REDUCING SUSCEPTIBILITY OF HEAT-TREATED SWEETGUM AND PINE TO MOLD COLONIZATION BY INCORPORATING TRADITIONAL BIOCIDES

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(Received February 2014)

Abstract. Heat treatment, an International Plant Protection Convention-approved measure for phytosanitation of wood packaging material, is achieved by maintaining a minimum core temperature of 56°C for 30 min. The heat treatment process is typically effective regarding phytosanitation, although there are concerns regarding the longevity of the protection provided by the heat treatment because the moisture content of the wood is not reduced enough to prevent insect reinfestation or mold colonization. Susceptibility of heat-treated wood to organisms may be mitigated by combining heat treatment with biocides. Commercial formulations consisting of didecyl-dimethylammonium chloride (DDAC) may be utilized separately or in combination with disodium octaborate tetrahydrate (DOT). To study mold growth following heat treatment, a modified mold test was conducted utilizing nonseasoned sweetgum (*Liquidambar styraciflua*) and southern pine (*Pinus* spp.) test samples to evaluate the efficacies of three biocide formulations applied in conjunction with the International Standards for Phytosanitary Measures No. 15 standardized heat treatment. The results of this study indicate that in a 4-wk test period conducted at 23.8°C and 85% RH, surface mold grew readily on heat-treated wood material, but surfaces treated with DDAC and/or DOT in conjunction with heat treatment significantly reduced surface mold growth.

Keywords: Heat treatment, ISPM 15, phytosanitation, surface mold, wood packaging material.

INTRODUCTION

The unintended spread of nonnative organisms threatens native biodiversity, affects native ecology, and causes substantial economic loss (Mack et al 2000; Mumford 2002; Pimentel et al 2005; Work et al 2005). International trade is the primary method by which nonnative organisms are dispersed, and wood packaging materials (WPM) such as pallets and crating are major contributors to the spread of many

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nonnative fungi and wood-destroying insects (Pasek et al 2000; U.S. Department of Agriculture [USDA] 2000; McCullough et al 2006; Colunga-Garcia et al 2009). To reduce the spread of nonnative organisms via WPM, an International Standard for Phytosanitary Measures (ISPM), "ISPM 15: Guidelines for Regulating Wood Packaging Material in International Trade," was developed by the Interim Commission on Phytosanitary Measures in 2002 (International Plant Protection Convention [IPPC] 2002; Molina-Murillo et al 2005). Approved measures in ISPM 15 for the phytosanitation of WPM include heat treatment (HT) and fumigation with methyl bromide (IPPC 2009). The use of methyl bromide has been reduced or replaced (IPPC 2008) because it readily depletes stratospheric ozone (Albritton and Watson 1992). Thus, National Plant Protection Organizations are encouraged to promote the use of alternative treatments (IPPC 2009). Because of this, HT is now the environmentally preferred method of phytosanitation for WPM.

HT schedules have been developed based on research data on insect mortality; as such, the HT specifications outlined in ISPM 15 require WPM be heated to a minimum core temperature of 56°C for at least 30 min, a time/temperature schedule commonly referred to as 56/30 (IPPC 2009). This schedule, however, is not sufficient for all pests such as the emerald ash borer, which requires a HT where the core temperature exceeds 60°C for 60 min (USDA 2011). While these schedules may be acceptable for phytosanitation, they are likely not adequate for kiln drying where the wood moisture content drops below 19% (Simpson 1991). Wood is susceptible to colonization by fungi at 27% MC and mold at 17% MC (Wernhoff 2001); thus, even without rewetting, HT wood is susceptible to reinfestation by insects as well as attack by decay and surface mold fungi because the wood moisture content could still be sufficiently high (Denig and Bond 2003). In fact, HT wood may be more susceptible to mold colonization due to the accumulation of surface moisture because of moisture migration from the core to the surface during HT along with the elevated surface temperatures (Denig and Bond 2003). This susceptibility is likely preventable with the use of a complete kiln dry cycle where the wood is dried below 19%, but the kiln dry process is expensive due to the associated energy costs and the time required is much longer (Simpson 1991). It is also important to note that mold can colonize kiln-dried material that has been rewetted (Clausen 2010). These issues as well as the heightened consumer perception of "toxic mold" (Pietrykowski et al 2008) create problems in the shipping industry with regard to customers' dissatisfaction when receiving shipments of molded WPM.

There is a need for an energy-efficient treatment method to provide long-term control over reinfestation by organisms in WPM. One potential solution is to combine the use of HT with biocide treatment to both sanitize WPM and help prevent reinfestation and mold growth; the crosstie industry has evaluated similar techniques utilizing hot borate solutions (Taylor and Lloyd 2009). Traditionally, in an attempt to control fungal growth on WPM manufactured from unseasoned wood, prophylactic fungicides (eg antisapstain treatments) are applied either by dipping or spraying directly to wood surfaces (Xiao and Kreber 1999). Many products currently marketed and labeled for use on wood to inhibit mold fungi (eg Boracare with Mold Care, Bardac 2280; NP-1, F2, Ecobrite III, and Timbercoat II) contain the bactericide/fungicide/biocide didecyl-dimethylammonium chloride, DDAC. Utilized extensively in the protection of freshly cut lumber from a host of organisms including mold, decay, and sapstain fungi as well as insects, DDAC is a key ingredient in 95% of the sapstain control products utilized in Canada (Chen et al 1995). Also a quaternary ammonium chloride, DDAC is a fungicidal component of the commercial wood preservative ammoniacal copper quat (Chen et al 1995; Hwang et al 2006). Studies have shown DDAC to successfully reduce surface mold on both southern pine (*Pinus* spp.) (SP) and aspen (Micales-Glaeser et al 2004). The goal of this study was to evaluate

the residual efficacy of the biocide treatments when exposed to HT per ISPM 15 standards for sweetgum (*Liquidambar styraciflua*) and SP.

MATERIALS AND METHODS

Wood materials utilized in this study consisted of unseasoned sweetgum (a hardwood) and unseasoned SP obtained from local lumber mills in Fulton, MS, and Ackerman, MS, respectively. The unseasoned stock was transported to the Mississippi State University Department of Forest Products and stored in an unsterile refrigeration unit prior to testing. Lower temperatures are not favorable for mold colonization and thus premature growth was inhibited (Clausen 2010). Test specimens were then cut from the unseasoned lumber with final dimensions measuring 17.78 cm × 1.90 cm × 1.90 cm (longitudinal × radial × tangential). The test specimens were marked with an ink line 7.62 cm from both ends, longitudinally, whereby one end was used as a control end and did not receive any treatment while the opposing end received treatment. The middle portion (2.54 cm) allowed for solution wicking and was not evaluated during testing. This specimen preparation was similar to glass slide tests conducted by Walters et al (1973) that measured algicidal growth in which the treatment method and control were at opposite ends of a single sample. The present study consisted of four treatment groups with five replicates per species (Table 1).

Two methods of chemical application were evaluated during this study to simulate application procedures likely to be utilized in industry (Xiao and Kreber 1999). Treatment groups 1 (DDAC dip), 3 (disodium octaborate tetrahydrate [DOT] and DDAC dip), and 4 (Bora-Care with Mold Care dip) (The use of trade names is solely for the convenience of the reader. Such use does not constitute endorsement by Mississippi State University of other products or services equally appropriate.) were dipped in chemical to simulate the utilization of dip tanks in a WPM manufacturing facility. Treatment group 2 (DOT and DDAC spray) was sprayed at 344 kPa with chemical to simulate the utilization of pneumatic chemical application during conveyance from one process to the next in a WPM manufacturing facility. Trial specimens were used to determine the appropriate dip time as well as to adjust the nozzle and spray pattern to achieve a uniform coating. The testing procedure for this study is separated into two cycles. Each cycle outlined in the study consists of a HT of the test specimens, subjection to accelerated mold growth conditions, evaluation of surface mold growth, and test specimen cleaning.

Cycle 1 Heat Treatment

Unseasoned, chemical-treated test samples were heat-treated in a small laboratory kiln. The surface and core temperatures were monitored on four samples throughout the HT process. For the sample receiving a thermocouple for core temperature monitoring, a hole equal to the diameter of the thermocouple wire was drilled half the depth into the sample along the midpoint of the sample length. The thermocouple wire was inserted into the drilled hole, sealed with silicone adhesive, and held in place by a push pin similar to Simpson et al (2003, 2005). For the samples used to monitor surface temperature, a thermocouple wire was pinned to the surface

Table 1. Treatment groups represented in testing, percent active ingredient used, and the application method for each group.

Treatment	Chemical[a]	Percent ai[b]	Application
1	DDAC	1.5	Dip
2	DOT and DDAC	10/1.5	Spray
3	DOT and DDAC	10/1.5	Dip
4	Bora-Care with Mold Care	10/1.5	Dip

[a] DOT, disodium octaborate tetrahydrate; DDAC, didecyl-dimethylammonium chloride. Bora-Care® with Mold Care™ provided by Nisus. DOT with DDAC is a laboratory-mixed solution.

[b] Percent solutions were calculated on a wt/wt basis.

of the sample along the midpoint of the length using a pushpin in such a way so that the exposed end of the thermocouple rested in direct contact with the surface of the test samples.

Test samples were stacked in the kiln on aluminum stickers to permit airflow along each sample surface. Samples with thermocouples were placed in random locations within the stack. The dry bulb temperature was achieved in the kiln via indirect heating with an electrical heating element and the wet bulb temperature was controlled via venting action. The target dry and wet bulb temperatures were 71 and 70°C, respectively, similar to HT schedules by Simpson et al (2003, 2005). The core temperature of the thermocouple-containing samples was monitored until 56°C was reached as per ISPM 15 (2009), after which time 40 min elapsed, 10 min longer than required in the standard to account for material variability and variability due to airflow in the stack. After the HT was conducted, samples were removed from the kiln.

Mold Growth Period

While standardized test methodologies exist for evaluating mold growth on wood substrates, they are intended to evaluate interior coatings and require an elaborate test apparatus utilizing cultured mold inocula (American Wood Protection Association Book of Standards [AWPA] 2010). For these reasons, a modified testing protocol was utilized in this study. The test setup consisted of 8 nonsterile plastic containers measuring 30.4 cm × 22.8 cm × 12.7 cm (length × width × depth) with 200 mL of deionized water and 400 g of sand dispersed evenly on the bottom of each container. The function of the sand was to collect runoff condensate from within the container and hold the water to help maintain an elevated humidity. A 2.54 cm × 7.62 cm × 20.32 cm (radial × tangential × longitudinal) unseasoned SP board was placed in each container in direct contact with the sand with the function being to serve as a uniform source of inoculums beneath all test samples. A single piece of screen mesh measuring 30.4 cm × 27.9 cm was placed in each container above the unseasoned pine to elevate the test samples approximately 2.54 cm from the sand and SP board. Five replicates of a single treatment group were then placed in each container (Fig 1). Lids were placed on the containers and the containers were placed in an environmental chamber at 85% RH and 23.8°C for 4 wk. This temperature was chosen in observance of a standardized method (AWPA 2010) and the RH was chosen because while mold can grow on organic substrates as low as 75% RH, it more commonly grows at 80-85% RH (Quarles 2008). After the 4-wk testing period, surface mold was evaluated on all treated and untreated surfaces of the test samples and was reported on a scale from 0% (no growth) to 100% (complete coverage). Test samples were then cleaned of surface mold with a mild detergent (Palmolive) mixture.

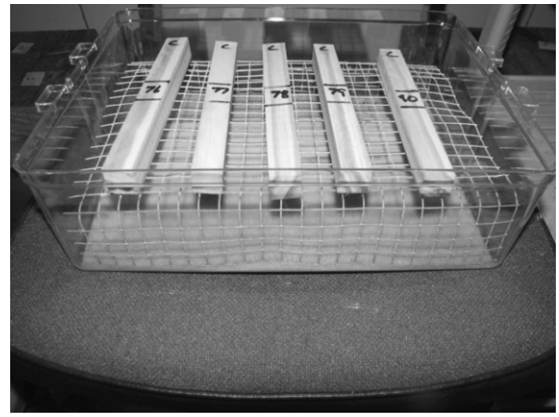

Figure 1. Southern pine (SP) sample placement for a single treatment group.

Statistical Analysis

Analysis of variance between the different control ends of the wood species as well as among the different containers was done in SAS 9.2 using PROC GLM. The difference between the control and treated ends of test samples was tested in SAS 9.2 (SAS Institute Inc. 2008) using PROC TTEST via a paired *t* test as well as using the nonparametric Wilcoxon signed-rank test in SAS 9.2 with PROC UNIVARIATE

to determine if each treatment significantly reduced mold growth.

Cycle 2 Heat Treatment

After cleaning, test samples were subjected to a second HT using the method outlined earlier. Following HT, the samples were replaced in the unsterile containers along with a new unseasoned SP board and an additional 100 mL of deionized water added to the sand layer. Containers were replaced in an environmental chamber at 85% RH and 24°C for 4 wk. At the end of the testing period, test samples were evaluated with the same procedure as the first cycle.

RESULTS AND DISCUSSION

Wood moisture content prior to chemical treatment was 31 and 66% (oven-dry basis) for SP and sweetgum, respectively. The time of the first HT schedule was 70 min with the core temperatures reaching 56°C 30 min into the cycle. The HT schedule went uninterrupted for 40 min after minimum core temperatures were reached with final core temperatures reaching 67°C and final surface temperatures approximately 69°C. Final wet bulb temperature in the kiln was 66°C at the end of the HT schedule. Moisture content calculated from extra test samples following the first HT were 21 and 55% (oven-dry basis) for SP and sweetgum, respectively. The temperature and time required for HT for cycle 2 were similar to cycle 1.

Cycle 1 Heat Treatment

There was no significant difference found between the control rating of the SP and sweetgum samples ($p = 0.1489$). There was also no significant difference found between the control rating among the different containers ($p = 0.0660$). However, the results point to some differences between the control ratings with the sweetgum control ends for the treatment group 4 (Bora-Care with Mold Care dip) where the control rating was 74% compared with the control ends being in the upper 80s to 90% range for the other treatment groups. This may indicate that the test setup could be improved with a single container for all treatment groups rather than individual containers such that variation among control groups could be decreased but it may also be attributed to wood variability. For cycle 1, all of the treatment group ends had significantly less mold growth than the control ends ($\alpha = 0.05$) (Table 2). The least difference between the control and the treated end was observed in treatment group 1 (DDAC dip) for SP where the treated end had only 27% reduction compared with the control end. This contrasted with the other treatment groups where the reductions were in the 70-90% range. Perhaps the samples in treatment group 1 (DDAC dip) were not treated with DDAC effectively or perhaps the DDAC treatment was not sufficient for a 4-wk mold growth cycle at 85% RH. As expected, treatment groups 3 (DOT and DDAC dip) and 4 (Bora-Care with Mold Care) performed similarly because they are laboratory

Table 2. Control and treated cycle 1 results for mold rating after 28 da and statistical analysis.

			Control		Treated		Reduction	Paired *t* test
Treatment	Chemical	Species	Rating mean (%)	Rating SD (%)	Rating mean (%)	Rating SD (%)	Rating (%)	*p* value
1	DDAC dip	Gum	94	13	19	8	75	0.0005
		Southern pine	97	4	70	14	27	0.0197
2	DOT and DDAC spray	Gum	96	7	11	13	85	0.0002
		Southern pine	92	10	18	11	74	0.0005
3	DOT and DDAC dip	Gum	86	18	0	0	86	0.0004
		Southern pine	94	5	4	4	90	<0.0001
4	Bora-Care® with Mold Care™ dip	Gum	74	15	0	0	74	0.0004
		Southern pine	90	12	1	2	89	<0.0001

SD, standard deviation.

and commercial formulations of the same chemicals. For all treatment groups, the nonparametric Wilcoxon signed rank test for significant differences among for mold rating between the control and treatment ends indicated no significant differences ($p = 0.0625$). This is likely the more appropriate statistical test because of skewness in the tails present in the data, but because of the limited number of samples per treatment group (n = 5), the power of the test was not high enough to indicate significant differences. However, the p value was reasonably close to $\alpha = 0.05$ so it may be reasonable to assume that with more samples the test would show significant differences. Refer to Figs 2 and 3 for an example of mold growth after the first cycle.

Figure 2. Example of sweetgum test samples after cycle 1.

Figure 3. Example of southern pine (SP) test samples after cycle 1.

Cycle 2 Heat Treatment

There was significant difference found between the control rating of the SP (97.5%) and sweetgum (81.75%) samples ($p = 0.0062$). There were also significant differences found between the control ratings among the different containers ($p < 0.0001$). This was seen in the Tukey grouping where the sweetgum control for the treatment group 1 (DDAC dip) had only 50% mold growth. The results for cycle 2 were much more variable than the results for cycle 1 (Table 3). Treatment group 1 (DDAC dip) did not have significant differences found between the treated and control ends ($p = 0.0547$ and 1.0, respectively) for sweetgum and SP. However, the sweetgum treated ends performed better than SP treated ends. The DOT and DDAC Dip (2) was more effective in cycle 2 than the spray treatment (3) was for both sweetgum and for SP. One interesting result was that treatment group 4 (Bora-Care with Mold Care dip) was not significantly different but treatment group 3 (DOT and DDAC dip) was significantly different for SP; however, for sweetgum, both treatments were significantly different. This was interesting because they are both formulas of the same compounds. It appears that most treatments lost efficacy during the second cycle; this could be the result of the extended exposure in the mold growth period or it could be attributed to the cleaning process. The nonparametric Wilcoxon signed rank test for significant differences among mold ratings was consistent with that found in cycle 1 such that the paired t tests that showed significant differences had a p value of 0.0625; and nonsignificant amounts of reduction using the paired t test showed p values > 0.0625.

CONCLUSIONS

The DDAC and/or DOT treatments utilized in this study showed promising results to inhibit or reduce surface mold growth on representative hardwood (sweetgum) and softwood (SP) species when combined with a HT cycle for phytosanitation. Analyzing the data using a paired t test, all treatments significantly reduced surface

Table 3. Control and treated cycle 2 results for mold rating after 4 wk and statistical analysis.

			Control		Treated		Reduction	Paired *t* test	Signed rank test
Treatment	Chemical	Species	Rating mean (%)	Rating SD (%)	Rating mean (%)	Rating SD (%)	Rating (%)	*p* value	*p* value
1	DDAC dip	Gum	50	28	24	11	26	0.0547	0.125
		Southern Pine	96	7	96	4	0	1.0	1.0
2	DOT and DDAC spray	Gum	94	11	62	18	32	0.0085	0.0625
		Southern Pine	100	0	89	7	11	0.0196	0.0625
3	DOT and DDAC dip	Gum	97	4	4	7	96	<0.0001	0.0625
		Southern Pine	98	3	55	16	43	0.0026	0.0625
4	Bora-Care® with Mold Care dip™	Gum	86	7	1	2	85	<0.0001	0.0625
		Southern Pine	96	4	75	18	21	0.0863	0.1875

SD, standard deviation.

mold growth compared with control ends during the cycle 1 exposure period. However, the nonparametric Wilcoxon signed rank test indicated no significant differences ($p = 0.0625$), likely because of lack of power because of the limited sample size. Because of this, it may be appropriate to increase the sample size in the future. Future testing could also include lengthening the exposure period to 6-8 wk and beyond to determine how long the treatments would work without subjecting them to a cleaning, which may have impacted the treatment. A lengthier exposure period may better simulate the time in a shipping container when WPM is shipped via ocean freight. Also, it may be appropriate to utilize standardized methods (AWPA 2010) as it would seem efficacy was lost during the cycle 2 exposure period. Although further research is needed, results obtained from this study may be useful in improving the modified mold test conducted and perhaps used to help standardize an alternative testing methodology for the evaluation of mold on unseasoned wood materials. Also, this work focused on relatively small samples that because of their size could be heat-treated relatively quickly. This study provided foundation data needed to establish a screening protocol for current and future WPM treatments to be utilized in conjunction with approved heat treatments to provide residual protection for the WPM. It may be appropriate to study the effect that a schedule utilizing a longer HT process may have on the efficacy of DDAC and/or DOT. Future studies will expand on the foundation data gathered in this initial project and will include larger sample size, full size samples, various biocides (traditional and nontraditional), and multiple species to broaden the knowledge base.

ACKNOWLEDGMENTS

This project was partially funded by the McIntyre-Stennis Formula Grant through the USDA-NIFA and the USDA Special Grant program for Wood Utilization Research.

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