Variation in mold susceptibility among hardwood species under laboratory conditions

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ABSTRACT

Wood packing materials (WPMs) utilized by the US Department of Defense (DOD) can be constructed with a diversity of wood species depending on end use. To extend service life, these materials are treated with preservatives to protect against insects and decay fungi. Recent issues have prompted the need to include moldicide additives with these treatments. The goal of this study was to evaluate mold susceptibility across a diversity of hardwood species with the purpose of identifying representative hardwood species that would be useful in differentiating efficacy of moldicide treatments in future studies. Conditioned hardwood lumber representing groups of species listed in DOD specifications were cut to obtain 7 × 20 × 70 mm specimens. The specimens were subjected to simulated rainfall to achieve the type of wetting that might occur in service, inoculated with mold spores, placed above moist paper in Petri dishes, and given visual ratings for mold growth at 2-week intervals. The results indicate that hickory, red oak sapwood, hard maple, white ash and white oak sapwood are all highly susceptible to mold growth. Mold growth was less consistent on aspen, cottonwood, and soft maple, and varied greatly between sapwood and heartwood for yellow poplar. Hickory, hard maple and white ash may be best suited for future WPM moldicide testing because of their availability and consistent mold susceptibility.

1. Introduction

The US Department of Defense (DOD) utilizes a diversity of materials wood packing materials (WPMs) (e.g. pallets, hinged wood boxes, wire bound boxes, dunnage). Acceptable wood materials, including approved wood species, are determined by whether the wood properties are sufficient to meet the requirements for the type of WPM being constructed. For example, many wood species, including most common North American hardwoods, can be used for assembly of wirebound boxes. In contrast, DOD specifications separate species used for pallets into four groups (Supplementary Table 1), with only the most dense and strongest hardwoods allowed for Group IV (ASTM D6199-18a, 2018).

Although WPMs are typically stored under cover, they are also expected to be able to resist biodeterioration during extended periods of outdoor exposure. Resistance to biodeterioration is imparted by dipping the WPMs in a wood preservative solution, typically zinc naphthenate. When zinc naphthenate recently became unavailable, research was conducted to select alternative preservatives based primarily on their efficacy against decay and termites (Lebow et al., 2015). Several preservatives were found to have sufficient efficacy in preventing decay and termite attack and were subsequently incorporated into military specifications. At the time the research was conducted, mold growth was not considered a high priority and mold resistance was not evaluated.

More recently concerns have arisen regarding observations of mold growth on WPMs in-service. Although specifications require wood products to be dried before WPM assembly, and re-dried after preservative treatment, they may be exposed to moisture during storage or transport. Exposure to precipitation could readily supply sufficient moisture for mold growth, and because the WPMs are tightly packed for shipment and storage, they would be slow to dry once wetted. It is also possible to have sufficient moisture for mold growth during storage, even without exposure to precipitation. Although the minimum moisture requirements for mold growth are not completely understood there is some agreement that sustained relative humidity in excess of 80–90% at the wood surface can allow mold to develop (Carll and Wiedenhoeft, 2009; Glass et al., 2017; Ojanen et al., 2010; Tsongas and Riordan, 2016;
Thus, tightly enclosed shipping containers or storage facilities with standing water could create humidity conditions sufficient to support mold growth. However, it is likely that some of the more extreme examples of mold growth observed in WPMs to date are a result of both wetting from precipitation and subsequent storage under conditions of high relative humidity.

Issues related to storage and mold contamination of WPMs have necessitated the evaluation of various moldicide additives to the current preservatives in use. Initial examination of moldicide additives was tested on solid southern yellow pine (Pinus spp.) and yellow poplar (Liriodendron tulipifera L.), as well as on plywood specimens comprised of thin, hard pine surface overlays and three inner hardwood veneers. These materials and species were selected to represent likely results across the spectrum of allowable materials. However, in early studies with yellow poplar we observed little to no mold growth even on water control specimens. Thus, these preliminary results were not useful in examining moldicide additive differences in hardwood samples. Fig. 1 illustrates this observation on water-treated control vs zinc naphthenate treated specimens, demonstrating that yellow poplar would be poor choice for differentiating among moldicide treatments.

Previous research has indicated that a number of factors, including wood species, can greatly influence extent of mold growth on wood substrates (Gobakken and Lebow, 2010; Viitanen et al., 2010; Lie et al., 2019a). Wood extractives have been suggested to play an important role in differences in mold susceptibility among wood species, either as a source of nutrients or as inhibitory toxicants (Theander et al., 1993; Johansson et al., 2017; Philip et al., 1995; Reinprecht et al., 2020; Schmidt et al., 2016; Feng et al., 2018; Xu et al., 2015). Moisture is a key factor in mold growth, and extent of water uptake during exposure has also been linked to intraspecies differences in extent of mold growth (Lie et al., 2019a,b).

Efforts to identify a hardwood species that could allow for differentiation of moldicide treatments revealed that little has been published about mold susceptibility among North American hardwood species. The objectives of this study were to examine differences in mold susceptibility among select hardwood species, and to identify species of hardwood that would be most informative for testing moldicides and/or moldicide additives in standard laboratory tests.

2. Materials and methods

2.1. Preparation of mold fungi

Growth of three mold strains, Aspergillus niger Tiegh. 1867 (strain 2.242), Penicillium chrysogenum Thom. (1910) (strain PH02), and Trichoderma atroviride P. Karst 1892 (strain ATCC20476) was initiated on 2% malt agar Petri plates in a controlled chamber set at 27 °C and 80% relative humidity (RH). Spore suspensions of test fungi were prepared from 2-week old fungal colonies by washing the surface of the malt agar Petri dish with 10–15 ml of sterile deionized water according to ASTM Standard D4445-10 (ASTM D4445-10, 2019). The wash water was then transferred to a spray bottle and diluted to approximately 100 ml of distilled water, to yield ~3 × 10^6 spores/ml. The spray bottle was adjusted to deliver 1 ml of inoculum per spray. This mixed spore suspension was used to maintain consistency with previous and future DOD WPM evaluations, and because previous research indicates that this approach generally produces the greatest mold growth across a range of wood substrates (Lie et al., 2019a).

2.2. Selection of species and preparation of wood samples

Wood species selection was based on availability and input from DOD personnel and WPM manufacturers on the species most commonly used in production of WPMs (Supplementary Table 1). Boards of the selected species were cut to a sample size of approximately 7 mm thick (T) by 20 mm wide (R) by 70 mm long (L). The specimens were primarily from quarter sawn boards; this facilitated the effort to separate sapwood from heartwood (where applicable). The exception was the western hemlock specimens, which were primarily flat sawn. In general, specimens of each species originated from the same source material with the exception of southern yellow pine, which was taken from pre-cut specimens that are used in a multitude of other tests. Softwood samples of western hemlock (sapwood and heartwood not differentiated) and southern yellow pine (sapwood only) were included as group II representatives for comparative purposes. Within the softwoods, pine in particular is commonly used in standard mold test methods and is frequently used by the DOD for fabrication of WPMs. Plywood (6 mm thickness with thin, hard pine surface overlays and three inner hardwood veneers) was also included as a positive control. The plywood, obtained from a WPM manufacturer who uses it to construct wirebound boxes, was included for comparison because it exhibited consistent extensive mold growth during earlier testing. For three hardwood species (denoted in Table 1), specimens of heartwood and sapwood were differentiated based on color and were tested separately. For all other hardwood species heartwood and sapwood were not evaluated separately either because of the lack of a true heartwood (i.e. maple and ash), or because the color of sapwood and heartwood was too difficult to distinguish (i.e. aspen and cottonwood). All specimens were allowed to equilibrate to between 7 and 10% moisture content under indoor laboratory conditions.

2.3. Mold testing – simulated rainfall

DOD specifications require that wood used in WPMs be dried to

![Fig. 1. Differences in zinc naphthenate moldicide efficacy on three different wood substrates. Y-axis represents visual ratings on a scale from 0 (no growth) to 5 (heavy growth). The growth/coverage rating is based on the extent of area covered while the aesthetic rating is based on the noticeability or appearance of the mold growth.](image-url)
The purpose of this simulated rainfall was to allow estimation of water uptake. WPMs can subsequently be exposed to precipitation at a rate of 10 mm/h, applied for five, 1-h intervals, each separated by a 5-h rest period for a total of 50 mm over the 24-h period immediately prior to mold exposure. The same surface that was subsequently inoculated with mold spores and rated for mold growth was immediately prior to mold exposure. The purpose of this simulated rainfall method is to increase wood moisture, which serves to enhance mold growth during the course of the test and to determine the leach resistance of moldicides when they are added in subsequent testing. A subset (4 replicates) of each type of specimen was weighed before and after growth during the course of the test and to determine the leach resistance of moldicides when they are added in subsequent testing. A subset (4 replicates) of each type of specimen was weighed before and after growth during the course of the test and to determine the leach resistance of moldicides when they are added in subsequent testing. A subset (4 replicates) of each type of specimen was weighed before and after growth during the course of the test and to determine the leach resistance of moldicides when they are added in subsequent testing.

2.4. Mold testing - petri dish test chamber

Mold susceptibility was evaluated by adapting the ASTM D4445-10 Standard Test Method, Fungicides for Controlling Sapstain and Mold on Unseasoned Lumber (Laboratory Method) (ASTM D4445-10, 2019). The choice of a laboratory mold test was difficult because of the wide range of conditions that might be encountered by DOD WPMs in service, and because the correlation of laboratory tests to outdoor exposures is not strong (Imken et al., 2020; Lie et al., 2019a). In our experience, ASTM D4445 provides a test that is less severe than agar plate testing but more severe and more reproducible than mold chamber tests. Our adaptation of the method also allowed us to directly compare wood species within the same Petri dish. ASTM D4445 was developed for mold and stain testing on unseasoned wood, but for WPM evaluations, rewetting of previously dried wood is more relevant. ASTM D4445 has been used extensively in our laboratory and previous researchers have reported that it can be successfully adapted for use on previously dried wood (Clausen and West, 2005; Karlal et al., 2020). Six test specimens were added to large Petri dishes (150 × 25 mm) containing 8 layers of sterile blotting paper saturated with 30 ml of sterile DI water and a polyethylene mesh to elevate test specimens (Fig. 2). Each test dish incorporated six of the 16 specimen types. A near balanced, incomplete block design, generated with SAS/QC® V14.1 (Cary, NC) optimal experimental design procedure (proc OPTEX; SAS Institute, 2015), was used with 40 total dishes so that each species occurred in 15 of the dishes and each pair of species mostly appearing together in five of the dishes. Plates containing blotting paper and test specimens were spray-inoculated with 1 ml mold spores and maintained at 27 °C, 70% RH for 12 weeks. At 2, 4, 6, 8, 10 and 12 weeks, specimens were assigned visual ratings for percent of mold coverage (i.e. growth/coverage rating) and noticeability of mold growth (i.e. aesthetic rating). Ratings were made without the use of magnification, but with a movable light source to allow for better visualization. In both cases ratings were on a scale of zero to five, with zero corresponding to no growth and 5 corresponding to complete coverage (growth/coverage rating) or highly visible mold growth (aesthetic rating) (Table 2) (Fig. 3). This two-rating system was developed to account for specimens that might have a high-density mold growth, but this growth is transparent or mainly microscopic and would likely be overlooked in a real-world setting.

2.5. Statistical analysis

Ratings were evaluated statistically using proportional odds (cumulative logit) generalized linear mixed effects models with fixed effects for species and weeks exposure and random effects for high and dish species (proc glimmix in SAS® V9.4) (Stroup, 2013). Proportional odds models estimate the probabilities of the rating trends (a multinomial ordinal variable) by fitting functions of their cumulative probabilities (log odds,
also called logits) to a linear functional of the predictors (species and weeks exposure). To capture the curvature over weeks, the fixed effects for weeks in the models were continuous second order polynomials of the logarithm of time (i.e. two terms). Species were ranked based on species effect contrasts from these fitted models.

3. Results and discussion

Mold rating results at 12-weeks exposure and initial percent water uptake for all wood species tested are shown in Fig. 4, grouped based on wood species classifications defined in ASTM standard D6199-18a. More detailed figures of these ratings over time are shown in Supplementary Fig. 1. Wood species with high levels of mold growth and little variation among test specimens included hickory and red oak sapwood. Hard maple and white ash also showed high mold resistance but had slightly more variation among the specimens tested. As observed previously, yellow poplar heartwood had higher levels of mold resistance than all other wood species tested, including heartwood samples from both red and white oak, making this species undesirable for moldicide testing purposes. Plywood, which served as the positive control in this study, consistently had high levels of mold growth.

For statistical comparison, wood types were assessed against southern yellow pine as it is the typical softwood utilized in standardized mold test methods (Fig. 5). The analysis indicates that red oak sapwood, hickory, hard maple, white oak sapwood, white ash and even white oak heartwood have a high probability of having more noticeable mold growth than that of southern yellow pine sapwood. Yellow poplar sapwood is added to this list when considering extent of mold coverage. There was also a gradual trend for the hardwood species to have greater mold growth, relative to pine, over time. Plywood, the positive control, was excluded from the statistical models as a lack of variation in ratings for these specimens caused computational problems.

As predicted, substantial differences in mold susceptibility were observed for the wood types evaluated in this study (Supplementary Fig. 1). Some findings, such as the greater extent of mold growth on white oak heartwood than on aspen was unexpected and demonstrates the complexity of predicting mold growth. Cursory examination of these data suggests that there is no observable connection between mold growth and group classification (Fig. 4). As these group assignments are largely based on wood density, these results suggest that high density

<table>
<thead>
<tr>
<th>Index</th>
<th>Growth/Coverage Rating</th>
<th>Aesthetic (~Noticeability) Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No detectable mold growth</td>
<td>No detectable mold growth</td>
</tr>
<tr>
<td>1</td>
<td>Slight to moderate growth detected covering ≤ 20% block surface</td>
<td>Not outwardly apparent by visual inspection</td>
</tr>
<tr>
<td>2</td>
<td>Growth detected covering 20%-40% of block surface</td>
<td>Sparse growth detected by visual inspection</td>
</tr>
<tr>
<td>3</td>
<td>Growth detected covering 40%-60% of block surface</td>
<td>Moderate levels of mold growth detected by visual inspection</td>
</tr>
<tr>
<td>4</td>
<td>Growth detected covering 60%-80% of block surface</td>
<td>Patchy areas with moderately-heavy to heavy growth, easily detected visually</td>
</tr>
<tr>
<td>5</td>
<td>Even growth covering 100% of the block surface</td>
<td>Heavy to very heavy and dense mold growth</td>
</tr>
</tbody>
</table>

* Includes both macro and microscopic growth.

Table 2
Visual rating systems used to characterize mold growth.

Fig. 1. Wood species with high levels of mold growth and little variation among test specimens included hickory and red oak sapwood. Hard maple and white ash also showed high mold resistance but had slightly more variation among the specimens tested. As observed previously, yellow poplar heartwood had higher levels of mold resistance than all other wood species tested, including heartwood samples from both red and white oak, making this species undesirable for moldicide testing purposes. Plywood, which served as the positive control in this study, consistently had high levels of mold growth.

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Fig. 3. Example blocks illustrating growth/coverage ratings (left) and aesthetic ratings (right).
does not imply high mold resistance. This conclusion is in agreement with other studies that have examined the relationship between wood density and mold growth (Brischke et al., 2006; Gobakken et al., 2011; Lie et al., 2019b).

Moisture availability is a key factor in mold growth and is often linked to differences between wood species (Lie et al., 2019a). However, in this study no statistical relationship was observed between water uptake and extent of mold growth (Fig. 6). For example, white oak sapwood, which had relatively low water uptake, exhibited some of the most consistent mold growth. The lack of moisture effect is likely attributable to the simulated rainfall conditioning step which ensured that all wood types initially had sufficient moisture for mold growth. The test conditions also ensured a high humidity environment. Water absorption is likely to play a more important role in wood species mold susceptibility under conditions where moisture availability is more limited.

Variation in wood chemistry, such as nutrient availability as well as extractive content (e.g. tannins, fatty acids, aldehydes, ketones), have also been linked to differences in mold susceptibility between species (Theander et al., 1993; Johansson et al., 2017; Philp et al., 1995; Reinprecht et al., 2020; Schmidt et al., 2016; Feng et al., 2018; Xu et al., 2015). Heartwood to sapwood ratio, for example, has been shown to influence mold growth, as the heartwood of certain wood species has been shown to contain high levels of extractives and reduced nutrient levels which directly impact susceptibility to biodegradation (Philp et al., 1995; Taylor et al., 2002; Gobakken and Westin, 2008). In this study, mold growth did tend to be greater on sapwood than heartwood of the same species. Regarding mold susceptibility and extractives, we did not observe a strong relationship between total extractive content and mold ratings ($R^2 = 0.13$ or 0.07 for coverage and aesthetic ratings, respectively). However, it should be noted that the extractive content of the specimens used in this study was not determined, and that the values shown in Table 1 are general species averages. In addition, it is likely that extractive composition, not just content, influences mold susceptibility. Feng et al. (2018) identified a diversity of chemical compounds from a total of nine hard- and softwood flours and found that wood species with high proportions of potentially anti-fungal compounds tended to have less mold growth. Xu et al. (2015) identified volatile compounds in extractives that were related to either increased or decreased mold growth on wood used in wood plastic composites. To best identify wood species for standard mold testing, more work is needed to examine how much variation there is in extractive content and composition within individual species.

In addition to wood chemistry, numerous physical characteristics such as surface structure (e.g. roughness, sawing pattern, etc.) have been shown to influence mold growth, in addition to a diversity of other variables (e.g. felling time, drying method, wood age, stand conditions, etc.) (Johansson et al., 2017). While it is impossible to account for all the variables that influence mold growth on wood, assessing mold resistance of various wood preservative additives necessitates the selection of representative wood species for testing purposes. This is particularly true in the case of WPM construction with such a diversity of allowable wood species. Recommendations based on results from this study are given in Table 3. Of the species evaluated in this study, hickory, white ash and hard maple appear best suited for evaluation of moldicides use to protect WPMs. These species were consistently given high ratings for both coverage and noticeability of mold growth. They are also Group IV species, and thus allowed for use in WPM pallets. Red and white oak

![Fig. 4. Boxplots showing 12-week ratings according to both rating systems organized by wood species group classification. Averages for each rating system are represented by open dots and water uptake for each wood species are represented as closed dots.](image-url)
sapwood also consistently earned high ratings, but the proportion of sapwood in these species is limited, making it more difficult to obtain sufficient comparative material for a large-scale testing program.

4. Conclusions

In this study we examined mold susceptibility across a variety of mainly hardwood species to identify which species would be useful in laboratory evaluation of moldicides. The results indicate that hickory, red oak sapwood, hard maple, white ash, and white oak sapwood are all highly susceptible to mold growth. Mold growth was less consistent on aspen, cottonwood, and soft maple, and varied greatly between sapwood and heartwood for yellow poplar. Hickory, hard maple, and white ash may be best suited for future WPM moldicide testing because of their availability and consistent mold susceptibility. While wood species with high levels of mold susceptibility and limited variation in susceptibility would likely be the best potential representative wood species for moldicide testing, a number of species would be acceptable. The results presented here are meant to provide a snapshot of mold susceptibility among hardwood species. A key parameter of this study is that the wood used was initially dry as required by DOD specifications. Thus, these results may not apply to mold or sapstain growth on green lumber. Broader conclusions about interspecies differences in mold susceptibility would require obtaining material from a wider range of sources and processing conditions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Fig. 5. Comparative differences in aesthetic rating (top) and growth/coverage rating (bottom) of specified hardwoods relative to southern yellow pine within the same exposure period based on species effect estimates (open dots give the estimated effect differences). The vertical lines at zero represent no difference between the given hardwood and pine. The intervals represent 95% confidence intervals for species effect differences (adjusted for multiplicity); differences are on the log scale since they represent changes in probabilities across the rating categories. A positive effect difference for a species means it has lower probability of a lower rating and higher probability of a higher rating than pine.
Fig. 6. Relationship between water uptake during simulated rainfall and average mold rating at 12 weeks. Average mold ratings restricted to a subset of specimens used to determine water uptake. Based on Pearson’s correlation coefficient, the coverage p-value = 0.6871 and the aesthetic p-value = 0.7026 (for Spearman’s rho the p-values are 0.9175 and 0.8357, respectively).

Table 3

<table>
<thead>
<tr>
<th>Wood Species</th>
<th>Recommendation</th>
<th>Notes based on results from this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow poplar</td>
<td>No</td>
<td>Heartwood has high natural mold resistance; sapwood could be useful for testing, but is difficult to differentiate from heartwood once specimens are cut</td>
</tr>
<tr>
<td>Sweet gum</td>
<td>No</td>
<td>Low mold susceptibility</td>
</tr>
<tr>
<td>Red oak</td>
<td>No</td>
<td>Although sapwood of this species was highly susceptible to mold, red oak has a low proportion of sapwood compared to heartwood so getting enough sapwood for testing would be difficult</td>
</tr>
<tr>
<td>White oak</td>
<td>No</td>
<td>Although sapwood of this species was susceptible to mold, white oak has a low proportion of sapwood compared to heartwood so getting enough sapwood for testing would be difficult</td>
</tr>
<tr>
<td>Eastern cottonwood</td>
<td>Yes/No</td>
<td>This species had moderate to high mold susceptibility, but with high variation</td>
</tr>
<tr>
<td>Aspen</td>
<td>Yes/No</td>
<td>This species is commonly used for mold testing so it may be useful to use for comparison purposes, but in this study it had low mold susceptibility</td>
</tr>
<tr>
<td>Hard maple</td>
<td>Yes</td>
<td>This species showed high susceptibility to mold with some variation among specimens</td>
</tr>
<tr>
<td>Hickory</td>
<td>Yes</td>
<td>This species was found to be highly susceptible to mold with slight variation among specimens</td>
</tr>
<tr>
<td>White ash</td>
<td>Yes</td>
<td>This species showed high susceptibility to mold with some variation among specimens</td>
</tr>
</tbody>
</table>

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibiod.2020.105082.

References