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## Assessment of decay risk of airborne wood-decay fungi

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**Abstract** The decay risk of airborne wood-decay fungi was investigated by using an air sampler. Japanese cedar disks 7.8 cm in diameter and about 3 mm in thickness with moisture content of about 100% were placed in a “BIOSAMP” air sampler and exposed to 1000 l air. Air sampling was carried out from June to September at the same sampling site in Tsukuba, Japan. The exposed disks were then incubated for 16 weeks in a damp container kept at  $26^{\circ} \pm 2^{\circ}\text{C}$ . During the incubation period, wood mass loss ranged from –15 to 807 mg with a mean mass loss of 244 mg. Factors affecting mass loss were explored. Wood moisture content and ratio of heartwood area proved to be significant factors. In addition, six weather factors were found to influence mass loss. Disks that were sampled on a cloudy day showed significantly higher mean mass loss compared to those sampled on a sunny day. Subculturing of filamentous fungi from 16-week incubated disks suggested one-third of the isolated fungi produced ligninolytic enzymes.

**Key words** Decay risk · Weather conditions · Spores · Airborne fungi · Air sampler

### Introduction

Wooden structures such as guardrails, noise barriers, and bridges can be considered as a carbon sink.<sup>1</sup> However, they are also a source of carbon dioxide emissions. Therefore, it is very important to prolong the service life of wooden structures and delay their emissions of carbon dioxide gas. One possible method for retarding gas emissions is through preservative treatments,<sup>2</sup> and many investigative studies on preservatives and preservative treatments have been conducted.<sup>3–5</sup>

On the other hand, research on the factors that accelerate carbon dioxide gas emission from wooden structures is also important, and such studies have revealed that one of the most important biological factors for accelerating emissions is wood-decay fungi.<sup>6,7</sup> Many studies have been conducted to investigate wood-decay fungi and the decay phenomenon, showing that the majority of wood-decay fungi are classified as Basidiomycota,<sup>7</sup> which form basidia that produce basidiospores. Basidiospores released from the basidia are transferred to wood by wind, water, and insects.<sup>8–10</sup> Fungal hyphae grow from the basidiospores and degrade the wood cell-wall components with enzymes and/or low molecular weight compounds.<sup>9,11</sup> Therefore, it was assumed that the decay risk of wood used for aboveground application might be affected by the number of living spores attached to the wood surface and the ability of their hyphae to degrade the wood cell-wall components.

The decay risk for aboveground applications was investigated through various methods, such as the lap joint test, L-joint test, and double-layer test.<sup>12</sup> However, because these studies required that wood samples be exposed to outdoor air for several months or years, the estimated decay risk was actually the mean decay risk for the entire exposure period. Attempts were also made to trap wood-decay fungi by using potato dextrose agar plates to count the number of living wood-rotting fungi in air.<sup>13</sup> However, these experiments seemed to be not efficient for estimating decay risk because additional experiments were required to assess the wood-decay activity of the trapped fungi.

To overcome these problems, a novel method was devised utilizing a wood disk and an air sampler. The method has several advantages for estimating the risk of a certain amount of air that will induce the decay of wood. In this article, we explain the process of assessing this risk, namely decay risk, and discuss factors affecting it.

### Materials and methods

Two hundred five Japanese cedar (*Cryptomeria japonica*) disks about 3 mm thick were prepared from logs about

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7.8 cm in diameter. The disks were dried at 60°C for 1 day and then weighed to determine their initial mass ( $M_0$ ). Their thickness and diameter and heartwood diameter were measured. Disks were randomly placed in individual Petri dishes and then placed in individual sealed bags for gas sterilization. Gas sterilization was carried out in an ethylene oxide gas sterilizer (model YS-A-C64E; Yuyama, Osaka, Japan) according to the product manual. Each disk was kept in its sterilized sealed bag until use. Some of the Petri dishes were weighed to determine their mean mass ( $M_p$ ).

A plate containing a Japanese cedar disk was removed from the sterilized bag just before air sampling. About 6 ml sterilized deionized water was aseptically added to the disk. The damp disk with the plate was weighed ( $M_1$ ) to calculate the initial moisture content of the disk according to the following equation:

$$\text{Initial moisture content (\%)} = \frac{\{(M_1 - M_p) - M_0\}}{M_0} \times 100 \quad (1)$$

All air sampling was carried out at about 100 cm in height using a BIOSAMP MBS-1000 air sampler (Midori Anzen, Tokyo, Japan) at the same place on the grounds of the Forestry and Forest Products Research Institute in Tsukuba, Japan. Each air sampling was run for 10 min between 0900 and 1700 on a sunny day or cloudy day, but not on a rainy day, from June 5 to November 28 in 2008. The sampling procedure followed that described in the product manual except for replacing the agar medium for the damp Japanese cedar disk. Each disk exposed to 1000 l air was kept on a plastic plate and incubated in a damp container at 26° ± 2°C for 16 weeks. In addition to these disks, four control disks without exposure to 1000 l air were also incubated in the container for the same period.

After the 16-week incubation period, filamentous fungi growing on the disk surface were collected and transferred to plates containing potato dextrose agar medium (Difco potato dextrose agar) with 100 ppm tetracycline-HCl, 50 ppm benzothiazol, and 0.05 % guaiacol. Each disk was dried at 60°C for 1 day and weighed to determine its dry mass after incubation ( $M_2$ ).

The fungi transferred onto the plates were subcultured repeatedly until visual examination confirmed the absence of any contamination. Each isolated fungus was allowed to grow on guaiacol wood-meal medium containing 0.05% guaiacol, 0.2% beech (*Fagus crenata*) wood meal, and 1.8% agar for about 5 days to determine the decay types.<sup>14</sup>

Weather data were collected by a C-CR1000 weather monitoring system (CLIMATEC, Tokyo, Japan) set at about 300 m southwest of the sampling point. Amount of cloud was visually checked at each sampling time. When less than 10% of the sky was covered with cloud, it was classified as sunny.

All statistical analyses were performed using JMP 8.0 software (SAS Institute, Cary, NC, USA) with the significance level set at 0.01.<sup>15</sup>

## Results

### Appearance of sample

Figure 1 shows two examples of typical Japanese cedar disks after the 16-week incubation period at 26° ± 2°C. Example A is a disk that did not show any mass loss. Small black spots suggested that there were mold spores in the 1000 l sampled air. However, no fungal hyphae were found on the disk surface by macroscopic observation. Among the 201 disks, 26 showed a similar appearance to example A; the other 175 disks showed white fungal hyphae on their surfaces. Example B is a disk that showed the highest mass loss (807 mg). White fungal hyphae were observed across the disk surface.

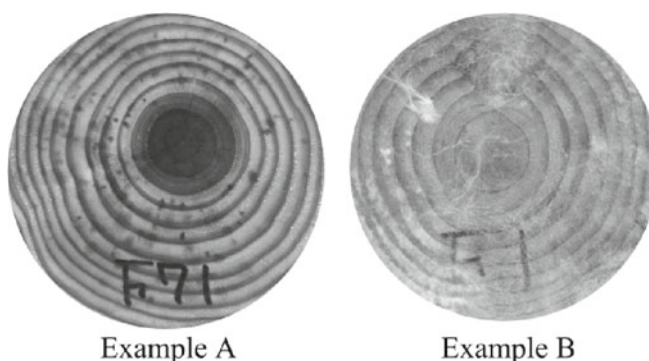
Mass loss determination revealed that the exposed disks without fungal hyphae showed mean mass loss of 23 mg with a standard deviation of 30 mg, and those with fungal hyphae showed 289 mg mean mass loss with a standard deviation of 164 mg.

The control samples showed mean mass loss of -6 mg with a standard deviation of 22 mg. No fungal hyphae and no black spots were observed on the surface of the control disks.

### Effect of heartwood proportion and initial moisture content on the decay risk of airborne fungi

The effect of the ratio of heartwood area on the mass loss of Japanese cedar disks during the 16-week incubation period is shown in Fig. 2. The plot of mass loss against percentage of heartwood area indicates that mass loss decreased by 8.0 mg with an increase of 1% in heartwood area. Correlation coefficient and *P* value between mass loss and percentage of heartwood area were 0.44 and <0.0001, respectively.

Figure 3 shows the effect of initial moisture content on mass loss. An increase of 1% of initial moisture content resulted in an increase of 7.4 mg in mass loss. Correlation coefficient and *P* value were 0.38 and <0.0001, respectively.



**Fig. 1.** Appearance of Japanese cedar disk exposed to 1000 l air followed by incubation for 16 weeks. *Example A:* Sample that did not show growth of fungal hyphae but showed black spots on disk surface. *Example B:* Sample that showed growth of fungal hyphae

Effect of sampling date on mass loss

All data on mass loss were plotted against the sampling date (Fig. 4). The maximum value of mass loss was 807 mg, which was obtained for the disk sampled on July 25.

Figure 5 shows the relationship between sampling month and mean mass loss. Mean mass losses with standard errors from June to December was  $228 \pm 28$ ,  $303 \pm 32$ ,  $235 \pm 41$ ,  $267 \pm 30$ ,  $270 \pm 27$ , and  $215 \pm 28$  mg, respectively. Statistical analysis by the Tukey–Kramer test showed no significant difference among these values.

Effect of weather factors on decay risk

Correlation coefficients and *P* values between mass loss and each weather factor are shown in Table 1. Six of 19 factors significantly affected mass loss on the sampling day ( $P < 0.01$ ). Sixteen weather factors 1 or 2 days before the sampling day were also investigated for their relationship with mass loss. In those cases, the number of factors significantly affecting mass loss decreased from 4 to 1. Correlation coef-

ficients also decreased from those values on the sampling day except for precipitation.

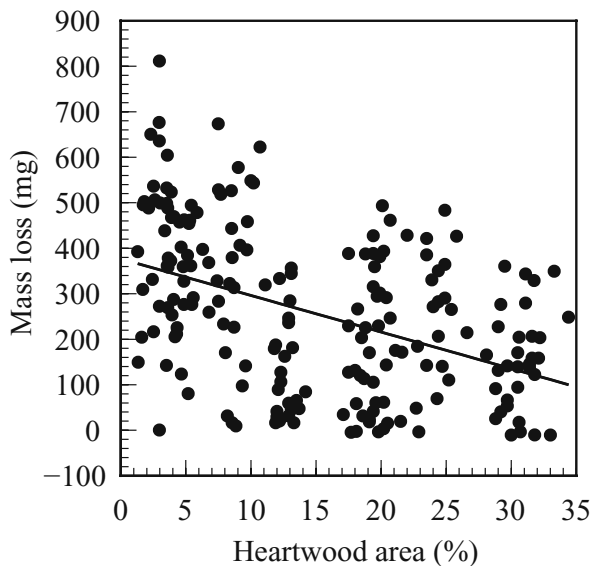
Isolated fungi and their phenol-oxidizing activity

Filamentous fungi grown on cedar disk surfaces during the 16-week incubation period were subcultured for isolation. Although many of the fungi transferred onto the PDA plates were lost due to lack of fungal growth, 293 strains of filamentous fungi were finally isolated. Visual observation of the guaiacol wood-meal medium revealed that 194 of the isolated strains changed the color of the medium to a dusky red.

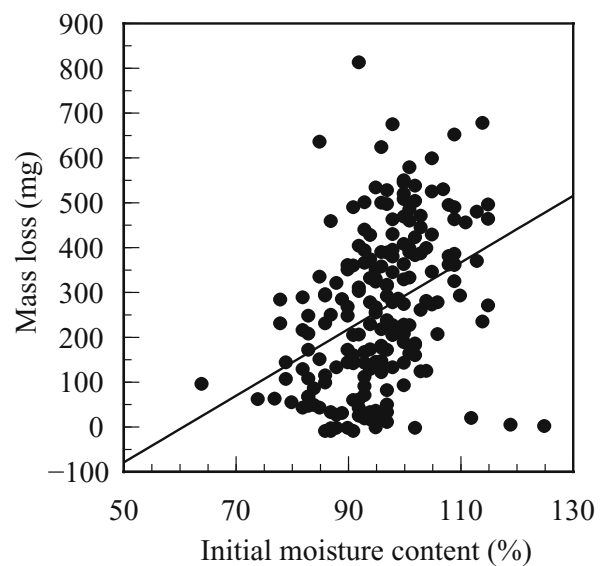
Discussion

Assessment of decay risk by airborne fungi

As mentioned in the Introduction, several methods were developed to estimate the decay risk for aboveground

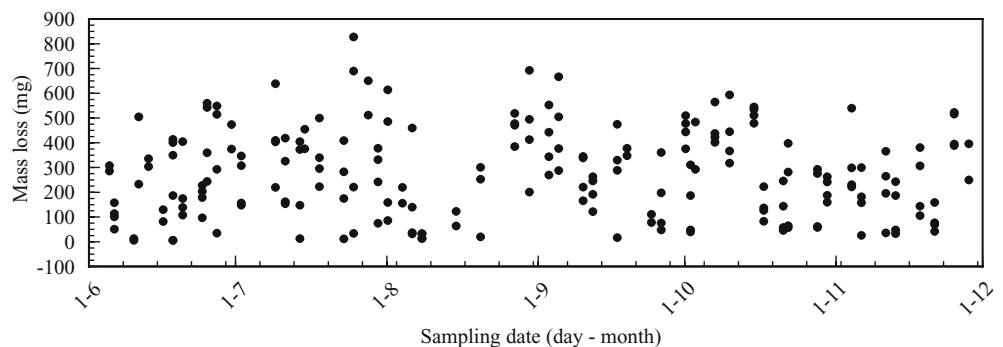


**Fig. 2.** Effect of percentage of heartwood area on mass loss caused by airborne wood-decay fungi. The *solid line* indicates the linear least square fit of mass loss against percentage of heartwood area given by the following equation:  $\text{Mass loss} = -8.0 \times \text{percentage of heartwood area} + 380$



**Fig. 3.** Effect of initial moisture content on mass loss caused by airborne wood-decay fungi. The *solid line* indicates the linear least square fit of mass loss against percentage of initial moisture content of samples given by the following equation:  $\text{Mass loss} = 7.4 \times \text{initial moisture content} - 450$

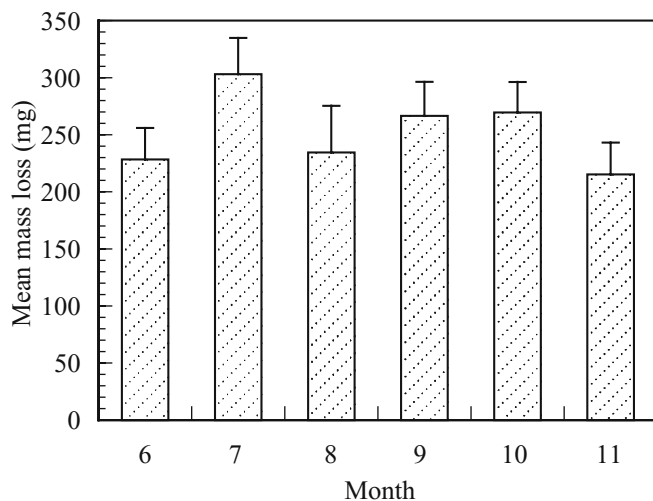
**Fig. 4.** Plot of mass loss caused by airborne wood-decay fungi against sampling date



**Table 1.** Correlation between weather factor and mass loss of Japanese cedar disks

| Weather factor                                 | On the sampling day     |                  | One day before the sampling day |                  | Two days before the sampling day |                |
|--|-------------------------|------------------|---------------------------------|------------------|----------------------------------|----------------|
|  | Correlation coefficient | <i>P</i> value   | Correlation coefficient         | <i>P</i> value   | Correlation coefficient          | <i>P</i> value |
| Temperature at sampling period (°C)            | 0.005                   | 0.942            | –                               | –                | –                                | –              |
| Relative humidity at sampling period (%)       | <b>0.320</b>            | <b>&lt;0.001</b> | –                               | –                | –                                | –              |
| Atmospheric pressure at sampling period (hPa)  | –0.014                  | 0.846            | –                               | –                | –                                | –              |
| Mean temperature (°C)                          | 0.079                   | 0.267            | 0.093                           | 0.191            | 0.088                            | 0.216          |
| Mean relative humidity (%)                     | <b>0.363</b>            | <b>&lt;0.001</b> | <b>0.281</b>                    | <b>&lt;0.001</b> | <b>0.219</b>                     | <b>0.002</b>   |
| Mean atmospheric pressure (hPa)                | –0.031                  | 0.658            | 0.000                           | 0.995            | –0.056                           | 0.430          |
| Mean wind speed (m/s)                          | 0.035                   | 0.620            | 0.100                           | 0.159            | –0.068                           | 0.340          |
| Amount of solar radiation (MJ/m <sup>2</sup> ) | <b>–0.724</b>           | <b>0.001</b>     | –0.138                          | 0.051            | –0.081                           | 0.250          |
| Sunshine duration (h)                          | –0.084                  | 0.238            | –0.148                          | 0.036            | –0.061                           | 0.392          |
| Maximum temperature (°C)                       | –0.014                  | 0.842            | 0.057                           | 0.416            | 0.060                            | 0.398          |
| Minimum temperature (°C)                       | 0.129                   | 0.068            | 0.135                           | 0.055            | 0.126                            | 0.074          |
| Maximum relative humidity (%)                  | –0.148                  | 0.036            | 0.046                           | 0.519            | 0.063                            | 0.373          |
| Minimum relative humidity (%)                  | <b>0.360</b>            | <b>&lt;0.001</b> | <b>0.226</b>                    | <b>0.001</b>     | 0.159                            | 0.024          |
| Maximum atmospheric pressure (hPa)             | –0.018                  | 0.795            | –0.009                          | 0.900            | –0.066                           | 0.350          |
| Minimum atmospheric pressure (hPa)             | –0.044                  | 0.535            | 0.002                           | 0.973            | –0.029                           | 0.679          |
| Daily range of temperature (°C)                | <b>–0.336</b>           | <b>&lt;0.001</b> | –0.182                          | 0.010            | –0.118                           | 0.096          |
| Daily range of relative humidity (%)           | <b>–0.369</b>           | <b>&lt;0.001</b> | <b>–0.222</b>                   | <b>0.002</b>     | –0.154                           | 0.029          |
| Daily range of atmospheric pressure (hPa)      | 0.063                   | 0.375            | –0.022                          | 0.751            | –0.091                           | 0.200          |
| Precipitation (mm)                             | 0.138                   | 0.051            | <b>0.195</b>                    | <b>0.006</b>     | 0.150                            | 0.034          |

Significant correlations ( $P < 0.01$ ) are indicated by boldface



**Fig. 5.** Relationship between mass loss caused by airborne wood-decay fungi and sampling month. Bars indicate standard errors of mean mass losses. The numbers of disks used for each month from June to November were 38, 36, 27, 28, 42, and 30, respectively

applications.<sup>11</sup> However, the decay risk estimated by these methods was the mean decay risk throughout the entire testing period. Therefore, a new method was needed to assess the decay risk in a certain amount of air during a specific period.

Detection of airborne fungi and identification of spore species has been well investigated in the indoor air of homes, hospitals, etc.<sup>16,17</sup> Air samplers were widely used in those studies because they can collect airborne fungi in a constant amount of air in a shorter period compared to a conventional outdoor exposure test. The main targets of these studies were molds, which are classified mainly as

Ascomycota and “Zygomycota.” We applied a sampling method using an air sampler to assess the decay risk of airborne wood-decay fungi in 1000 l of air.

Our preliminary experiment suggested inability to collect Basidiomycota with potato dextrose agar (PDA) containing antimicrobial agents without interference of other microorganisms such as mold (unpublished data). Therefore, we tried to adapt Japanese cedar disks as a substrate that would allow the growth of wood-decay fungi and inhibit the growth of mold.

The appearance of Japanese cedar disks after the test (see Fig. 1) is shown by these two typical disks exposed to 1000 l air followed by a 16-week incubation period. When the 1000 l of sampled air did not contain spores of wood-decay fungi, the fungi did not come out on the disk surface during the incubation period. However, there would still be many other types of airborne fungi, such as molds, present. Therefore, traces of mold growth were seen on the disk as black spots (see Fig. 1, example A). On the other hand, when the sampled air contained spores of wood-decay fungi, the spores attached themselves to the disk surface, and then started growing, assimilating wood components and other microorganisms.<sup>18</sup> Consequently, fungal hyphae elongated beyond the black spots and partially covered the disk surface (see Fig. 1, example B). Japanese cedar disks are concluded to be a good substrate for screening wood-decay fungi from molds.

The novel method using an air sampler and Japanese cedar disk was considered to be advantageous for assessing decay risk according to the advantages and disadvantages listed in Table 2.<sup>19,20</sup> Therefore, we decided to assess the decay risk directly by air sampling of 1000 l air, followed by investigating the mass loss of Japanese cedar disks during the 16-week incubation period.

**Table 2.** Advantages and disadvantages of the novel method

|                       | Novel method <sup>a</sup>                  | Conventional method <sup>b</sup>   |
|-----------------------|--|--|
| Sampling period       | Short <sup>c</sup>                         | Long   |
| Process               | Two-step process<br>Sampling<br>Incubation | Four-step process<br>Sampling<br>First incubation<br>Transferring<br>Second incubation |
| Quantitative analysis | Possible                                   | Impossible   |
| Apparatus             | Necessary                                  | Not necessary  |

<sup>a</sup>Method using air sampler and wood disk

<sup>b</sup>Method used in conventional outdoor exposure test using potato dextrose agar (PDA) medium<sup>19,20</sup>

<sup>c</sup>Longer sampling period can be also acceptable

### Effect of heartwood proportion and initial moisture content on the decay risk of airborne fungi

Figure 2 shows two significant characteristics that affected mass loss. One was the percentage of heartwood area. The plot of mass loss against the percentage of heartwood area indicates that the mass loss decreased by 8.0 mg with an increase of 1% in heartwood area. The correlation coefficient between mass loss and percentage of heartwood area was low ( $r = 0.44$ ), but the  $P$  value ( $<0.0001$ ) clearly indicates a significant correlation between them. It was revealed by accelerated decay tests and field tests that Japanese cedar heartwood is more durable than is its sapwood.<sup>21,22</sup> The results of this study showing that the percentage of heartwood area affected mass loss can be explained by the difference in durability between heartwood and sapwood of Japanese cedar.

Another factor significantly affecting mass loss was the moisture content of the disk during the air-sampling period. An increase in moisture content during the sampling period resulted in an increase of mass loss (see Fig. 3) if initial moisture content ranged between 63% and 124%. Statistical analysis revealed a correlation between moisture content and mass loss ( $r = 0.38$ ,  $P < 0.0001$ ). Increase in initial moisture content probably resulted in an increase in moisture content during the incubation period. Therefore, the relationship observed in Fig. 3 may be attributed to the integral effect of high moisture content during both sampling period and incubation period. Our next task is to estimate the effect of individual moisture content during the sampling period and an incubation period on mass loss caused by airborne decaying fungi.

### Effect of sampling month on decay risk

Figure 4 shows all data on mass loss plotted against the sampling date. The figure suggests that the sampling season did not affect mass loss. To clarify this hypothesis, the average mass loss in each sampling month was compared. As shown in Fig. 5, there was almost no difference in mean mass loss. Statistical analysis by the Tukey–Kramer test also indicated that there was no significant difference. It is concluded that, in the case of this study, decay risk was almost the same for each month from June to December in 2008.

**Table 3.** Effect of weather on fungal growth and mass loss of Japanese cedar disks

|            | Number of disks                 |                             | Mean mass loss (mg) |
|------------|---------------------------------|-----------------------------|---------------------|
|            | Fungal hyphae were not observed | Fungal hyphae were observed |                     |
| Sunny day  | 21                              | 101                         | 217 (171)           |
| Cloudy day | 5                               | 74                          | 312 (173)           |

Standard deviation is shown in parentheses

### Effect of weather factors on decay risk

The effect of weather factors such as temperature on the mass loss of the Japanese cedar disks was also investigated. To estimate the contribution of each weather factor to mass loss, correlation coefficients and  $P$  values were calculated between mass loss and each weather factor. As shown in Table 1, relative humidity during the sampling period and five weather factors, i.e., mean relative humidity, amount of solar radiation, maximum relative humidity, daily range of temperature, and daily range of relative humidity on the sampling day, significantly affected mass loss. In contrast, temperature and atmospheric pressure during the sampling period, and mean, maximum, and minimum temperature and atmospheric pressure as well as maximum relative humidity on the sampling day proved to have no effect on mass loss.

After confirming that six weather factors on the sampling day affected the decay risk, the contribution of each significant weather factor was estimated in detail using the data from Table 1. Considering whether correlation coefficients of the six weather factors are positive or negative, it is speculated that the decay risk caused by airborne wood-decay fungi was high on cloudy days.

To investigate the foregoing speculation that decay risk is likely to be higher on cloudy days, the number of disks that did not show fungal hyphae after the 16-week incubation was counted on a cloudy day and on a sunny day. As shown in Table 3, Fisher's exact test proves that the decay risk was significantly higher on the cloudy day compared to the sunny day. Student's  $t$  test using data on mass loss during the 16-week incubation period also showed that the cloudy day air induced a significantly higher mean mass loss on the damp disk compared to that of the sunny day. The relationship between the release of basidiospores and the weather factors have been well examined in phytopathogenic fungi.<sup>23,24</sup> These studies also revealed that an increase in relative humidity increased the release of spores. In addition to these findings, this study revealed that the decay risk is significantly affected not by maximum relative humidity but by minimum relative humidity.

Comparing the correlation between mass loss and weather factors, the correlation coefficient and  $P$  value both decrease with an increase in duration from the sampling period, except in the case of precipitation. These facts indicate that the influence of weather factors diminished with an increase in time from the sampling period. However, there is one exception. Precipitation positively affected mass loss only on the day before the sampling. It was

reported that rainfall prompts phytopathogenic fungi to produce and release their spores.<sup>25</sup> Wood-decay fungi are also prompted to release their basidiospores as shown by the phytopathogenic fungi.

#### Decay type of airborne fungi

Wood-rotting Basidiomycota are divided into two groups, namely, white-rot fungi and brown-rot fungi.<sup>26</sup> One of the major differences between the two groups is the ability to produce lignin-degrading enzymes. In this study, the presence of ligninolytic enzyme activity was checked using a wood meal medium containing guaiacol that is oxidized by ligninolytic enzymes produced by white-rot fungi to form dusky-red compounds.<sup>27</sup> The ratio of strains showing the presence of phenol oxidase activity was 66.2% of 293 isolated strains. Although it is unclear whether all isolated strains belong to the wood-decay fungi, experimental results indicate that about one-third of the isolated fungi were probably classified as white-rot fungi. To confirm this figure, identification of fungal species has been proceeding in our laboratory using the polymerase chain reaction (PCR) method.<sup>28</sup> The relationship between each fungal species and the weather factors will be clarified according to the progress of this investigation.

The novel method was shown to reveal the decay risk in a certain amount of air. The method was also found to be useful for screening airborne wood-decay fungi. In addition, it is worth mentioning that the novel method can be expanded, such as for simulating decay in nature. Microbial succession that might occur in the field can be simulated on a wood disk on which airborne wood-decay fungi and mold are trapped. Another application is for simulating decay process in nature, which is affected by both decay risk and incubation conditions. By controlling incubation conditions according to natural conditions, the decay process in nature caused by airborne fungi trapped at a certain time can be estimated.

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