Introduction

In 2004 a study of the effectiveness of the available popular commercial moldicides was conducted. Two of the active ingredients that were tested were Disodium Octaborate Tetrahydrate (DOT) and Didecyl Dimethyl Ammonium Chloride DDAC), **the active ingredients in Bora-Care and Mold-Care, respectively**.

Bora-Care and Mold Care were tested alone and in combination by the **USDA** Forest Products lab as a mold preventive on both **wood and sheet rock** and results were published at the International Research Group on wood Protection (Micales-Glaser et al., 2004).

Tests were carried out against 4 of the EPA-listed **health effect mold fungi,** including the '**Toxic Mold**' fungi *Stacybotrys chartarum*.

Treatments were effective in reducing both mold **growth and sporulation**.

A **combination of the two** was most effective and **totally prevented** even the most difficult-to-control fungus (*Cladosporium cladosporoides*) at concentrations below those used in **Bora-Care with Mold-Care**.

The authors conclude that by using products during construction or **after water damage, the problems associated with the growth of common molds and their potential health effects can be avoided**.

Entire study follows:

IRG/WP 04-30338

THE INTERNATIONAL RESEARCH GROUP ON WOOD PRESERVATION

Section 3 Wood protecting chemicals

Efficacy of Didecyl Dimethyl Ammonium Chloride (DDAC), Disodium Octaborate Tetrahydrate (DOT), and Chlorothalonil (CTL) against Common Mold Fungi

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Abstract

The fungitoxic properties of four fungicides, alone and in combination, against four different mold fungi commonly associated with indoor air quality problems were evaluated on two different wood species and sheetrock. The fungicides were chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) (CTL) in a 40.4% aqueous dispersion, disodium octaborate tetrahydrate (DOT) in two different forms - a 40% glycol solution and a 98% wettable powder, and didecyl dimethyl ammonium chloride (DDAC) in an 80% solution. The fungi were *Aspergillus niger, Cladosporium cladosporioides, Penicillium brevicompactum,* and *Stachybotrys chartarum*. All fungicide treatments on wood reduced growth, sporulation and discoloration of the mold fungi when compared to nontreated specimens. No single fungicide provided total control of all four fungi on wood. CTL provided the best single-agent protection by totally preventing the growth of *C. cladosporioides* and *S. chartarum* and reducing growth of *A. niger* and *P. brevicompactum* to low levels. DOT in both forms was very effective against *A. niger*, but provided only sporadic protection against other fungi. DDAC provided good protection against *S. chartarum* but was not as effective against the other molds. Combinations of the different biocides were more effective than any single agent. DOT + DDAC totally prevented or greatly reduced growth of *A. niger*, *P. brevicompactum* and *S. chartarum*. *Cladosporium cladosporioides* was the most difficult organism to control, but even this was achieved when DDAC was increased to 1.0% with DOT. The most consistent control of discoloration, sporulation, and growth of the fungi on wood was obtained with the combination of DOT and CTL. DOT, alone or in combination with DDAC or CTL, was also very effective against the fungi on sheetrock. The results suggest that by using appropriate products, during construction or after water damage, problems associated with the growth of common molds and their potential health effects can be avoided.

[A]The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

Key Words: mold, chlorothalonil, DOT, DDAC, borates, Bora-Care®, Cellu-Treat®, Mold-Care®, Clortram® F-40, indoor air quality, IAQ, antisapstain, quaternary ammonium compounds, mildewcide.

Introduction

Recent public concern about potentially adverse health effects of molds growing in houses, schools, and the work environment shows that there is an important need for mold-resistant products. The potential health effects associated with mold has been a controversial topic in the United States in recent years and has gained much attention. Large insurance settlements, celebrity involvement, media scrutiny and rapid communication *via* the Internet have exacerbated the fears of the public of "toxic mold." Initial concerns about exposure to mycotoxins from molds such as *Stachybotrys chartarum* have not held up to scientific scrutiny (Redd, 2002; Center for Disease Control, 2004). It has been established that molds are associated with allergies in sensitive individuals and can trigger asthmatic attacks in those prone to asthma (Dales and Miller, 2001; Day and Ellis, 2001). People with compromised immune systems can suffer additional other mold-induced pathology (Summerbell, 2001).

The best way to prevent mold growth is to eliminate water in the wood, and the removal of moisture sources is always the first recommendation that should be made for fungal control (US EPA, 2002). Unfortunately there are many ways in which wood comes into contact with water. Many of these pathways, such as plumbing and roofing leaks, are avoidable and can be prevented by good construction practices and the diligence of homeowners. Other situations, such as excessive rain during construction, flooding, high humidity and temperature extremes that result in condensation, often cannot be avoided. There is always the potential for building materials to become wet! If vulnerable materials could be protected during construction and in situations where exposure to moisture cannot be controlled, the rapid growth of mold and decay fungi following a moisture event could be prevented. This is especially important to building occupants with known mold sensitivities or who have a heightened awareness and fear of possible adverse health affects from exposure to mold.

Most common molds are Deuteromycetes - the imperfect forms of Ascomycetes - and are controllable with commercially available fungicides and mildewcides. The large reproductive capacity of these imperfect fungi easily allows them to develop fungicide resistance, so treatments that display several different modes of action are more likely to have long-term success. Several groups of fungicides are commercially available to prevent the growth of the related sapstain fungi in lumber. Didecyl dimethyl ammonium chloride (DDAC) is frequently used to treat freshly cut logs and lumber before kiln-drying to prevent the development of bluestain (or sapstain) discoloration (Forest Products Laboratory, 1999). The fungicide chlorothalonil has also been proven to be effective against mold fungi (Micales et al., 1989) and is used in paints as a mildewcide among many other agricultural and industrial uses. Many construction materials are treated with borates for non-biodeterioration goals, including fire retardancy and insect

control. Studies have shown that these borate treatments can also reduce the amount of mold growth (Fogel and Lloyd, 2002). The objective of this paper was to evaluate the efficacy of these treatments, alone and in combination, against four common mold fungi frequently associated with indoor air quality problems: *Aspergillus niger, Cladosporium cladosporioides, Penicillium brevicompactum*, and *Stachybotrys chartarum*.

Materials and Methods

Four different fungicides, alone and in combination, were used. Bora-Care® (Nisus Corporation) contains 40% disodium octaborate tetrahydrate (DOT) in solution. Cellu-Treat® (Nisus Corporation) is a wettable powder that contains 98% DOT. Mold-Care® (Nisus Corporation) consists of an 80% solution of didecyl dimethyl ammonium chloride (DDAC). Clortram® F-40 (Sostram Corporation, Roswell, GA) contains 40.4% chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) (CTL) in an aqueous dispersion. The seventeen specific treatments are listed below:

		Test Concentrations (% w/w)		
No.	Treatment	CTL	DOT	DDAC
1	Water Control			
2	CTL	0.5		
3	CTL	1.0		
4	DDAC			0.5
5	DDAC			1.0
6	40% DOT		8.5	
7	40% DOT		15.0	
8	98% DOT		8.5	
9	98% DOT		15.0	
10	40% DOT + DDAC		8.5	0.5
11	40% DOT + DDAC		8.5	1.0
12	40% DOT + CTL	0.5	8.5	
13	40% DOT + CTL	1.0	8.5	
14	98% DOT + DDAC		8.5	0.5
15	98% DOT + DDAC		8.5	1.0
16	98% DOT + CTL	0.5	8.5	
17	98% DOT + CTL	1.0	8.5	

Southern yellow pine and aspen sapwood blocks (70 X 19 X 6 mm, the long axis parallel to the grain) were cut from untreated kiln-dried boards obtained commercially, as representatives of susceptible softwood and susceptible hardwood species. Similarly sized pieces of sheetrock, supplied by Nisus Corporation, were also prepared. Borates are regularly used in the manufacturing process of sheetrock to improve strength and other characteristics. This material was analyzed prior to use to determine the absence of borate.

Samples of aspen, pine, and sheetrock were autoclaved for 25 minutes in glass deep-dished Petri plates. Further work was conducted aseptically to prevent unwanted contamination. Samples to be treated were dipped in a preparation of fungicide for a minimum of 15 seconds. They were then placed on dry blotting paper within 150 mm-diameter Petri plates and allowed to dry overnight in a laminar air-flow hood Treatments are listed above. A total of 6 replications of each fungicide-fungus combination was prepared for the specimens of aspen and pine; 4 replications were made for each sheetrock treatment.

After the drying period, the filter papers were moistened with 15 ml sterile distilled water. The moisture chambers were equilibrated for 24 hours before inoculation, thus allowing the wood and sheetrock specimens to become thoroughly wetted. Immediately before inoculation, an additional 5 ml of sterile distilled water was added to the blotting paper to make up for moisture absorbed by the specimens.

Each specimen was inoculated with 0.5 ml of a spore/mycelial suspension. The suspensions were made by blending 2 flasks of 14-day-old fungal cultures (growing in 25 ml of 2% malt extract broth) with 100 ml sterile distilled water for a minimum of 15 seconds. The following fungi were used:

Cladosporium cladosporioides (JAG 1001-04)
Penicillium brevicompactum (JAG 1002-04)
Aspergillus niger (JAG 1003-04)
Stachybotrys chartarum (JAG 1004-04)

The cultures are routinely stored in the culture collection of the Center for Forest Mycology Research, Forest Products Laboratory, Madison, WI, USA. After inoculation, the plates were sealed with Parafilm®, placed in plastic bags, and incubated at 25°C and 80% humidity. Samples were rated for fungal growth using a modified ASTM method D 3273 – 94 after 2, 4, 8, and 12 weeks of growth. Both macroscopic and microscopic growth assessments were made. An Olympus stereoscopic binocular microscope was used for the microscopic examination. Three different ratings were given to each specimen: a) amount (degree of coverage and thickness) of mycelial growth; b) amount of sporulation; and c) degree of macroscopic discoloration of the specimen. Each evaluation consisted of a 4 point scale: 0 – none; 1 – light; 2 – moderate; and 3 – extensive; using the following criteria:

Fungal growth:

0 – no visible fungal mycelia observed microscopically
1 – fungal mycelia observed on specimen surface microscopically but growth is scanty and appressed against the specimen surface. Little or no aerial hyphae.
2 – fungal mycelium is more extensive and covers a significant percent of the specimen surface (more than 50%). Aerial mycelia also present.
3 – extensive fungal growth over most of specimen surface. Aerial hyphae thick.

Sporulation:

0 – no sporulation observed
1- sporulation light. Only certain areas exhibit fruiting structures or few fruiting structures are observed in any area.
2- sporulation moderate, both in the percent of specimen area showing fruiting bodies and in the number of fruiting bodies formed in any area.
3- sporulation extensive

Macroscopic discoloration

0 – no macroscopic discoloration
1 – discoloration present but light
2 – discoloration moderate. Less than 50% of specimen surface discolored.
3 – discoloration extensive. More than 50% of specimen discolored.

This is a more aggressive evaluation than is mandated by the standard but provides a much better indication of the amount of fungal growth and sporulation on each sample. Samples that are even slightly discolored frequently had a large spore load that could affect sensitive individuals.

Results and Discussion:

Pine and aspen:

Successful colonization occurred on all control specimens of pine and aspen. The amount of colonization on treated specimens varied with the fungicide application and the species of fungus. Results after 12 weeks are summarized in Figures 1 – 4. All values are the mean of 6 replications. Ratings for "sporulation" and "mycelial growth" were frequently identical except when the target fungus was able to form vegetative mycelium but could not sporulate due to the presence of the fungicide. In some cases, the fungicide had unique impacts on the development of the fungal colonies. Treatments of chlorothalonil, at final concentrations of 0.5% and 1.0%, greatly restricted colony formation so that only a few discreet colonies were formed on the surface of the specimens. The colonies were compact and brush-like, and did not spread as in the other treatments. This colony morphology was not observed on specimens treated with other fungicide combinations.

Often a sample was evaluated as being "lightly colonized" (both microscopically and macroscopically) because small fungal colonies were able to form around the remnants of larger, thick pieces of mycelial inoculum. The surfaces of these inoculum "chunks" were not in direct contact with the treated specimen and were thus protected from direct contact with the fungicide. A surface was rated as lightly colonized (i.e., "1") if the fungal colony grew onto the surface of the wood in the immediate vicinity of the inoculum. In most cases, this occurred on only one or two specimens of a particular treatment and did not influence the average treatment rating.

The macroscopic discoloration in the control specimens was often quite light, especially for specimens inoculated with *A. niger* and *S. chartarum*. Discoloration by these organisms is due solely to the production of pigmented spores, and even a heavily colonized surface may not result in a spore density sufficient for extensive discoloration. For this reason, it is important to include a microscopic examination of the surface. A simple macroscopic estimation, as described in the ASTM standard D 3274 – 95 may allow a large number of fungal spores and extensive fungal colonization to go undetected. This was not a problem with specimens of *C. cladosporioides*, which produces a dark pigment in the substrate, nor with *P. brevicompactum*, which produces a dense vegetative mycelium.

Growth on control and treated specimens gradually increased until the 8-week sampling. There were few changes between the 8-week and 12-week readings (data not shown). Readings at 2- and 4- weeks did not accurately reflect the growth potential of the organisms. Test results could be accelerated by growing the fungi at a slightly higher temperature, however this was not done so that the test would reflect temperatures found in the indoor air environment.

CTL treatments at 0.5% and 1.0% alone (treatments #2 and 3) were effective at preventing the growth and sporulation of *C. cladosporioides* and *S. chartarum*, but did not totally control *A. niger* or *P. brevicompactum*, although the growth of the latter two fungi was reduced with the treatments. DDAC at 1.0% (treatment #5) controlled the growth of *S. chartarum*, but was not as effective against the other fungi; the treatment at 0.5% (treatment #4) failed to control the growth and sporulation of any of the fungi completely although reductions in growth were observed. The two different forms of DOT (treatments #6,7,8, and 9) provided total control against *A. niger* at both concentrations. Fungal growth and sporulation of the other three species were reduced, especially at 15% (treatments 7 and 9).

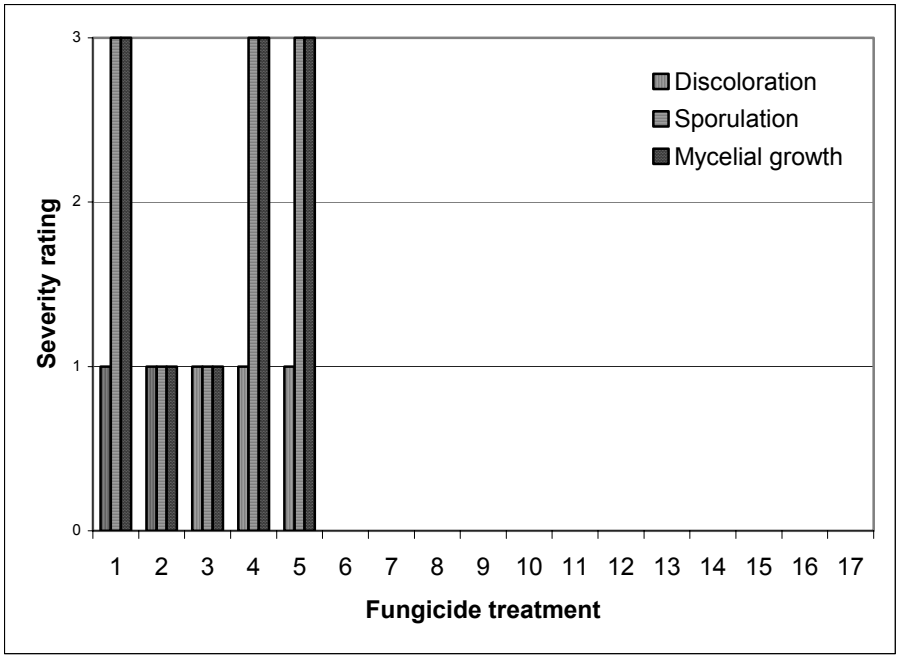
Combinations of the different fungicides were generally more effective than the individual treatments alone. DOT + DDAC (treatments # 10, 11,14 and 15) totally prevented or greatly reduced growth of *A. niger*, *P. brevicompactum* and *S. chartarum*. *Cladosporium cladosporioides* was the most difficult organism to control, but even this was achieved when DDAC was increased to 1.0% and DOT was used (treatment #15). The most consistent control of discoloration, sporulation, and growth was obtained with the combination of DOT and CTL at both concentration levels (treatments #12, 13, 16, and 17). The growth of all four fungi was totally prevented with these treatments - indeed, no growth was observed on any single specimen.

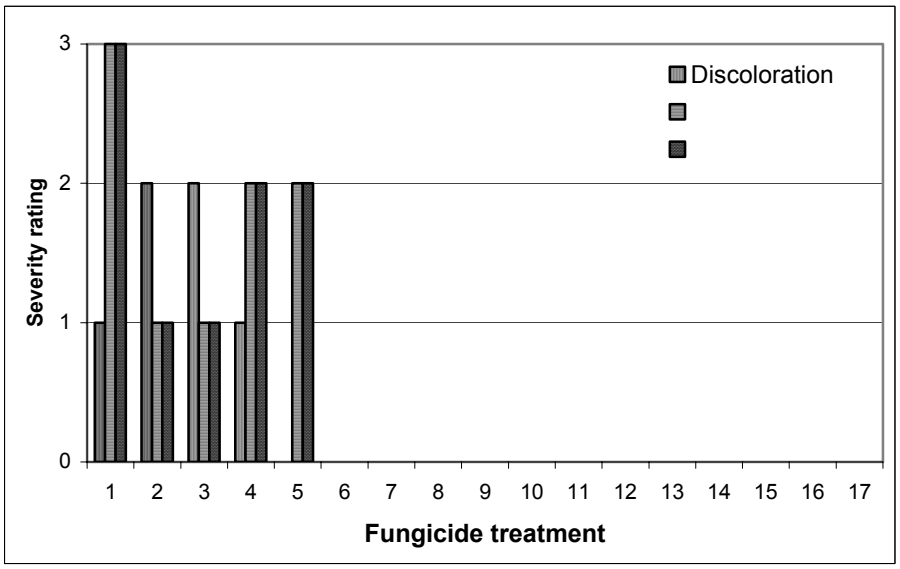
Sheetrock

Results of tests using sheetrock instead of wood are presented in Figure 5. *Aspergillus niger* did not successfully colonize the sheetrock, even in the control specimens, so no results are reported for this organism.

The two concentrations of CTL (treatments #2 and 3) failed to control the growth of *S. chartarum*, although they did reduce colony formation into discreet units that were uniformly dispersed over the surface of the specimen. Curiously, CTL at 0.5% (treatment #2) prevented the growth of *P. brevicompactum*, while the higher concentration of 1.0% (treatment #3) did not. Both concentrations were effective against *C. cladosporioides*. The two treatments of DDAC (treatments #4 and 5) actually seemed to stimulate the growth of all three fungi, perhaps by altering the pH of the sheetrock into a more optimal range for fungal growth. All other treatments that contained DOT were effective against all three fungi. Control of the fungi on sheetrock was easier to achieve than on pine or aspen.

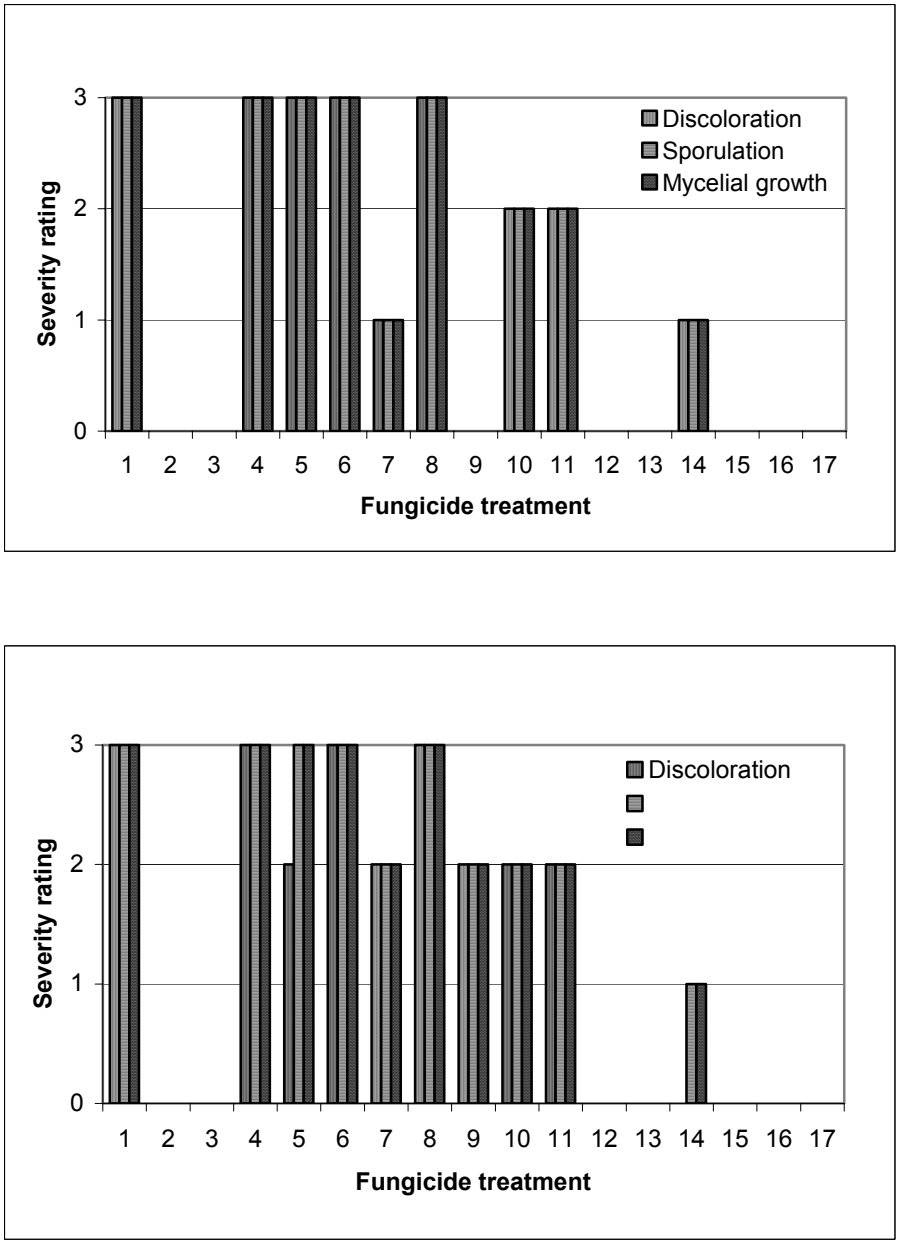
In this highly aggressive test, all fungicide treatments reduced the growth, sporulation and discoloration of the four fungi as compared to the nontreated specimens. No single fungicide provided total control of all four fungi on wood. CTL provided the best single-agent protection on wood by totally preventing growth of *Cladosporium cladosporioides* and *Stachybotrys chartarum* and reducing the growth of *Aspergillus niger* and *Penicillium brevicompactum* to low levels. Combinations of the different biocides were more effective than any single agent. The most consistent control of discoloration, sporulation, and growth was obtained with the combination of DOT and CTL. The results of this test suggest that properly treated building materials could be used in situations where moisture cannot be avoided without the development of mold infestations and their resultant health effects in sensitive individuals.





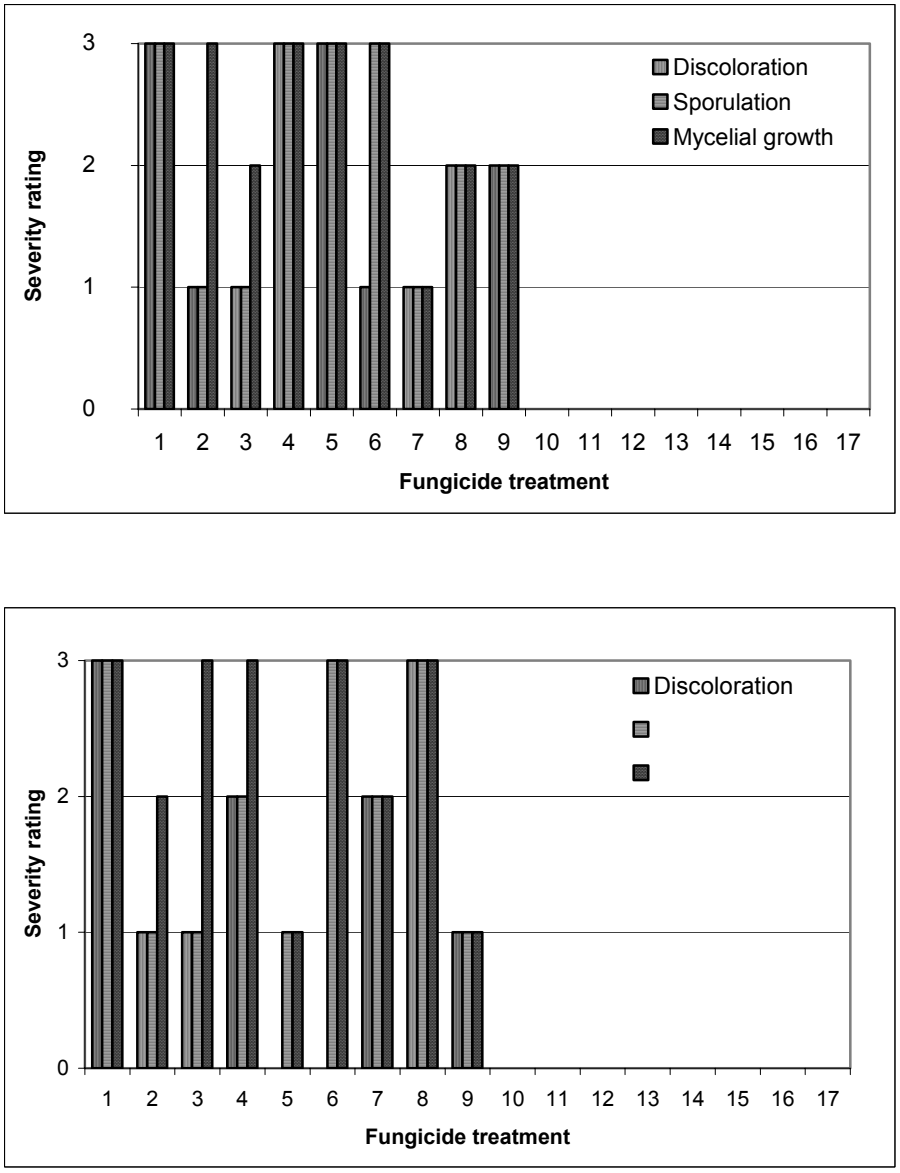
1 – Control
2 - 0.5% CTL
3 - 1.0% CTL
4 - 0.5% DDAC
5 - 1.0% DDAC
6 - 8.5% DOT (solution)
7 - 15% DOT (solution)
8 - 8.5% DOT (WP)
9 - 15% DOT (WP)
10 - 0.5% DDAC + 8.5% DOT (solution)
11 - 1.0% DDAC + 8.5% DOT (solution)
12 - 0.5% CTL + 8.5% DOT (solution)
13 - 1.0% CTL + 8.5% DOT (solution)
14 - 0.5% DDAC + 8.5% DOT (WP)
15 - 1.0% DDAC + 8.5% DOT (WP)
16 - 0.5% CTL + 8.5% DOT (WP)
17 - 1.0% CTL + 8.5% DOT (WP)

Figure 1: Effect of fungicide treatments on the discoloration, sporulation, and mycelial growth of *Aspergillus niger* on pine (above) and aspen (below) after 12 weeks. Each point is the average of six replicate samples. Fungicide concentrations and fungal growth severity ratings are as described in the text.



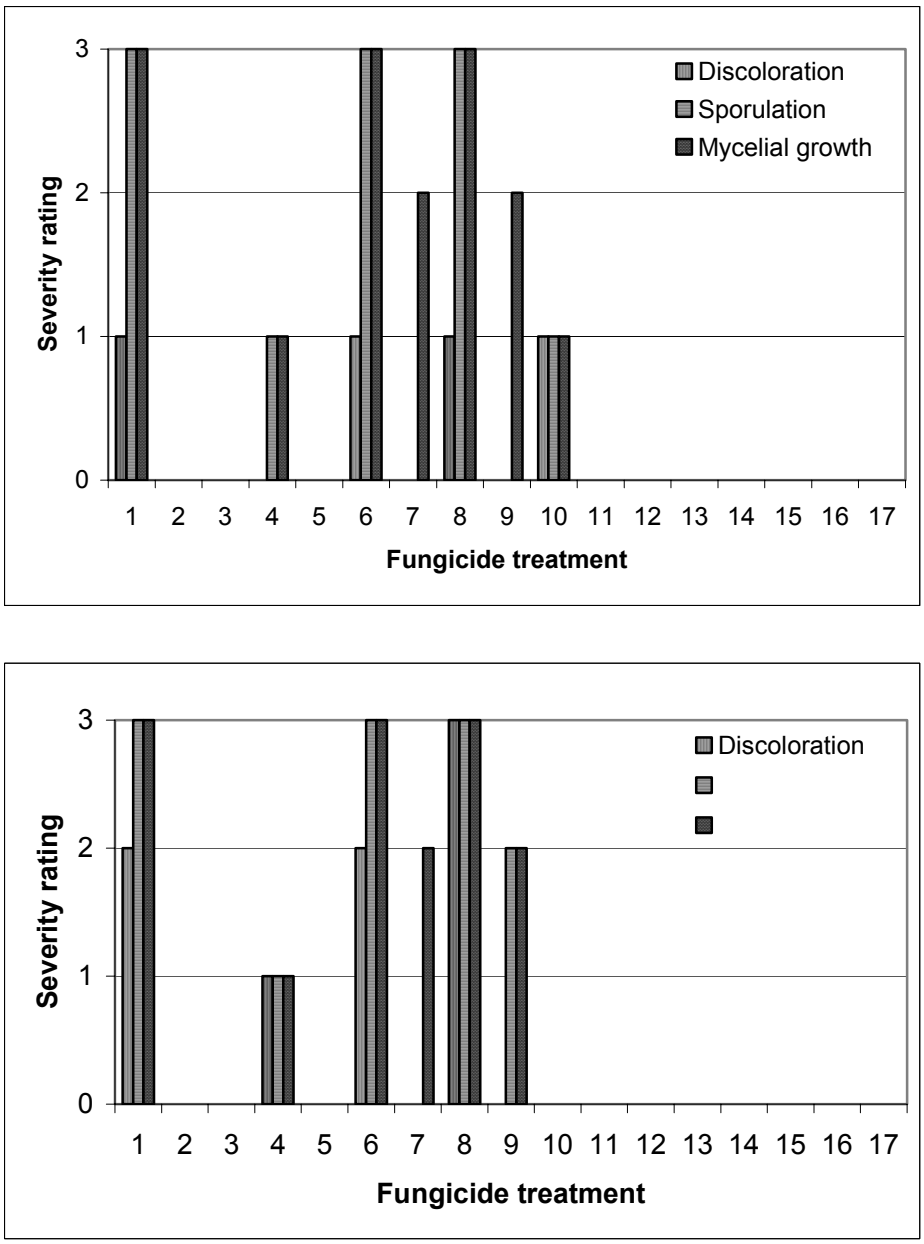
1 – Control
2 - 0.5% CTL
3 - 1.0% CTL
4 - 0.5% DDAC
5 - 1.0% DDAC
6 - 8.5% DOT (solution)
7 - 15% DOT (solution)
8 - 8.5% DOT (WP)
9 - 15% DOT (WP)
10 - 0.5% DDAC + 8.5% DOT (solution)
11 - 1.0% DDAC + 8.5% DOT (solution)
12 - 0.5% CTL + 8.5% DOT (solution)
13 - 1.0% CTL + 8.5% DOT (solution)
14 - 0.5% DDAC + 8.5% DOT (WP)
15 - 1.0% DDAC + 8.5% DOT (WP)
16 - 0.5% CTL + 8.5% DOT (WP)
17 - 1.0% CTL + 8.5% DOT (WP)

Figure 2: Effect of fungicide treatments on the discoloration, sporulation, and mycelial growth of *Cladosporium cladosporioides* on pine (above) and aspen (below). Each point is the average of six replicate samples after 12 weeks. Fungicide concentrations and fungal growth severity ratings are as described in the text.



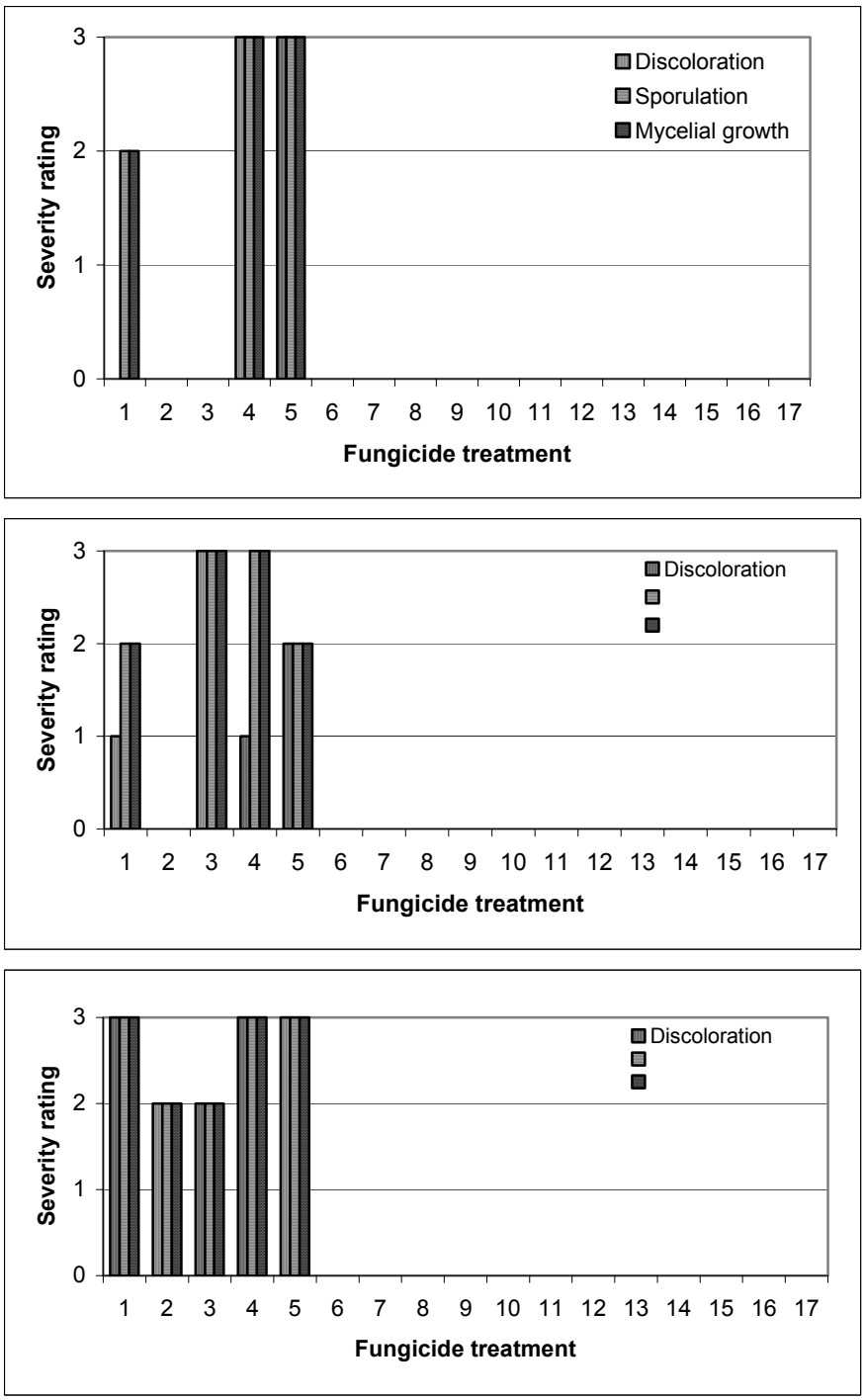
1 – Control
2 - 0.5% CTL
3 - 1.0% CTL
4 - 0.5% DDAC
5 - 1.0% DDAC
6 - 8.5% DOT (solution)
7 - 15% DOT (solution)
8 - 8.5% DOT (WP)
9 - 15% DOT (WP)
10 - 0.5% DDAC + 8.5% DOT (solution)
11 - 1.0% DDAC + 8.5% DOT (solution)
12 - 0.5% CTL + 8.5% DOT (solution)
13 - 1.0% CTL + 8.5% DOT (solution)
14 - 0.5% DDAC + 8.5% DOT (WP)
15 - 1.0% DDAC + 8.5% DOT (WP)
16 - 0.5% CTL + 8.5% DOT (WP)
17 - 1.0% CTL + 8.5% DOT (WP)

Figure 3: Effect of fungicide treatments on the discoloration, sporulation, and mycelial growth of *Penicillium brevicompactum* on pine (above) and aspen (below) after 12 weeks. Each point is the average of six replicate samples. Fungicide concentrations and fungal growth severity ratings are as described in the text.



1 – Control
2 - 0.5% CTL
3 - 1.0% CTL
4 - 0.5% DDAC
5 - 1.0% DDAC
6 - 8.5% DOT (solution)
7 - 15% DOT (solution)
8 - 8.5% DOT (WP)
9 - 15% DOT (WP)
10 - 0.5% DDAC + 8.5% DOT (solution)
11 - 1.0% DDAC + 8.5% DOT (solution)
12 - 0.5% CTL + 8.5% DOT (solution)
13 - 1.0% CTL + 8.5% DOT (solution)
14 - 0.5% DDAC + 8.5% DOT (WP)
15 - 1.0% DDAC + 8.5% DOT (WP)
16 - 0.5% CTL + 8.5% DOT (WP)
17 - 1.0% CTL + 8.5% DOT (WP)

Figure 4: Effect of fungicide treatments on the discoloration, sporulation, and mycelial growth of *Stachybotrys chartarum* on pine (above) and aspen (below) after 12 weeks. Each point is the average of six replicate samples. Fungicide concentrations and fungal growth severity ratings are as described in the text.



1 – Control
2 - 0.5% CTL
3 - 1.0% CTL
4 - 0.5% DDAC
5 - 1.0% DDAC
6 - 8.5% DOT (solution)
7 - 15% DOT (solution)
8 - 8.5% DOT (WP)
9 - 15% DOT (WP)
10 - 0.5% DDAC + 8.5% DOT (solution)
11 - 1.0% DDAC + 8.5% DOT (solution)
12 - 0.5% CTL + 8.5% DOT (solution)
13 - 1.0% CTL + 8.5% DOT (solution)
14 - 0.5% DDAC + 8.5% DOT (WP)
15 - 1.0% DDAC + 8.5% DOT (WP)
16 - 0.5% CTL + 8.5% DOT (WP)
17 - 1.0% CTL + 8.5% DOT (WP)

Figure 5: Effect of fungicide treatments on discoloration, sporulation, and mycelial growth of *C. cladosporioides* (top), *P. brevicompactum* (middle), and *S. chartarum* (bottom) on sheetrock after 12 weeks. Each point is the average of four replicate values. Fungicide concentrations and fungal growth severity ratings are as described in the text.

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