerably slower than that induced by ground contact, it is a certain eventuality, and a serious lacuna of information exists in this regard. In addition, as the amount of wood used as material for the construction of exterior elements of buildings is increasing recently, it is necessary to investigate the growth of fungi on wood used in the above-ground parts of buildings.

The present study sought to determine fungal diversity associated with above-ground wood as well as unravel the sequential changes that occur during the process. Towards this end, three types of wood with different susceptibilities to fungi were analyzed. These included naturally durable wood, preservative-treated wood, and untreated wood. Naturally durable wood and preservative-treated wood are frequently used for the construction of exterior structures in Korea on account of being protected against fungal activity by organic compounds and/or heavy metals, respectively. Although the use of naturally durable species has increased due to being perceived as alternative replacements for preservative-treated wood, little is known about the biodiversity of various fungal species on the same, in spite of considerable research efforts. Similarly, the type of fungal diversity and succession on preservative-treated wood is largely unknown. The present study, therefore, aimed to characterize fungal diversity in the three above-mentioned types of wood during a four-year period of above-ground exposure.

EXPERIMENTAL

Preparation of Wood Samples

Fungal communities were compared on three types of wood samples, namely tropical hardwoods, copper-based preservative-treated wood, and untreated wood. Samples of three tropical hardwoods, namely Apitong, Bangkirai, and Merbau, of 2×8 cm^2 cross-sectional area and 30 cm length were prepared. All of the above-mentioned hardwoods are

naturally durable species that belong to different durability classes. Among the four durability classes: very durable, durable, moderately durable, and nondurable, Bangkirai is classified as a very durable species (Yatagai and Takahashi 1980), and Apitong and Merbau may be durable or moderately durable, depending on the wood species (Meniado *et al.* 1975; Chudnoff 1984; Yamamoto and Hong 1989) since several wood species often share the same trade name (Scheffer and Morrell 1998). The preservative-treated wood samples were prepared by cutting radiata pine sapwood to the same size as the tropical hardwood samples, followed by vacuum-impregnation with either ACQ-2 (8.0% CuO and <8.0% dodecyldimethylammonium chloride) or CUAZ-3 (9.25% CuO, 0.19% propiconazole, and 0.19% tebuconazole) to achieve the target retentions of 2.6 kg/m^3 and 0.96 kg/m^3, respectively. Additionally, untreated radiata pine sapwood, commonly regarded as decay susceptible, was included as a non-durable species in the context of fungal attack. Post sample preparation, six wood samples were installed on a 1 m high above-ground deck at Korea University, Seoul, and four replicates were used for each condition.

Fungal Isolation

Fungal surveys were conducted on the above-ground exposed wood samples at 1, 2, and 4 years. Fungi were isolated from three randomly removed wood chips from each of the samples, which were subsequently placed individually on a 2% malt extract agar medium (20 g malt extract, 15 g agar, and 1 L distilled water) supplemented with 100 ppm streptomycin, prior to incubation at room temperature. Individual mycelia were sub-cultured in fresh media until a pure culture was obtained, and all isolates were grouped on the basis of their morphological features. Molecular methods were employed to identify representative samples from each group.

Fungal Identification

Fungal DNA was extracted using the AccuPrep Genomic DNA Extraction Kit (Bioneer, Republic of Korea). Internal transcribed spacer (ITS) sequences for analysis of ascomycetes and zygomycetes, and 28S rDNA sequences for analysis of basidiomycetes were amplified by PCR using the following two primer pairs: ITS 4 (5′-TCCTCCGCTTATT GATATGC-3′) / ITS 5 (5′-GGAAGTAAAAGTCG TAACAAGG-3′) for ITS (White et al. 1990), and LROR (5′-ACCGCGTGAACTTAAGC-3′) / LR3 (5′-GGTCCGTGTTTCAAGAC-3′) for 28S rDNA (Vilgalys and Hester 1990). PCR amplification was conducted as previously described by Kim *et al* (2009). The PCR products were sequenced at the Macrogen DNA Synthesis and Sequencing Facility (Seoul, Republic of Korea), and data analysis was conducted via BLAST searches against GenBank reference strain sequences (Wheeler *et al*. 2007).

RESULTS

A total of 420 fungal isolates were obtained from three tropical hardwoods (Apitong, Bangkirai, and Merbau), two copper-based preservative-treated wood (ACQ-treated wood and CUAZ-treated wood), and untreated radiata pine samples in the three surveys (Tables 1–6). The total number of isolates and species abundance differed for each wood species according to exposure time, which is indicated in the table by the number of isolates by fungal species according to exposure time, and blanks, indicating no isolates. While ascomycetes were found to be most abundant in all three surveys, the proportion of

basidiomycetes increased marginally in the third survey. Consequently, although wood surface discoloration gradually worsened throughout the study period, appreciable decay hardly occurred prior to the third survey.

Apitong

The 88 isolates obtained comprised 20 genera and 34 species of ascomycetes, two genera and two species of zygomycetes, and four genera and five species of basidiomycetes (Table 1). The first survey resulted in the isolation of 14 strains, including 13 species, with *Phoma* sp. being the most frequently recorded. The most prevalent species among the 16 strains obtained in the second survey included five ascomycetes, namely *Alternaria alternata, Diaporthe* sp., *Paraconiothyrium brasiliense*, *Phoma glomerata*, and *Phomopsis velata*. The last survey that resulted in the isolation of 58 strains, saw the addition of a zygomycete, *Rhizopus oryzae* to the list of dominant species present on Apitong.

Bangkirai

A total of 78 isolates were obtained, including 21 genera and 26 species of ascomycetes, three genera and three species of zygomycetes, and four genera and five species of basidiomycetes (Table 2). Of the eight strains obtained in the first survey, the ascomycete, *Alternaria tenuissima* was the most frequently isolated. The ascomycetes, *A. alternata* and zygomycetes, *Umbelopsis isabellina* were the most frequently isolated species among the eight strains obtained in the second survey. Additionally, seven ascomycetes, namely *A. tenuissima*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Nectria balansae*, *Paraphaeosphaeria verruculosa*, *Phoma glomerata*, and *Trichoderma atroviride*, and a basidiomycete, *Coprinellus radians* were the most frequent species among the 59 strains obtained from the third survey.

Merbau

The 78 isolates obtained, comprised of 23 genera and 29 species of ascomycetes, one zygomycete species, and five genera and six species of basidiomycetes (Table 3). Six ascomycetes, namely *Fusarium avenaceum*, *Penicillium spinulosum*, and *Sydowia polyspora* among the 13 strains obtained from the first survey, and three ascomycetes, *P. glabrum*, *T. atroviride*, and *T. harzianum* among the 15 strains obtained from the second survey were the most dominant species. Of the 50 strains obtained in the third survey, *A. tenuissima*, *C. cladosporioides*, *E. nigrum*, *P. glomerata*, and *T. atroviride* were most frequently recorded.

ACQ-treated Wood

The 52 isolates obtained, comprising 15 genera and 19 species of ascomycetes and three genera and four species of basidiomycetes (Table 4). Only five and six strains were obtained in the first and second survey, respectively, among which *Aureobasidium pullulans* was the only species to be frequently recorded. The dominant species in the third survey included two ascomycetes, *A. tenuissima* and *P. glomerata*, and a basidiomycete, *C. radians*. Further, the establishment of their dominance was accompanied by an increase in the total number of isolates to 41.

Table 1. Fungi Isolated from Above-ground Exposed Apitong

Closest matched fungal species in BLAST (GenBank acc. no.)	No. of Isolates			Similarity (%)[a]	Fungal Identity
	12 months	24 months	48 months		
Ascomycetes					
Alternaria alternata (GQ241273)	1	2*	3*	530/530 (100.0)	*Alternaria alternata*
Alternaria tenuissima (KT223327)			11*	511/511 (100.0)	*Alternaria tenuissima*
Aplosporella prunicola (KF766147)			1	550/550 (100.0)	*Aplosporella prunicola*
Aureobasidium pullulans (AY225167)		1		571/571 (100.0)	*Aureobasidium pullulans*
Bipolaris sorokiniana (KP174682)			1	524/524 (100.0)	*Bipolaris sorokiniana*
Bipolaris sorokiniana (KT192201)			1	532/532 (100.0)	*Bipolaris sorokiniana*
Cladosporium cladosporioides (KM979883)	1		5*	496/496 (100.0)	*Cladosporium cladosporioides*
Colletotrichum acutatum (KT215297)		1		565/565 (100.0)	*Colletotrichum acutatum*
Colletotrichum sp. (DQ300348)	1			573/573 (100.0)	*Colletotrichum* sp.
Curvularia geniculata (KF498864)			1	534/534 (100.0)	*Curvularia geniculata*
Curvularia spicifera (KP899109)			1	514/514 (100.0)	*Curvularia spicifera*
Diaporthe sp. (KC145887)		3*		571/571 (100.0)	*Diaporthe* sp.
Epicoccum nigrum (HM037925)			6*	489/489 (100.0)	*Epicoccum nigrum*
Epicoccum nigrum (KR095197)			1	490/490 (100.0)	*Epicoccum nigrum*
Epicoccum sp. (FJ176473)			1	499/499 (100.0)	*Epicoccum sp.*
Fusarium asiaticum (AB289554)			1	485/485 (100.0)	*Fusarium asiaticum*
Fusarium avenaceum (AY147285)	1			549/550 (99.8)	*Fusarium avenaceum*
Fusarium equiseti (HQ332532)	1	1	3	533/534 (99.8)	*Fusarium equiseti*
Fusarium lateritium (HM061323)	1		1	545/545 (99.8)	*Fusarium lateritium*
Fusarium lateritium (GU480949)		1		549/550 (99.8)	*Fusarium lateritium*
Fusarium solani (JQ277276)	1			550/551 (99.8)	*Fusarium solani*
Nectria balansae (JN995620)	1		1	557/558 (99.8)	*Nectria balansae*
Paraconiothyrium brasiliense (JX496034)		2*		579/579 (100.0)	*Paraconiothyrium brasiliense*
Penicillium commune (KC009833)		1		575/575 (100.0)	*Penicillium commune*
Pestalotiopsis sp. (HQ832806)			1	549/551 (99.6)	*Pestalotiopsis* sp.
Didymella glomerata (KT192202)		2*	2	486/486 (100.0)	*Phoma glomerata*
Phoma sp. (KT336520)	2*			512/512 (100.0)	*Phoma* sp.

Table 1. (continued)

Closest matched fungal species in BLAST (GenBank acc. no.)	No. of Isolates			Similarity (%)[a]	Fungal Identity
	12 months	24 months	48 months		
Diaporthe eres (KC343081)		2*		567/567 (100.0)	*Phomopsis velata*
Pleurostomophora richardsiae (AB364704)	1			559/559 (100.0)	*Pleurostomophora richardsiae*
Setosphaeria rostrata (KT265238)			1	542/542 (100.0)	*Setosphaeria rostrata*
Spencermartinsia viticola (KJ561170)			1	491/494 (99.4)	*Spencermartinsia viticola*
Trichoderma atroviride (GQ241294)			6*	587/588 (99.8)	*Trichoderma atroviride*
Trichoderma harzianum (KT336515)			2	600/600 (100.0)	*Trichoderma harzianum*
Zygomycetes					
Mucor circinelloides (DQ118990)	1			626/627 (99.8)	*Mucor circinelloides*
Rhizopus oryzae (KT899481)			3*	604/604 (100.0)	*Rhizopus oryzae*
Basidiomycetes					
Coprinellus radians (KM246027)			2	612/614 (99.7)	*Coprinellus radians*
Coprinellus xanthothrix (FJ755223)			1	607/609 (99.7)	*Coprinellus xanthothrix*
Porostereum spadiceum (JF416686)			1	577/579 (99.7)	*Porostereum spadiceum*
Trametes sanguinea (HM595619)	1			616/616 (100.0)	*Trametes sanguinea*
Trametes versicolor (KC176313)	1			615/616 (99.8)	*Trametes versicolor*
Total isolates	**14**	**16**	**58**		

[a] Similarity (%) displayed by matched /comparable nucleotides.

* Dominant species. A species is regarded as a dominant species if Pi>1/S, where Pi (frequency of species i/total frequency for all species) is the proportion of the total sample represented by species i, and S (species richness) is the number of competing species (Camargo *et al.* 1993).

Table 2. Fungi Isolated from Above-ground Exposed Bangkirai

Closest matched fungal species in BLAST (GenBank acc. no.)	No. of isolates			Similarity (%)[a]	Fungal identity
	12 months	24 months	48 months		
Ascomycetes					
Alternaria alternata (GQ241273)		3*	2	508/508 (100.0)	*Alternaria alternata*
Alternaria tenuissima (KT223327)	3*		11*	511/511 (100.0)	*Alternaria tenuissima*
Botrytis cinerea (KM840848)			1	492/492 (100.0)	*Botrytis cinerea*
Cladosporium cladosporioides (KM979883)			3*	532/532 (100.0)	*Cladosporium cladosporioides*
Curvularia lunata (HM008930)		1		562/562 (100.0)	*Curvularia lunata*
Dothiorella gregaria (KT192425)			1	499/499 (100.0)	*Dothiorella gregaria*
Epicoccum nigrum (HM037925)			5*	489/489 (100.0)	*Epicoccum nigrum*
Epicoccum sp. (FJ176473)			1	499/499 (100.0)	*Epicoccum* sp.
Fusarium equiseti (HQ332532)			2	496/496 (100.0)	*Fusarium equiseti*
Nectria balansae (JN995620)			3*	532/532 (100.0)	*Nectria balansae*
Neopestalotiopsis clavispora (GQ415344)			1	512/512 (100.0)	*Neopestalotiopsis clavispora*
Paecilomyces variotii (KC254066)	1			603/603 (100.0)	*Paecilomyces variotii*
Paraconiothyrium brasiliense (JX496034)		1		577/579 (99.7)	*Paraconiothyrium brasiliense*
Paraphaeosphaeria verruculosa (JX496080)			3*	530/530 (100.0)	*Paraphaeosphaeria verruculosa*
Penicillium spinulosum (HQ608158)	1	1		567/567 (100.0)	*Penicillium spinulosum*
Pestalotiopsis microspora (JX436801)			1	551/551 (100.0)	*Pestalotiopsis microspora*
Pestalotiopsis sp. (HQ832806)			1	564/567 (99.5)	*Pestalotiopsis* sp.
Didymella glomerata (KT192202)			4*	492/492 (100.0)	*Phoma glomerata*
Setosphaeria rostrata (KT265238)			1	557/557 (100.0)	*Setosphaeria rostrata*
Spencermartinsia viticola (KJ561170)			1	499/502 (99.4)	*Spencermartinsia viticola*
Sporothrix variecibatus (JX028591)		1		580/580 (100.0)	*Sporothrix variecibatus*
Talaromyces verruculosus (HM469420)	1			555/555 (100.0)	*Talaromyces verruculosus*
Trichoderma atroviride (GQ241294)			7*	587/588 (99.8)	*Trichoderma atroviride*
Trichoderma citrinoviride (KC009820)		1		622/622 (100.0)	*Trichoderma citrinoviride*
Trichoderma harzianum (KT336515)	1		2	591/591 (100.0)	*Trichoderma harzianum*
Fungal sp. (HM999913)			1	509/509 (100.0)	Unknown ascomycete sp.

Table 2. (continued)

Closest matched fungal species in BLAST (GenBank acc. no.)	No. of Isolates			Similarity (%)[a]	Fungal Identity
	12 months	24 months	48 months		
Zygomycetes					
Mucor circinelloides (JF439684)		1		598/598 (100.0)	*Mucor circinelloides*
Rhizopus oryzae (KT899481)			2	604/604 (100.0)	*Rhizopus oryzae*
Umbelopsis isabellina (JN206400)		2*		561/561 (100.0)	*Umbelopsis isabellina*
Basidiomycetes					
Coprinellus radians (KM246027)			3*	614/616 (99.7)	*Coprinellus radians*
Coprinellus xanthothrix (FJ755223)			1	612/614 (99.7)	*Coprinellus xanthothrix*
Porostereum spadiceum (JF416686)			1	577/579 (99.7)	*Porostereum spadiceum*
Schizophyllum commune (AY858374			1	582/582 (100.0)	*Schizophyllum commune*
Trametes versicolor (JX290580)	1			582/582 (100.0)	*Trametes versicolor*
Total isolates	**8**	**11**	**59**		

[a] Similarity (%) displayed by matched /comparable nucleotides.

* Dominant species. A species is regarded as a dominant species if Pi>1/S, where Pi (frequency of species i/total frequency for all species) is the proportion of the total sample represented by species i, and S (species richness) is the number of competing species (Camargo *et al.* 1993).

Table 3. Fungi Isolated from Above-ground Exposed Merbau

Closest matched fungal species in BLAST (GenBank acc. no.)	No. of Isolates			Similarity (%)[a]	Fungal Identity
	12 months	24 months	48 months		
Ascomycetes					
Alternaria alternata (GQ241273)			2	508/508 (100.0)	*Alternaria alternata*
Alternaria tenuissima (KT223327)			11*	511/511 (100.0)	*Alternaria tenuissima*
Ascochyta medicaginicola (KF293988)		1		553/553 (100.0)	*Ascochyta medicaginicola*
Choanephora infundibulifera (KP724997)			1	571/571 (100.0)	*Choanephora infundibulifera*
Cladosporium cladosporioides (KM979883)			6*	532/532 (100.0)	*Cladosporium cladosporioides*
Curvularia geniculata (KF498864)			1	534/534 (100.0)	*Curvularia geniculata*
Curvularia sp. (JF742784)			1	505/505 (100.0)	*Curvularia* sp.
Dothiorella gregaria (KT192425)			2	499/499 (100.0)	*Dothiorella gregaria*
Epicoccum nigrum (HM037925)			6*	489/489 (100.0)	*Epicoccum nigrum*
Fusarium avenaceum (AY147285)	2*			548/548 (100.0)	*Fusarium avenaceum*
Fusarium equiseti (HQ332532)			1	484/485 (100.0)	*Fusarium equiseti*
Jattaea mookgoponga (HQ878589)		1		540/540 (100.0)	*Jattaea mookgoponga*
Nectria balansae (JN995620)			1	532/532 (100.0)	*Nectria balansae*
Neopestalotiopsis clavispora (KJ677242)			1	499/499 (100.0)	*Neopestalotiopsis clavispora*
Paecilomyces variotii (KC254066)	1			603/603 (100.0)	*Paecilomyces variotii*
Paraconiothyrium brasiliense (JX496034)		1		579/579 (100.0)	*Paraconiothyrium brasiliense*
Penicillium glabrum (JX421727)		3*		549/549 (100.0)	*Penicillium glabrum*
Penicillium spinulosum (HQ608158)	4*			567/567 (100.0)	*Penicillium spinulosum*
Talaromyces purpurogenus (JX965238)	1			541/541 (100.0)	*Penicillium purpurogenus*
Pestalotiopsis microspora (HM595547)			1	557/557 (100.0)	*Pestalotiopsis microspora*
Didymella glomerata (KT192202)			5*	486/486 (100.0)	*Phoma glomerata*
Phoma sp. (FJ176472)		1		515/515 (100.0)	*Phoma* sp.
Phomopsis sp. (JF288552)	1			569/569 (100.0)	*Phomopsis* sp.
Pyrenochaeta cava (JF740260)		1		528/531 (99.4)	*Pyrenochaeta cava*
Sydowia polyspora (JN944640)	3*	1		577/578 (99.8)	*Sydowia polyspora*
Trichoderma atroviride (KC008065)		1		609/609 (100.0)	*Trichoderma atroviride*
Trichoderma atroviride (GQ241294)		2*	3*	587/588 (99.8)	*Trichoderma atroviride*

Table 3. (continued)

Closest matched fungal species in BLAST (GenBank acc. no.)	No. of Isolates			Similarity (%)[a]	Fungal Identity
	12 months	24 months	48 months		
Trichoderma harzianum (EU280079)		2*		608/608 (100.0)	*Trichoderma harzianum*
Trichoderma harzianum (KT336515)			1	600/600 (100.0)	*Trichoderma harzianum*
Valsa leucostoma (GU062285)		1		572/573 (99.8)	*Valsa leucostoma*
Xylariales (GQ906967)			1	535/536 (99.8)	Unknown xylariales sp.
Zygomycetes					
Rhizopus oryzae (KT899481)			1	604/604 (100.0)	*Rhizopus oryzae*
Basidiomycetes					
Bjerkandera adusta (KC176332)			1	604/610 (99.0)	*Bjerkandera adusta*
Coprinellus radians (KM246027)			1	612/614 (99.7)	*Coprinellus radians*
Coprinellus xanthothrix (FJ755223)			1	612/614 (99.7)	*Coprinellus xanthothrix*
Gelatoporia subvermispora (FN907911)			1	615/615 (100.0)	*Gelatoporia subvermispora*
Phlebia tremellosa (JF416676)			1	604/604 (100.0)	*Phlebia tremellosa*
Trametes versicolor (KC176313)	1			582/582 (100.0)	*Trametes versicolor*
Total isolates	**13**	**15**	**50**		

[a] Similarity (%) displayed by matched /comparable nucleotides.

* Dominant species. A species is regarded as a dominant species if Pi>1/S, where Pi (frequency of species i/total frequency for all species) is the proportion of the total sample represented by species i, and S (species richness) is the number of competing species (Camargo *et al.* 1993).

Table 4. Fungi Isolated from Above-ground Exposed ACQ-treated Wood

Closest matched fungal species in BLAST (GenBank acc. no.)	No. of Isolates			Similarity (%)[a]	Fungal Identity
	12 months	24 months	48 months		
Ascomycetes					
Alternaria alternata (GQ241273)			2	508/508 (100.0)	*Alternaria alternata*
Alternaria tenuissima (KT223327)			9*	511/511 (100.0)	*Alternaria tenuissima*
Aplosporella longipes (KM030583)			1	546/546 (100.0)	*Aplosporella longipes*
Aspergillus tubingensis (KF624772)		1		580/580 (100.0)	*Aspergillus tubingensis*
Aureobasidium pullulans (HG532077)	3*	3*		570/570 (100.0)	*Aureobasidium pullulans*
Cladosporium cladosporioides (KM979883)			1	496/496 (100.0)	*Cladosporium cladosporioides*
Dothiorella gregaria (KT192425)			1	499/499 (100.0)	*Dothiorella gregaria*
Epicoccum nigrum (HM037925)			2	489/489 (100.0)	*Epicoccum nigrum*
Fusarium oxysporum (KC304806)			1	488/488 (100.0)	*Fusarium oxysporum*
Paraconiothyrium brasiliense (JX496058)			1	557/557 (100.0)	*Paraconiothyrium brasiliense*
Penicillium rubrum (JX965243)	1			556/558 (99.6)	*Penicillium rubrum*
Pestalotiopsis microspora (HM595547)			2	551/551 (100.0)	*Pestalotiopsis microspora*
Didymella sp. (KP127999)			1	477/477 (100.0)	*Phoma* sp.
Didymella glomerata (KT192202)			10*	486/486 (100.0)	*Phoma glomerata*
Phoma herbarum (KF251212)		1		521/521 (100.0)	*Phoma herbarum*
Psathyrella candolleana (DQ110874)			1	616/616 (100.0)	*Psathyrella candolleana*
Trichoderma atroviride (GQ241294)			1	587/588 (99.8)	*Trichoderma atroviride*
Trichoderma harzianum (JN108918)	1			598/598 (100.0)	*Trichoderma harzianum*
Ascomycota sp. (JN835204)			1	492/497 (99.0)	Unknown ascomycete sp.
Basidiomycetes					
Bjerkandera adusta (KC176332)			1	610/610 (100.0)	*Bjerkandera adusta*
Coprinellus radians (KM246027)		1	4*	607/609 (99.7)	*Coprinellus radians*
Coprinellus xanthothrix (FJ755223)			1	612/614 (99.7)	*Coprinellus xanthothrix*
Gelatoporia subvermispora (FN907911)			1	609/610 (99.8)	*Gelatoporia subvermispora*
Total isolates	**5**	**6**	**41**		

[a] Similarity (%) displayed by matched /comparable nucleotides.

* Dominant species. A species is regarded as a dominant species if Pi>1/S, where Pi (frequency of species i/total frequency for all species) is the proportion of the total sample represented by species i, and S (species richness) is the number of competing species (Camargo *et al.* 1993).

Table 5. Fungi Isolated from Above-ground Exposed CUAZ-treated Wood

Closest matched fungal species in BLAST (GenBank acc. no.)	No. of Isolates			Similarity (%)[a]	Fungal Identity
	12 months	24 months	48 months		
Ascomycetes					
Alternaria alternata (GQ241273)			3*	508/508 (100.0)	*Alternaria alternata*
Alternaria tenuissima (KT223327)			6*	511/511 (100.0)	*Alternaria tenuissima*
Arthrinium phaeospermum (FJ462766)			1	573/573 (100.0)	*Arthrinium phaeospermum*
Aureobasidium pullulans (JF439462)			2	553/553 (100.0)	*Aureobasidium pullulans*
Chaetomium aureum (JX186515)			1	549/549 (100.0)	*Chaetomium aureum*
Cladosporium cladosporioides (KM979883)			4*	496/496 (100.0)	*Cladosporium cladosporioides*
Cladosporium sp. (LN808884)			1	526/526 (100.0)	*Cladosporium* sp.
Epicoccum nigrum (HM037925)			2	489/489 (100.0)	*Epicoccum nigrum*
Neopestalotiopsis clavispora (GQ415344)			1	512/512 (100.0)	*Neopestalotiopsis clavispora*
Paraconiothyrium brasiliense (JF439492)	1			594/594 (100.0)	*Paraconiothyrium brasiliense*
Penicillium fellutanum (AF033399)		1		542/542 (100.0)	*Penicillium fellutanum*
Penicillium ochrochloron (AF178516)		1		519/519 (100.0)	*Penicillium ochrochloron*
Didymella glomerata (KT192202)			8*	486/486 (100.0)	*Phoma glomerata*
Phoma herbarum (KF251212)		1	1	521/521 (100.0)	*Phoma herbarum*
Psathyrella candolleana (DQ110874)			1	608/609 (99.8)	*Psathyrella candolleana*
Pteris tremula (GQ241293)			1	523/525 (99.6)	*Pteris tremula*
Trichoderma atroviride (GQ241294)			2	587/588 (99.8)	*Trichoderma atroviride*
Trichoderma harzianum (KT336515)			3*	600/600 (100.0)	*Trichoderma harzianum*
uncultured Pleosporales (FJ552933)	1			627/645 (97.2)	Unknown ascomycete sp. 1
uncultured Pleosporales (GU055987)			2	536/540 (99.3)	Unknown ascomycete sp. 2
Basidiomycetes					
Bjerkandera adusta (KC176332)			1	618/618 (100.0)	*Bjerkandera adusta*
Coprinellus radians (KM246027)			3*	612/614 (99.7)	*Coprinellus radians*
Coprinellus xanthothrix (FJ755223)			2	607/609 (99.7)	*Coprinellus xanthothrix*
Total isolates	**2**	**3**	**45**		

[a] Similarity (%) displayed by matched /comparable nucleotides.

* Dominant species. A species is regarded as a dominant species if Pi>1/S, where Pi (frequency of species i/total frequency for all species) is the proportion of the total sample represented by species i, and S (species richness) is the number of competing species (Camargo *et al.* 1993).

Table 6. Fungi Isolated from Above-ground Exposed Radiata Pine

Closest matched fungal species in BLAST (GenBank acc. no.)	No. of Isolates			Similarity (%)[a]	Fungal Identity
	12 months	24 months	48 months		
Ascomycetes					
Alternaria alternata (GQ241273)		1		530/530 (100.0)	*Alternaria alternata*
Alternaria tenuissima (KT223327)	1		11*	511/511 (100.0)	*Alternaria tenuissima*
Aschersonia sp. (HQ172896)		2*		527/529 (99.6)	*Aschersonia* sp.
Choanephora infundibulifera (KP724997)			1	571/571 (100.0)	*Choanephora infundibulifera*
Cladosporium cladosporioides (KM979883)		2*	6*	496/496 (100.0)	*Cladosporium cladosporioides*
Coniochaeta ligniaria (AY198390)		1		566/566 (100.0)	*Coniochaeta ligniaria*
Curvularia intermedia (GU073102)			1	566/566 (100.0)	*Curvularia intermedia*
Curvularia lunata (FJ792584)	1			587/587 (100.0)	*Curvularia lunata*
Curvularia spicifera (KP899109)			1	514/514 (100.0)	*Curvularia spicifera*
Epicoccum nigrum (HM037925)			5*	499/500 (99.8)	*Epicoccum nigrum*
Fusarium culmorum (JX125046)			1	490/490 (100.0)	*Fusarium culmorum*
Fusarium equiseti (HQ332532)	1			534/534 (100.0)	*Fusarium equiseti*
Fusarium lateritium (GU480949)		1		547/550 (99.5)	*Fusarium lateritium*
Fusarium lateritium (HM061323)	1		1	501/501 (100.0)	*Fusarium lateritium*
Fusarium verticillioides (JX914478)		1		547/547 (100.0)	*Fusarium verticillioides*
Didymella glomerata (EU098115)	2*			508/508 (100.0)	*Paraconiothyrium fuckelii*
Penicillium griseofulvum (GQ241285)	1			534/534 (100.0)	*Penicillium griseofulvum*
Penicillium oxalicum (KF997090)		1		551/551 (100.0)	*Penicillium oxalicum*
Penicillium purpurogenum (JX965237)	2*	1		541/541 (100.0)	*Penicillium purpurogenum*
Didymella glomerata (KT192202)		1	1	486/486 (100.0)	*Phoma glomerata*
Phoma herbarum (JQ936276)	1			521/523 (99.6)	*Phoma herbarum*
Ascochyta medicaginicola (EU167575)	1	1		530/530 (100.0)	*Phoma medicaginis*
Spencermartinsia viticola (KJ561170)			1	497/500 (99.4)	*Spencermartinsia viticola*
Trichoderma atroviride (GQ241294)		1	4*	587/588 (99.8)	*Trichoderma atroviride*
Trichoderma harzianum (AY605739)		1		610/610 (100.0)	*Trichoderma harzianum*
Trichoderma harzianum (KT336515)			1	600/600 (100.0)	*Trichoderma harzianum*
Trichoderma viride (EU280079)	1			593/593 (100.0)	*Trichoderma viride*

Table 6. (continued)

Closest matched fungal species in BLAST (GenBank acc. no.)	No. of Isolates			Similarity (%)[a]	Fungal Identity
	12 months	24 months	48 months		
Trichoderma viridescens (KT336515)		3*		593/593 (100.0)	*Trichoderma viridescens*
Zygomycetes					
Rhizopus oryzae (KT899481)			4*	604/604 (100.0)	*Rhizopus oryzae*
Umbelopsis isabellina (JN206400)		1		561/561 (100.0)	*Umbelopsis isabellina*
Basidiomycetes					
Bjerkandera adusta (KC176332)	1			617/617 (100.0)	*Bjerkandera adusta*
Coprinellus radians (KM246027)		1	2	614/616 (99.7)	*Coprinellus radians*
Coprinellus xanthothrix (FJ755223)			2	612/614 (99.7)	*Coprinellus xanthothrix*
Total isolates	**13**	**19**	**42**		

[a] Similarity (%) displayed by matched /comparable nucleotides.

* Dominant species. A species is regarded as a dominant species if Pi>1/S, where Pi (frequency of species i/total frequency for all species) is the proportion of the total sample represented by species i, and S (species richness) is the number of competing species (Camargo *et al.* 1993).

CUAZ-treated Wood

A total of 50 isolates were obtained, including 15 genera and 20 species of ascomycetes, and two genera and three species of basidiomycetes (Table 5). There were no dominant species among the two strains obtained in the first, and 3 strains obtained in the second survey. However, five ascomycetes, namely *A. alternata*, *A. tenuissima*, *C. cladosporioides*, *P. glomerata*, and *T. atroviride*, and one basidiomycete, *C. radians* were found to be dominant among the 45 strains obtained in the third survey.

Radiata Pine

The 74 isolates obtained, included 13 genera and 26 species of ascomycetes, two genera and two species of zygomycetes, and two genera and three species of basidiomycetes (Table 6). *P. glomerata* and *P. purpurogenus* emerged as the dominant species among the 13 strains obtained in the first survey. In contrast, the dominant strains switched to three ascomycetes, namely *Aschersonia* sp., *C. cladosporioides*, and *T. viridescens* among the 19 strains obtained in the second survey, which further changed to the four ascomycetes, *A. tenuissima*, *C. cladosporioides*, *E. nigrum*, and *T. atroviride*, and one zygomycete, *R. oryzae* among the 42 strains obtained in the third survey.

DISCUSSION

Fungal diversity and succession were observed to be most strongly influenced by substrate type, as has been previously reported (Kebli *et al*. 2011). Unsurprisingly, the composition of fungal species was found to differ during each survey, with dramatic shifts in fungal succession being observed without any evidence of previous colonization. These findings are corroborated by several previous studies (Jönsson *et al*. 2008; Kirker *et al*. 2012). While the first and second surveys resulted in low frequencies of isolates with poor species richness, greater frequency and diversity were recorded in the third survey in all wood samples. The low frequencies at the beginning of the investigation may have been caused by two factors. First, the initial resistance was consistent with the natural durability and antiseptic effect associated with tropical hardwood extract and preservatives, respectively. Second, the moisture content of wood above ground is not higher than the water content of the wood in contact with the ground. Further, the samples were not visibly affected by the dominant species in the initial survey, and discoloration was only visible from the second survey onwards in the tropical hardwood and radiata pine samples (Fig. 1). The extent of discoloration was more obvious in Bangkirai and radiata pines than that observed in the other wood types. While the Bangkirai samples developed a dark gray discoloration due to *A. tenuissima*, the radiata pine samples were discolored in part, to dark gray and green by *A. tenuissima* and *T. harzianum*, respectively. *A. tenuissima* is known to commonly colonize and cause discoloration of wood and wood products (Lee *et al*. 2014), and *T. harzianum*, a green spore producer, is one of the most common wood-inhabiting species (Huh *et al*. 2011). The Apitong and Merbau samples were also found to undergo a moderate degree of discoloration. The dominant species isolated from Apitong, namely *A. alternata*, *Diaporthe* sp., *Paraconiothyrium brasiliense*, *P. glomerata*, and *Phomopsis velata* produced dark brown and gray spores or mycelia that changed wood color to dark brown. Of these, *A. alternata*, which was differentiated as a mold by Seifert (Seifert 1999), is a well-known dark mold, and *P. brasiliense* has been identified in various wood types by Damm *et al*. (2008), via GenBank sequence database searches.

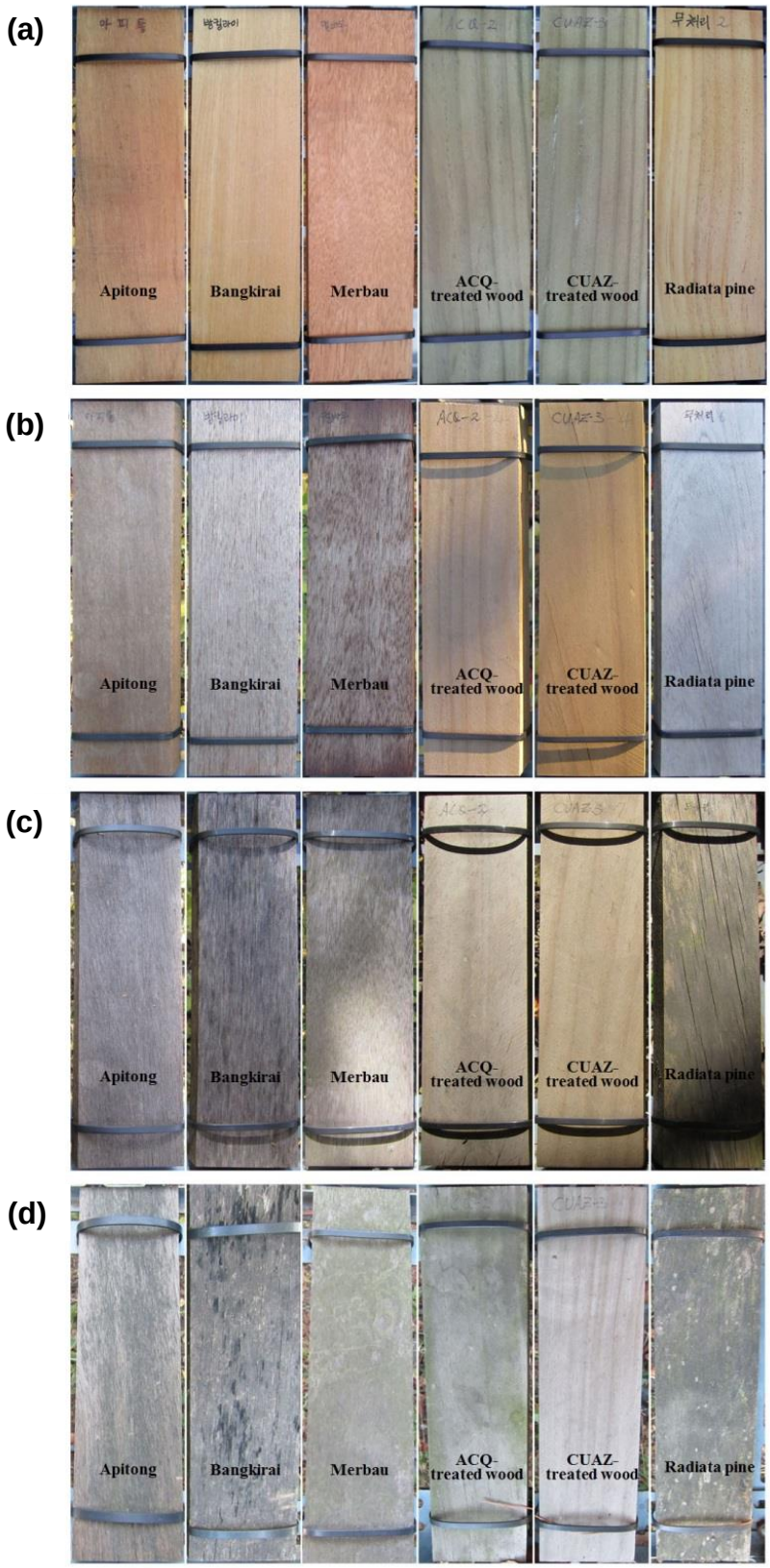

Fig. 1. The changes on wood surfaces as a function of time spent above-ground (a) after installation, (b) after one year of exposure (c) after two years of exposure, and (d) after four years of exposure

Additionally, the pathogenic species, *Phomopsis* species and *P. glomerata*, have been previously found to occupy discolored portions of wood (Hosford 1975; Baumgartner *et al*. 2013). In contrast, the three fungi isolated from Merbau, namely *P. glabrum*, *T. atroviride*, and *T. harzianum* are abundant surface producers of blue-green spores. *P. glabrum*, which is ubiquitously present in forests (Lee *et al*. 2003). It is known to cause considerable discoloration of the Japanese red pine (Jang *et al*. 2011). On the other hand, the extent of wood discoloration caused by the two *Trichoderma* species was moderate in comparison, on account of the species being sparse spore producers (Huh *et al*. 2011). Notably, fungal growth on copper-based preservative-treated wood samples became evident only during the second survey.

All of the wood types, except for the CUAZ-treated samples harbored similar dominant species and exhibited severe surface discoloration (Fig. 1). The highest frequency in tropical hardwoods and radiata pine were recorded for *A. tenuissima*, while *P. glomerata* was most abundant in the copper-based preservative-treated wood. The genus *Phoma* was commonly present in the radiata pine samples. *P. glomerata* has been previously readily isolated from western hemlock samples that were treated with chromated copper arsenate (Choi 2004, Kim *et al*. 2007). While a proportion of wood samples in the present study were treated with preservatives to prevent fungal growth, is possible that *P. glomerata* is insensitive to the active ingredients. Notably, *C. cladosporioides*, that causes significant dark khaki wood discoloration, as assessed by Lee *et al*. (2012), was frequently obtained from all of our samples, except for the ACQ-treated wood samples.

Notably, basidiomycetes, usually the main participants in wood decay, were recorded to have a low frequency in the initial survey; however, their numbers were observed to increase to six genera and seven species by the third survey (*Bjerkandera adusta*, *C. radians*, *C. xanthothrix*, *Gelatoporia subvermispora*, *Phlebia tremellosa*, *Porostereum spadiceum*, and *Schizophyllum commune*). In particular, basidiomycetes were obtained primarily from tropical hardwoods and preservative-treated wood samples that contained natural or artificial antifungal materials, and not from untreated radiata pine, a decay-susceptible species. Among the basidiomycetes, the brown rot fungus *C. radians*, which has been previously isolated from various wood types, was found to be the dominant species in the Bangkirai, ACQ-, and CUAZ-treated wood samples (Casieri *et al*. 2009; Jang *et al*. 2015).

Outstanding shifts in the fungal communities, as evidenced by greater species richness, increased numbers of isolates, and the emergence of basidiomycetes was seen to occur during the third survey. While we did not observe a marked decline in natural durability or the antifungal effects of preservatives during the initial survey, a simultaneous decrease in durability and an increased susceptibility to fungi was readily apparent during the third survey. This may be a consequence of exposure to rain or other types of moisture that potentially erode protective components. We therefore infer that the early stages of wood decomposition may have commenced around the time of the third survey since fungal succession during wood decay typically shifts from non-decaying fungi to soft-rot, to brown-rot, and finally to white-rot fungi (Hyde and Jones 2002). Even though the fungal communities identified differed between wood types between surveys, the diversity of dominant species in the third survey was minimally influenced by wood type in comparison to that observed in the initial survey. Above-ground fungal wood deterioration is generally initiated either by the surface landing and germination of atmospheric spores and hyphae, or by the growth of fungi already present in the wood. Therefore, the type of dominant

species established in the substrate is often a result of the characteristics of the surrounding environment, but diversity is also affected.

In conclusion, wood that is not in contact with the ground typically experiences delayed fungal attack in comparison to wood that is in contact with the ground. This is readily explained by the inability of moisture and microorganisms to migrate from the soil to above-ground wood. A previous study established that while the early stages of decomposition for various wood types in contact with the ground commences within four months of exposure (Prewitt *et al*. 2014), the initiation of decomposition of above-ground wood took around four years. It is therefore obvious that the establishment of basidiomycetes species, particularly white rot fungi, as the predominant species, results in accelerated wood decay. Consequently, further studies that monitor fungal isolates during the later stages of wood decomposition are essential for better comprehension of wood decay. Additionally, an in-depth analysis of control measures to counter strains that cause major discoloration is necessary.

CONCLUSIONS

1. Fungal diversity differed according to wood type, and it was observed to be entirely shifted at every survey time point. The results of the first and second surveys revealed substantially low species richness and diversity values in all wood types, particularly in those that had been treated with copper-based preservatives.
2. The number of fungal isolates was seen to increase after two years, and higher species richness was recorded after four years of exposure. Most isolates were found to be ascomycetes, which are early colonizers that cause wood decay.
3. Although the surface effects of fungal communities on tropical hardwoods and preservative-treated wood samples were suppressed to a greater extent than that evident on radiata pine samples, up to two years of exposure, surface discoloration was evident in all wood types except for CUAZ-treated wood after four years of exposure. Notably, the genus *Alternaria* was found to discolor naturally durable species within two years.
4. In wood samples that were not in contact with the ground, initiation of decay may be delayed up to four years, prior to subsequent acceleration. The number of isolates was observed to increase after four years of exposure, since hardly any basidiomycetes were recorded during the initial survey. These comprised mainly brown rot fungi that are early wood decay causing colonizers. Consequently, appropriate preventive measures are of paramount importance to avoid wood decay.

ACKNOWLEDGMENTS

This work was supported by a research grant from Korea University.

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Article submitted: August 11, 2022; Peer review completed: September 11, 2022; Revised version received: October 13, 2022; Accepted: October 15, 2022; Published: October 19, 2022.
DOI: 10.15376/biores.17.4.6740-6760