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Experimental investigation of the humidity effect on wood discoloration by selected mold and stain fungi for a proper conservation of wooden cultural heritages

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Abstract

Unfinished interior wood members in wooden cultural heritages undergo severe discoloration by fungal attack during the summer rainy season. The prevention of fungal discoloration is crucial in the conservation of wooden cultural heritages. It is important to investigate discoloration properties of major mold and stain fungi under different relative humidity conditions to prevent discoloration damage. Among the 54 ascomycetes isolated from wooden cultural heritages in Korea, *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *Coniochaeta velutina,* and *Graphiopsis chlorocephala* were selected as test fungi because of their particularly strong discoloration intensity on wood. For each fungal species, the minimum relative humidity and exposure period for discoloration were investigated. It was found that *A. pullulans* and *Cl. cladosporioides* started to grow and discolor at 75% relative humidity within 3 weeks to 4 weeks, and *Co. velutina* and *G. chlorocephala* grew and discolored at relative humidity values greater than 95%. Therefore, the prevention of fungal discoloration of interior wood members by *A. pullulans* and *Cl. cladosporioides* for the proper conservation of wooden cultural heritages can be achieved by maintaining an indoor relative humidity of 75% or less during the summer rainy season.

Keywords: Fungal discoloration, Relative humidity, Wooden cultural heritages

Introduction

Unfinished interior wood members in wooden cultural heritages (WCHs) in Korea are often discolored severely by fungal attack during the summer rainy season (Fig. 1). This fungal discoloration has been ongoing, but more discoloration damage is expected in the future due to the increase in atmospheric humidity caused by recent increases in precipitation [1]. Although it has little effect on wood strength, fungal discoloration should be prevented because it greatly reduces the esthetic value of wood [2]. Several factors affect fungal attack on wood surfaces. The most important factor is the indoor relative humidity (RH). The critical limit of the RH and exposure period necessary for fungal attack depend on the fungal species. Some fungal species may begin to grow and discolor if the critical RH is maintained for a certain period, even when the moisture content of the wood is maintained below the fiber saturation point [3]. Investigations of annual changes in indoor RH in some WCHs showed that indoor RH in the summer rainy season increased to above 75% and this increase lasted for more than 4 weeks [4]. These results suggest that serious fungal discoloration of interior wood by some mold and stain fungi might occur in the summer. Kim et al. [4] observed severe discoloration of unfinished interior wood of existing WCHs during their RH investigation. Fungal discoloration is expected to become more severe in the future as the

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Fig. 1 Severe fungal discoloration of unfinished interior wood members

duration period of RH values exceeding 75% increases because of projected increases in the precipitation and temperature caused by climate change [5]. Proper conservation of WCHs to prevent fungal discoloration will require identifying the mold and stain fungi associated with heavy discoloration and determine the minimum RH values and exposure periods needed for growth and discoloration.

In this study, test fungi that caused heavy discoloration were selected from 54 ascomycetes isolated from WCHs in a previous study [6] and the discoloration characteristics of each test fungus were assessed under various RH conditions.

Materials and methods

Selection of test fungi

Fifty-four fungi isolated in previous study [6] were screened for ability to develop intensive dark staining. Fresh sapwood specimens of Japanese red pine (5 mm × 20 mm × 60 mm), commonly used in WCHs, were prepared and autoclaved at 121 °C for 20 min. The discoloration intensity test was performed according to the procedure described by Huh et al. [7]. Four replicates were placed on U-shaped glass rod in a Petri dish for each fungal strain. To maintain high humidity in the Petri dishes, two layers of moistened filter papers were placed on the bottom of each dish. One end of each wood specimen was inoculated with each fungal species grown on malt extract agar and incubated at room temperature for 4 weeks. Discoloration intensity was visually assessed on each wood specimen using a scale of 1 to 3 (1 = slight discoloration, 2 = moderate discoloration, and 3 = heavy discoloration), as shown in Fig. 2. In addition, coloration of the wood surface was determined using the Munsell Soil Color Charts (Munsell Color, Baltimore, MD, USA).

Nineteen species caused discoloration, but only *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *Coniochaeta velutina,* and *Graphiopsis chlorocephala* produced discoloration intensities of 3. The four test fungi produced black or very dark grayish-green to grayish olive discoloration of the wood and were selected for further study (Table 1).

Discoloration testing

Sapwood specimens (5 mm × 20 mm × 60 mm) were cut from air-dried Japanese red pine boards to examine the degree of surface discoloration by selected test fungi under various RH conditions at 25 °C. The sapwood specimens were autoclaved at 121 °C for 20 min before the discoloration test. The test temperature was selected because the average temperature over the last 30 years in Seoul (capital of Korea) was 24.9 °C in July and 25.7 °C in August [8]. The humidity experiments were conducted using small humidity chambers that consisted of airtight plastic boxes with wood specimens supported on a

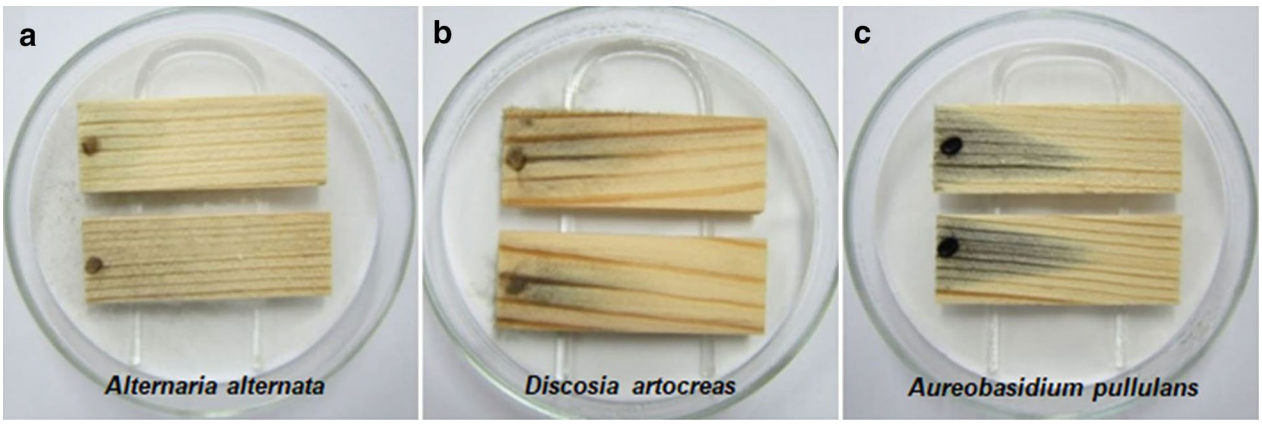

Fig. 2 Discoloration intensity of wood surface by mold and stain fungi; the discoloration was rated as: **a** 1 = slight discoloration; **b** 2 = moderate discoloration; and **c** 3 = heavy discoloration

Table 1 Wood discoloration and color of wood surface 4 weeks after inoculation with selected ascomycetes previously isolated from WCHs

Fungus	Isolate no.	Discoloration intensity[a]	Coloration of the wood surface
Alternaria alternata	KUC5301	1	Dark gray
Aspergillus niger	KUC5302	1	Black
Aureobasidium pullulans	KUC5305	3	Black
Botryosphaeria dothidea	KUC5306	1	Very dark gray
Cladosporium cladosporioides	KUC5307	3	Very dark grayish-green
Coniochaeta velutina	KUC5310	3	Dark olive gray
Coniothyrium fuckelii	KUC5311	1	Pale olive
Discosia artocreas	KUC5313	2	Dark gray
Graphiopsis chlorocephala	KUC5321	3	Very dark grayish olive
Penicillium brevicompactum	KUC5333	1	Grayish-green
Penicillium chermesinum	KUC5334	1	Light gray
Penicillium herquei	KUC5336	1	Yellow
Penicillium purpurogenum	KUC5338	2	Dark grayish olive
Penicillium paneum	KUC5339	1	Dark olive gray
Penicillium simplicissimum	KUC5340	2	Grayish-green
Penicillium sp. 1	KUC5341	1	Brownish yellow
Phoma exigua	KUC5347	2	Dark gray
Phoma glomerata	KUC5348	2	Dark gray
Trichorderma harzianum	KUC5358	1	Olive green

[a] 1 = slight discoloration; 2 = moderate discoloration; 3 = heavy discoloration

plastic mesh platform. Aqueous glycerol solutions were used to adjust RH (75%, 90%, and 95%) within chambers [9]. The 100% RH (saturated) was reached by using a closed chamber containing distilled water. For each RH treatment, two chambers containing three wood specimens each were used. The surface of wood specimens was inoculated with 1 mL of the suspension of colony forming units (CFU) of each fungus containing approximately 10^7 CFU/mL by spraying so that the entire surface was moistened with the suspension. The degree of discoloration for each specimen was visually rated from 0 (no discoloration) to 5 (100% discolored) every week during the 12-week incubation period based on the percentage of the discolored area over total area (Table 2).

Table 2 Rating scale for the discoloration of specimen surface

Rating	Macroscopic discoloration level
0	No discoloration
1	A little discoloration, but light on surface
2	Less than 10% of specimen surface discolored
3	10% or more and less than 50% of specimen surface discolored
4	50% or more and less than 100% of specimen surface discolored
5	100% of specimen surface discolored

Results and discussion

Fungal growth and resultant discoloration are a result of complex interactions among RH, incubation period, and fungal species (Fig. 3). These interactions must be considered for the development of strategies to prevent fungal discoloration on WCHs in humid environments. RH was the key environmental factor determining fungal discoloration, and the degree of discoloration increased rapidly in high RH conditions. The lowest RH and shortest incubation period required for growth differed among the test fungi. The discoloration by *Co. velutina* was only observed at 100% RH, while *G. chlorocephala* began to discolor at RH values greater than 95%. *A. pullulans* and *Cl. cladosporioides,* discolored at RH values greater than 75%. Findings with *A. pullulans* and *Cl. cladosporioides* were similar to those of Viitanen [10] who found that the risk for fungal growth in pine and spruce sapwood exists in continual humidity exposures above 80% RH. Fungal discoloration by *A. pullulans* and *Cl. cladosporioides* was detected within 3 weeks to 4 weeks at 75% RH and within at least 2 weeks at RH values greater than 90%. However, these two fungi did not cause discoloration exceeding 1 (a little fungal discoloration) during incubation for 12 weeks at 75–90% RH, except in the case of *Cl. Cladosporioides* at a 90% RH. The degree

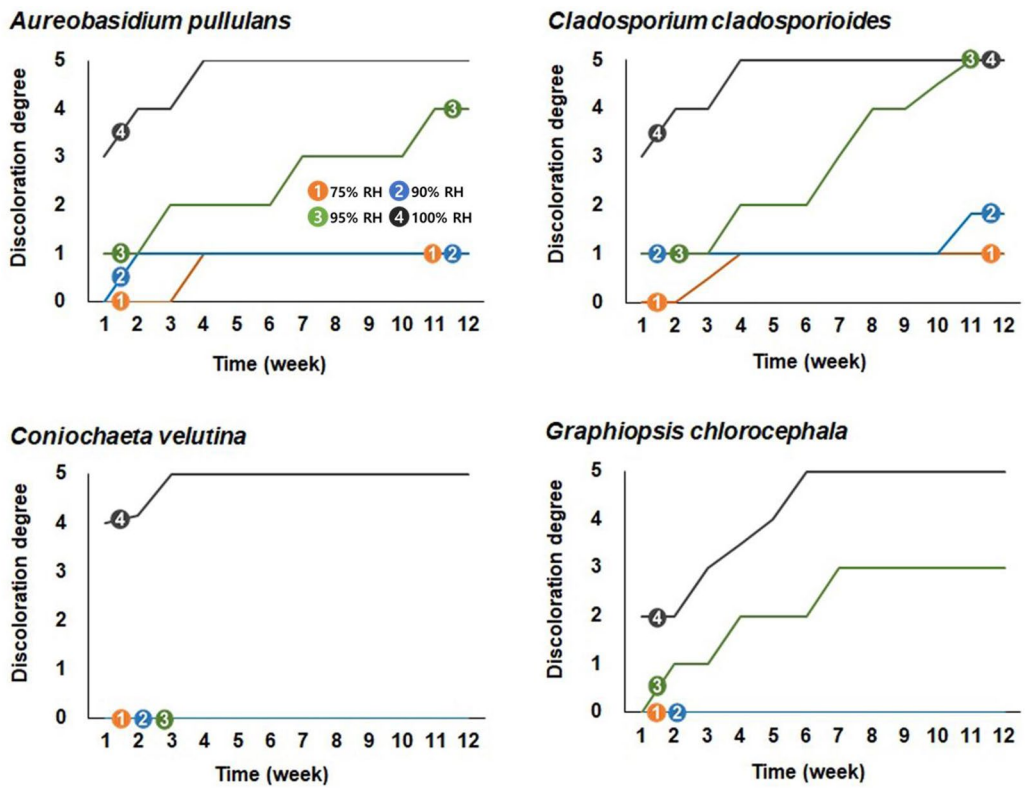

Fig. 3 Effect of the RH on wood discoloration by mold and stain fungi at 25 °C

of discoloration for *Cl. cladosporioides* increased to 2 (fungal discoloration of more than 10%) at 90% RH after 11 weeks of incubation. Maximum discoloration (fungal discoloration around 100%) at 100% RH was achieved, although the incubation period required to reach the maximum discoloration differed among the fungal species.

Prevention of fungal discoloration on wood surfaces is very crucial for the conservation of WCHs because of their heavy discoloration intensity, regardless of their degree of discoloration. The indoor RH of WCHs in Korea in the summer rainy season increases to above 75% and remains elevated for more than 4 weeks [4].

Thus, greater emphasis should be placed on discoloration by *A. pullulans* and *Cl. cladosporioides*, which start to grow at 75% RH, than on discoloration by *Co. velutina* and *G. chlorocephala*, which require at least 95% RH for growth. *A. pullulans* and *Cl. cladosporioides* were isolated at a low frequency [6], and the discoloration degree was only 1 at 75% RH. However, these taxa should not be overlooked because they are dark stain and mold fungus, respectively, and have a prominent effect on the appearance of wood. Also, *Cl. cladosporioides* has been reported to cause more severe discoloration in Japanese red pine than radiata pine [11]. Considering that the majority of domestic WCHs have been built using Japanese red pine, the discoloration problems caused by *Cl. cladosporioides* are a serious concern.

Therefore, the indoor RH of WCHs should be maintained at below 75% or even lower to prevent discoloration caused by *A. pullulans* and *Cl. cladosporioides* all year round, regardless of outdoor RH. If the outdoor RH is lower than 75%, the indoor RH of WCHs can be easily controlled by opening doors in the daytime. In contrast, if the outdoor RH exceeds 75% due to continuous rain especially in the summer rainy season, the indoor RH can rise to above 75% even if the doors are closed, in which case the dehumidifier can be used to control the indoor RH.

Because *A. pullulans* and *Cl. cladosporioides* are distributed worldwide and are common environmental fungi found in moisture-damaged buildings [12, 13], the results of this study can be applied to prevent mold and stain development in various wooden buildings located in humid climates.

Conclusions

Fungal growth and resultant discoloration of wood surfaces is a complicated process that depends on interactions between RH, fungal species, and incubation period.

The discoloration by *Co. velutina* and *G. chlorocephala* was only detected at 100% RH and RH values greater than 95%, respectively. In contrast, *A. pullulans* and *Cl. cladosporioides* began to grow and discolor within 3–4 weeks at 75% RH, although the discoloration degree was low. Fungal attack by *A. pullulans* and *Cl. cladosporioides* can be controlled by maintaining an indoor RH of 75% or less during the summer rainy season.

Abbreviations
WCHs: Wooden cultural heritages; RH: Relative humidity.

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Authors' contributions
GHK and MJK designed and planned the experiment. MJK, YSC, and JJO performed the experiments. MJK and YSC analyzed data, interpreted data, and wrote the manuscript with contribution of JJO. GHK supervised the experiment and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

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