

Mold Solutions MMR Product Testing Report

Purpose of testing:

The purpose of this testing was to determine what effect MMR mold stain remover, hereby referred to as MMR had on three different types of fungi (molds) on two different types of wood based building materials. MMR was also compared to the effects of ordinary bleach (6% sodium hypochlorite) as well as another brand name disinfectant, hereby referred to as BND. Three different organisms were used in the testing. The organisms were *Paecilomyces variotii, Chaetomium globosum* and *Ulocladium chartarum*. These are common molds and often recovered from water damaged building materials. The viability of the organisms after treatment was evaluated as well as any morphological changes of treated fungi as compared to a control sample. Viability was assessed by culturing post-treated samples and any morphological changes were documented photographically. There is a glossary at the end of the report which explains many of the terms used in the report.

Summary of testing procedures:

Small pieces of plywood and 2X4's were autoclaved. This process sterilized the surface of the wood. One piece of plywood and one piece of 2x4 were not inoculated and used as negative controls to check the sterility of the wood after the autoclave treatment. Each of the other samples was inoculated with spore suspensions of known concentration, then placed in a moist chamber at room temperature and allowed to develop. Samples were checked periodically to monitor development. When the samples reached maturity, defined as confluent growth or nearly confluent growth with heavy sporulation over the surface of the wood, the samples were then removed from the moist chambers and allowed to dry at room temperature for 48 hours. A 1 in² area from the surface of each sample was then swabbed and cultured via serial dilutions on Malt Extract Agar (MEA) to determine the number of cfu/in² as a pretreatment baseline. The samples were then treated with MMR, BND or bleach using spray bottles until the samples appeared saturated. One piece of plywood and one piece of 2x4 were not treated. These two pieces were used as positive controls for the morphological evaluations. 24 hours after treatment, a 1 in² area from each sample was swabbed and cultured via serial dilutions on Malt Extract Agar (MEA) to determine viability of the fungi after treatment. Slide preparations of surface growth from each sample were evaluated microscopically to assess any morphological variations of treated samples vs. the untreated positive control samples and were documented photographically in the morphological evaluation section of the report. Slide preparations of the wood below the surface growth were evaluated to determine any morphological changes that occurred in the mycelium that penetrated the wood and documented photographically in the morphological evaluation section below.



Viability Trials:

Testing procedure and timeline

The fungi used in the study were isolated from environmental sources and specific identifications were made using the literature referenced at the end of the study.

The fungi were grown up on Malt Extract Agar (MEA). When the colonies were mature with heavy sporulation, the surface of the fungal colonies of each different species was swabbed and the swabs vortexed in 5 mL of sterile water. These suspensions were used to inoculate the wood pieces. The concentrations of each inoculate suspension was determined by serial dilution on malt extract agar (MEA).

Concentration of inoculants:

Paecilomyces variotii1,200,000 cfu/mLChaetomium globosum310,000 cfu/mLUlocladium chartarum240,000 cfu/mL

Each piece of wood was inoculated with 1 mL of inoculate suspension and the inoculum spread across the surface of the wood with a sterile spreader.

The *Ulocladium chartarum* portion of testing was initiated on 7/9/2012. Confluent or nearly confluent growth was observed by 7/27/2012. The fungus was allowed to continue to develop until 08/06/2012. The *Ulocladium chartarum* was then removed from the moist chamber and allowed to dry at room temperature for 48 hours. At 48 hours a 1 in² portion of the sample was swabbed and a serial dilution performed to determine the viability of the fungi pretreatment. The MMR, BND or bleach was then applied and allowed to work for 24 hours. At 24 hours post-treatment another 1 in² section was swabbed and a serial dilution was performed to assess the viability post-treatment.

The *Chaetomium globosum* portion of the testing was initiated on 7/9/2012. Confluent or nearly confluent growth was observed by 08/06/2012. The *Chaetomium globosum* was removed from the moist chamber and allowed to dry at room temperature for 48 hours. At 48 hours a 1 in² portion of the sample was swabbed and a serial dilution performed to determine the viability of the fungi pretreatment. The MMR, BND or bleach was then applied and allowed to work for 24 hours. At 24 hours post-treatment another 1 in² section was swabbed and a serial dilution was performed to assess the viability post-treatment.

The *Paecilomyces variotii* portion of the testing was initiated on 7/30/2012. Confluent or nearly confluent growth was observed by 08/06/2012. The *Paecilomyces variotii* was removed from the moist chamber and allowed to dry at room temperature for 48 hours. At 48 hours a 1 in² portion of the sample was swabbed and a serial dilution performed to determine the viability of the fungi pretreatment. The MMR, BND or bleach was then applied and



allowed to work for 24 hours. At 24 hours post-treatment another 1 in² section was swabbed and a serial dilution was performed to assess the viability post-treatment.



Table 1: Pre and Post-treatment viability

		MMR Via	bility Trials		
	1 in ² c	of sample was swabbed for	pre and post application sar	npling	
MMR Trials	1	1	1		1
Plywood: <i>Ulocladium</i> <i>chartarum</i> Inoculation / Post-treatment with MMR	Plywood: Chaetomium globosum inoculation / Post-treatment with MMR	Plywood: Paecilomyces variotii inoculation / Post- treatment with MMR	2X4: Ulocladium chartarum Inoculation / Post-treatment with MMR	2X4: Chaetomium globosum inoculation / Post-treatment with MMR	2X4: Paecilomyces variotii inoculation / Post-treatment with MMR
Count = < 10 cfu/in ²	Count = < 10 cfu/in ²	Count = < 10 cfu/in ²	Count = < 10 cfu/in ²	Count = < 10 cfu/in ²	Count = < 10 cfu/in ²
Plywood: Ulocladium chartarum Inoculation / Pre-treatment	Plywood: <i>Chaetomium</i> <i>globosum</i> Inoculation / Pre-treatment	Plywood: Paecilomyces variotii Inoculation / Pre- treatment	2X4: Ulocladium chartarum Inoculation / Pre-treatment	2X4: Chaetomium globosum Inoculation /Pre-treatment	2X4: Paecilomyces variotii Inoculation / Pre-treatment
Count = 130,000 cfu/in ²	Count = 190,000 cfu/in ²	Count = 820,000 cfu/in ²	Count = 80,000 cfu/in ²	Count = 60,000 cfu/in ²	Count = 1,750,000 cfu/in ²
Percent Reduction= > 99.9%	Percent Reduction= > 99.9%	Percent Reduction= > 99.9%	Percent Reduction= > 99.9%	Percent Reduction= > 99.9%	Percent Reduction= > 99.9%
BND Trials					
Plywood: <i>Ulocladium</i> <i>chartarum</i> Inoculation / Post-treatment with BND	Plywood: <i>Chaetomium</i> <i>globosum</i> inoculation / Post-treatment with BND	Plywood: Paecilomyces variotii inoculation / Post- treatment with BND	2X4: Ulocladium chartarum Inoculation / Post-treatment with BND	2X4: Chaetomium globosum inoculation / Post-treatment with BND	2X4: Paecilomyces variotii inoculation / Post-treatment with BND
Count = 9,200 cfu/in ²	Count = 300 cfu/in ²	Count = 5,400 cfu/in ²	Count =1,800 cfu/in ²	Count =240 cfu/in ²	Count =6,800 cfu/in ²
Plywood: <i>Ulocladium</i> <i>chartarum</i> Inoculation / Pre-treatment	Plywood: <i>Chaetomium</i> <i>globosum</i> Inoculation / Pre-treatment	Plywood: Paecilomyces variotii / Inoculation Pre- treatment	2X4: <i>Ulocladium</i> <i>chartarum</i> Inoculation / Pre-treatment	2X4: Chaetomium globosum Inoculation / Pre-treatment	2X4: <i>Paecilomyces</i> <i>variotii</i> Inoculation / Pre-treatment
Count = 210,000 cfu/in ²	Count = 180,000 cfu/in ²	Count = 880,000 cfu/in ²	Count = 150,000 cfu/in ²	Count = 70,000 cfu/in ²	Count = 1,100,000 cfu/in ²
Percent Reduction=95.6%	Percent Reduction= 99.8%	Percent Reduction= 99.4%	Percent Reduction=98.8%	Percent Reduction=99.7%	Percent Reduction=99.4%
Bleach Trials					
Plywood: <i>Ulocladium</i> <i>chartarum</i> Inoculation / Post-treatment with Bleach	Plywood: <i>Chaetomium</i> <i>globosum</i> inoculation / Post-treatment with Bleach	Plywood: Paecilomyces variotii inoculation / Post- treatment with Bleach	2X4: Ulocladium chartarum Inoculation / Post-treatment with Bleach	2X4: Chaetomium globosum inoculation / Post-treatment with Bleach	2X4: Paecilomyces variotii inoculation / Post-treatment with Bleach
Count = 240 cfu/in ²	Count = 4000 cfu/in ²	Count = 24,000 cfu/in ²	Count = 110 cfu/in ²	Count = 70 cfu/in ²	Count = 6,700 cfu/in ²
Plywood: <i>Ulocladium</i> <i>chartarum</i> Inoculation / Pre-treatment	Plywood: <i>Chaetomium</i> <i>globosum</i> inoculation / Pre-treatment	Plywood: Paecilomyces variotii inoculation / Pre- treatment	2X4: Ulocladium chartarum Inoculation / Pre-treatment	2X4: Chaetomium globosum inoculation / Pre-treatment	2X4: Paecilomyces variotii inoculation / Pre-treatment
Count = 340,000 cfu/in ² Percent Reduction= 99.9% Negative Control Plywood	Count = 310,000 cfu/in ² Percent Reduction=98.7%	Count = 710,000 cfu/in ² Percent Reduction=96.6%	Count = 80,000 cfu/in ² Percent Reduction=99.9% Negative Control 2x4 = Nc	Count = 24,000 cfu/in ² Percent Reduction=99.7%	Count = 710,000 cfu/in ² Percent Reduction=99.1%



Summary of viability trials:

MMR was the most effective at reducing the viability of the fungi. The recovery of fungi from the wood post-treatment with MMR was < 10 cfu/in². Bleach was the second most effective at reducing the viability of the fungi. BND was the least effective at reducing the viability of the fungi.

Table 2: Overall Average Percent Reduction of Viability per Type of Solution Used to Treat the Wood

MMR Percent Reduction	BND Percent Reduction	Bleach Percent Reduction	
> 99.9%	98.8%	99.0%	

Morphological evaluation:

The morphological evaluation consisted of the examination of the surface fungal growth and subsurface fungal growth both pre and post-treatment. The morphologies of the post-treated samples are compared to positive controls that were prepared at the same time as the samples that were treated.

Characteristics of the fungi evaluated in morphological study:

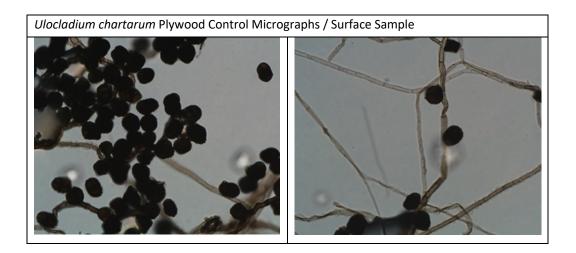
- **Hyphae** are tubular vegetative structures that fungi send out to exploit nutrient sources and water. Secretion of enzymes and absorption of nutrients occurs in this part of the fungi.
- **Spores** are a type of reproductive propagule fungi produce. They are often resistant to conditions that would kill the hyphae.
- **Conidiophores** are a specialized type of hyphae where spores called conidia are formed. Both *Paecilomyces variotii* and *Ulocladium chartarum* produce their spores from conidiophores.
- **Ascocarps** are spore producing structures produced by certain groups of fungi. The spores produced by this type of structure are called ascospores. *Chaetomium globosum* produce their spores in ascocarps.

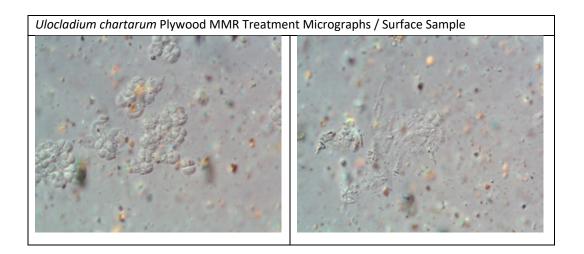
*Please note the same positive control micrographs are used to compare to each of the treated samples. All micrographs were taken at 400x magnification using an Olympus BX50 microscope and an Olympus DP10 camera. Brightfield or Differential Interference Contrast (DIC) microscopic techniques were used for best results.



MMR Treatment - Plywood – Ulocladium chartarum				
Pre-treatment	Post-treatment			

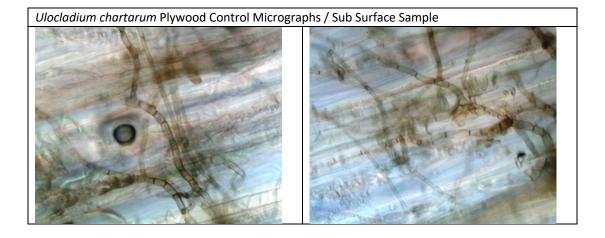






• **Observations of post-treated sample:** Extensive destruction and bleaching of the hyphae and conidiophores was observed. Spore morphology has been distorted and completely bleached out.

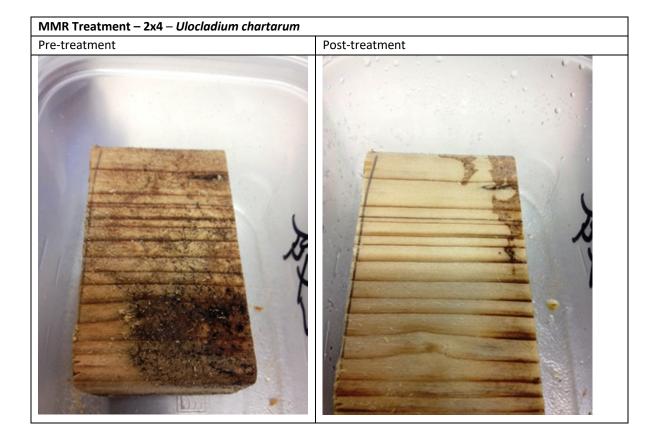




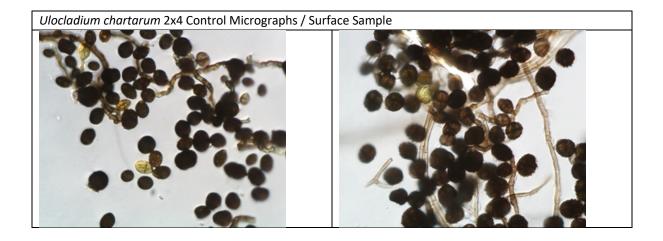


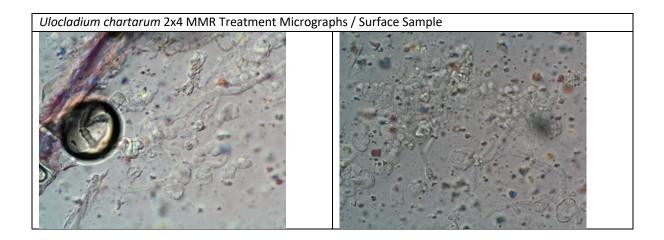
• **Observations of post-treated sample:** Extensive destruction of hyphae was observed. Most hyphae not destroyed was bleached out.





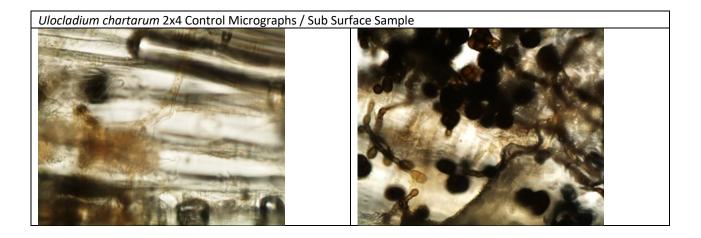


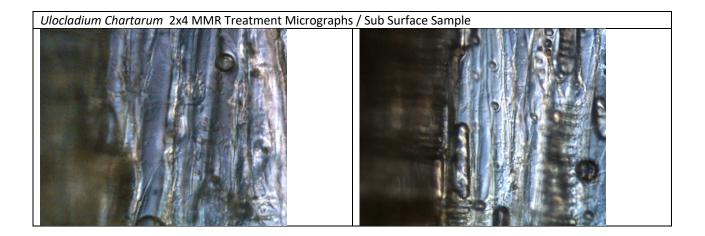




• **Observations of post-treated sample:** Extensive destruction and bleaching of hyphae and conidiophores was observed. Spore morphology was distorted and completely bleached out.

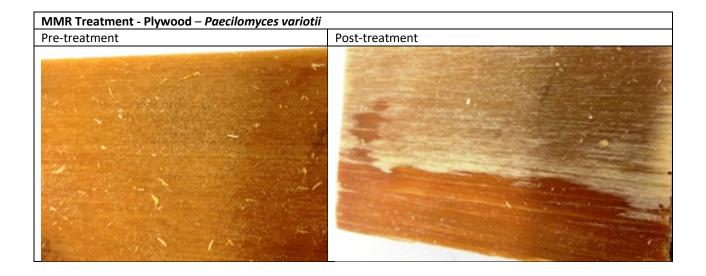




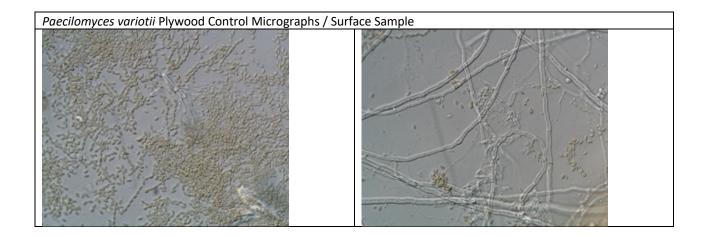


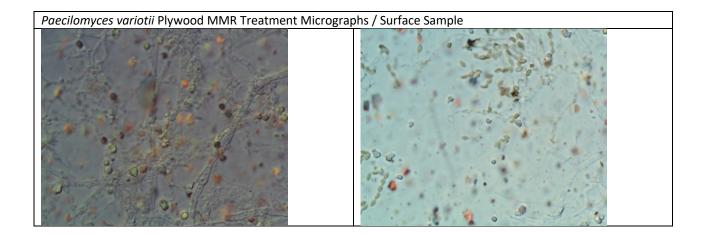
• Observations of post-treated sample: Could not detect any subsurface hyphae.





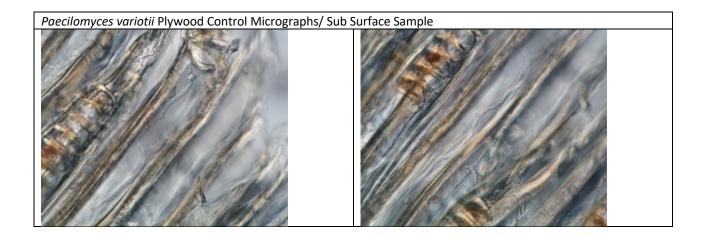


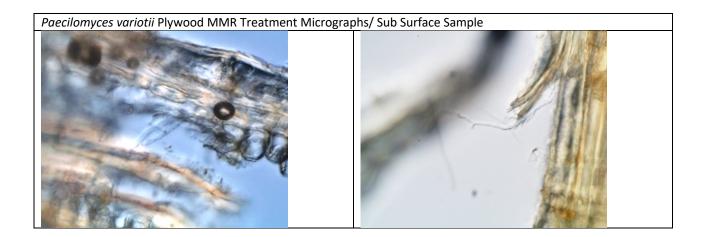




• **Observations of post-treated sample:** Extensive destruction of hyphae and conidiophores was observed. Hyphae and conidiophores that did remain intact showed a distorted morphology. Spore morphology remained consistent with the control.

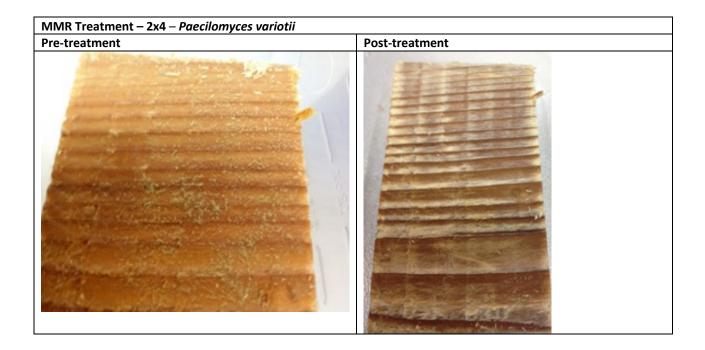




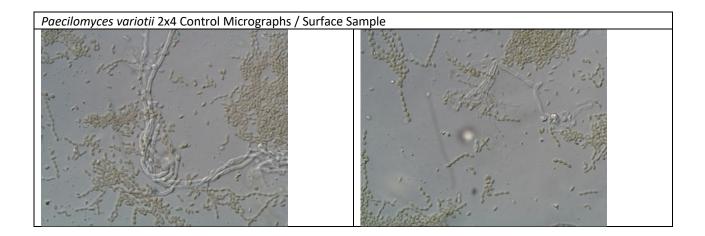


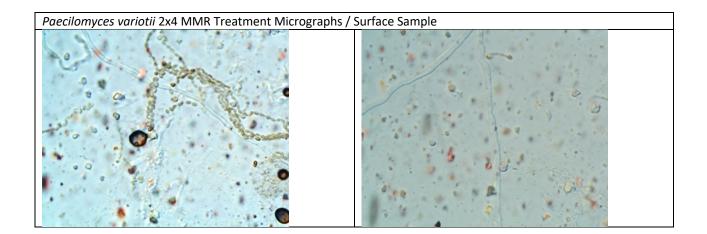
Observations of post-treated sample: Subsurface hyphae was hard to detect on post-treated sample. The hypha that was detected appeared fragmented.





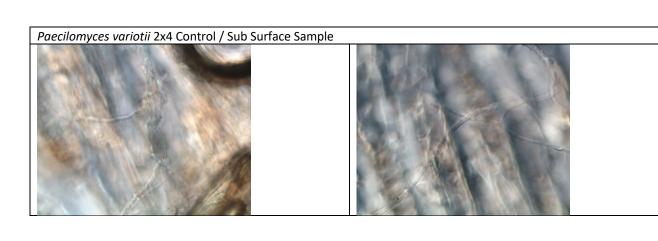


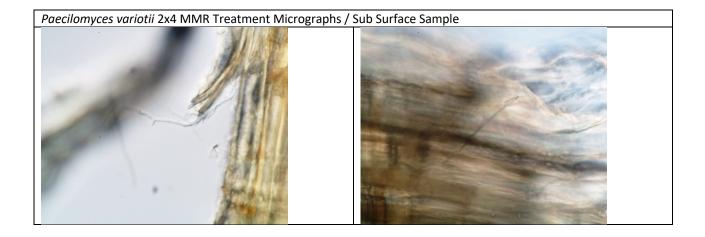




• **Observations of post-treated sample:** Extensive destruction of the hyphae and conidiophores was observed. Hyphae and conidiophores that did remain intact showed a distorted morphology. Spore morphology remained consistent with the control.





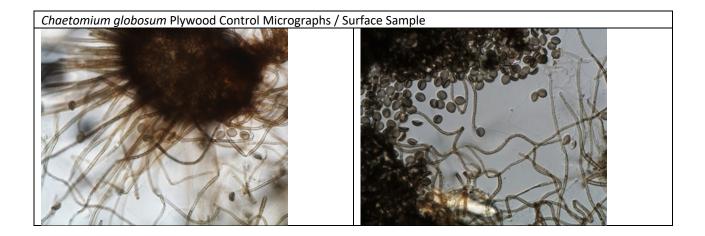


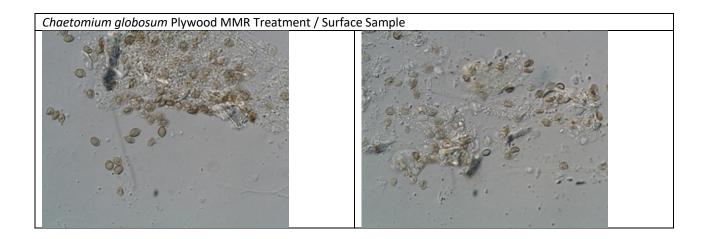
Observations of post-treated sample: Subsurface hyphae was hard to detect on post-treated sample. The hypha that was detected appeared fragmented.





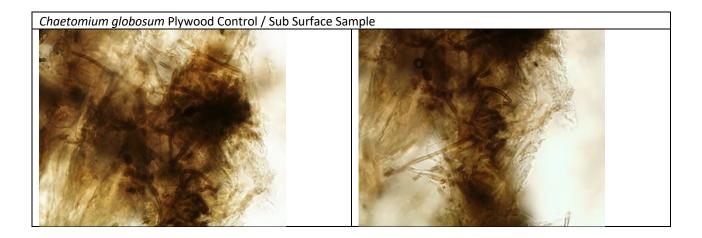


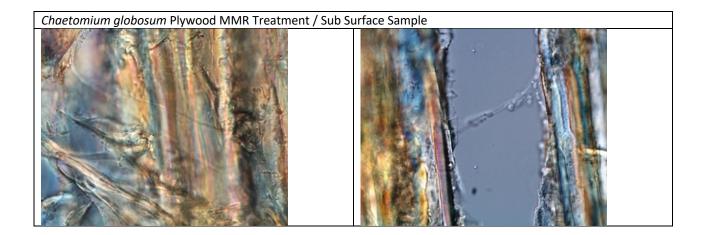




Observations of post-treated sample: Extensive destruction of ascocarps and hyphae was observed. Most spores were partially to completely bleached out and many spores showed an atypical morphology.





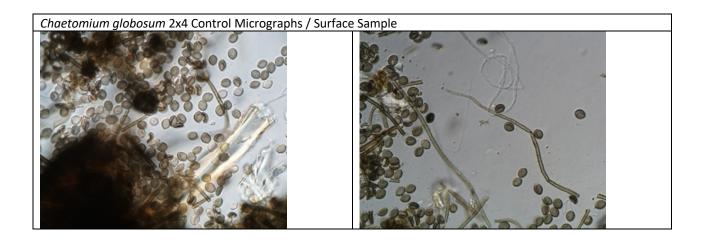


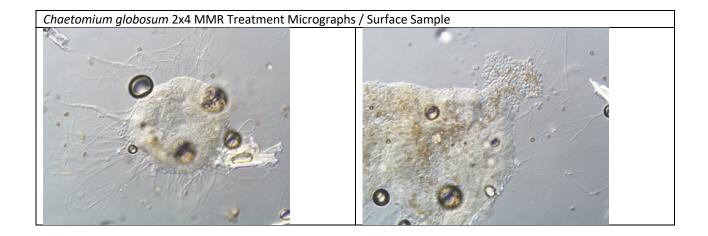
Observations of post-treated sample: Extensive destruction of hyphae was observed. The hyphae that remained showed fragmentation. Ascocarps embedded in the wood below the surface were completely bleached out.





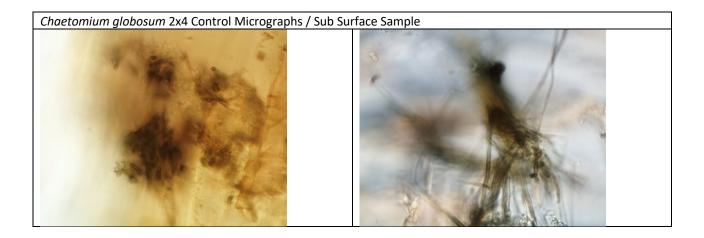






Observations of post-treated sample: Extensive destruction and bleaching of ascocarps and hyphae was observed. The spores showed extensive bleaching and extremely atypical morphologies.

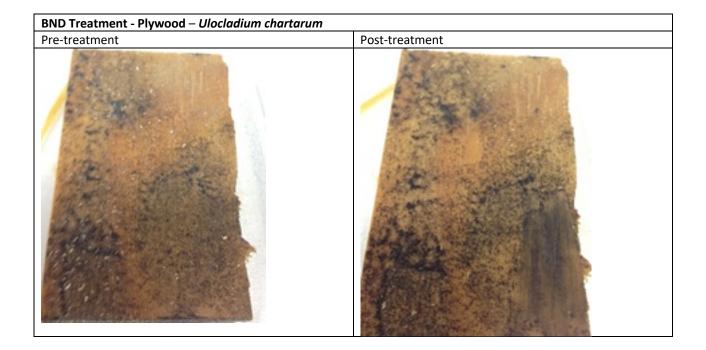






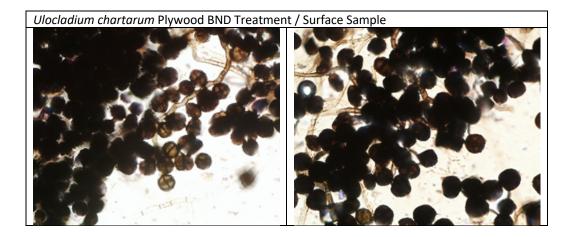
• Observations of post-treated sample: Could not detect any subsurface hyphae.



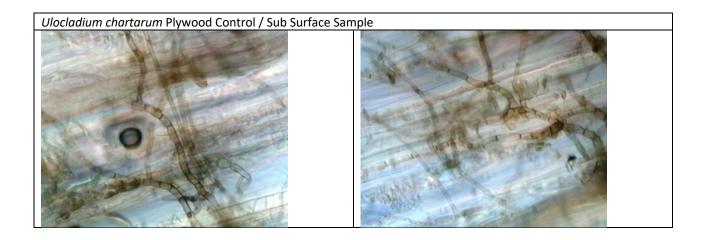




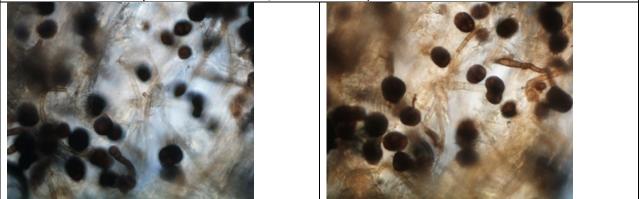




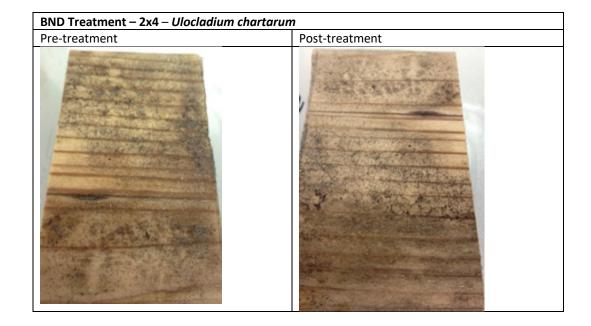




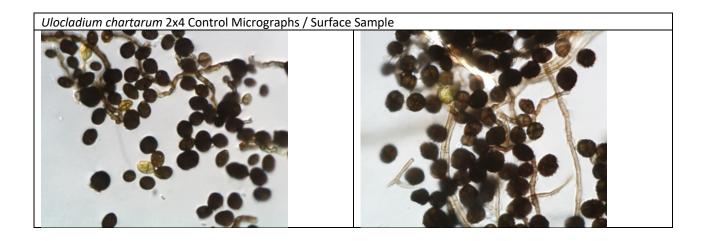
Ulocladium chartarum Plywood BND Treatment / Sub Surface Sample

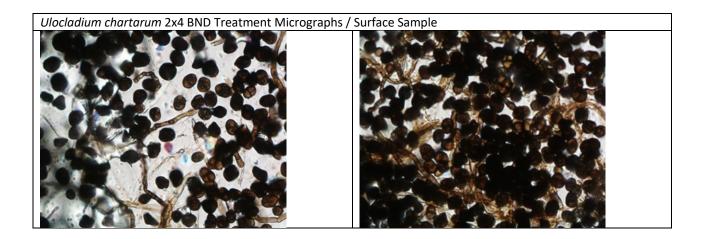




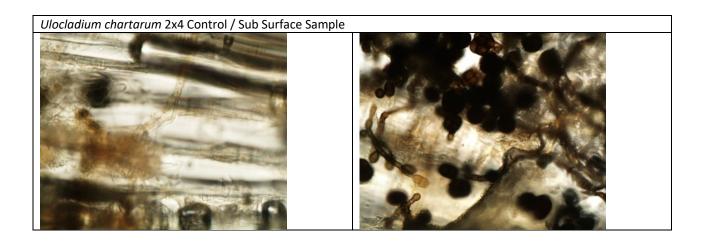


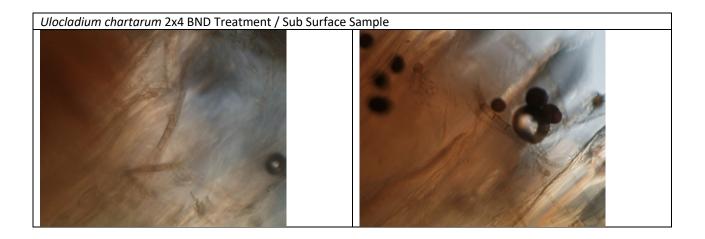




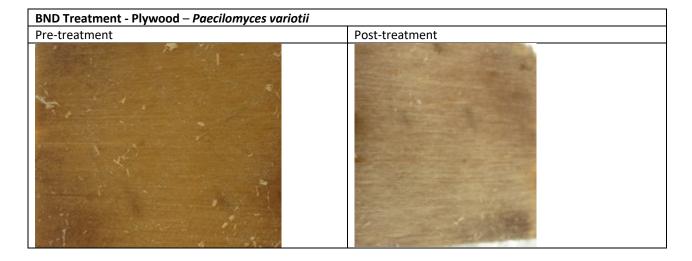




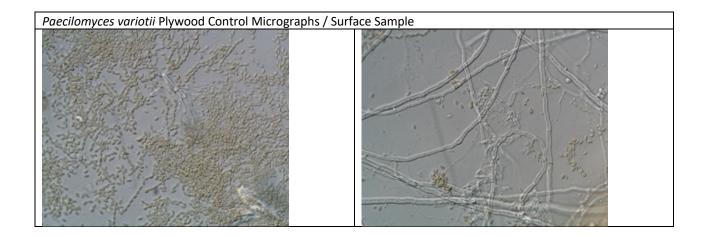


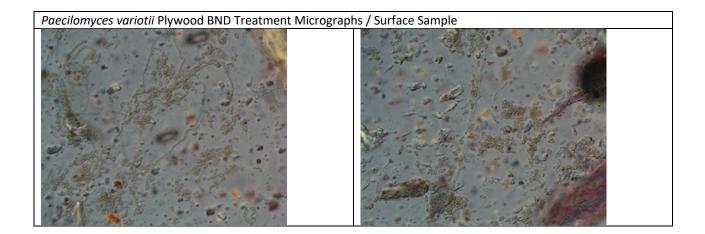






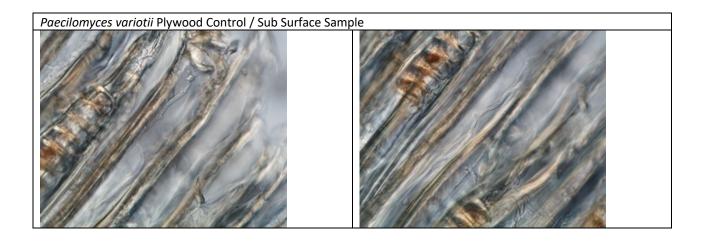


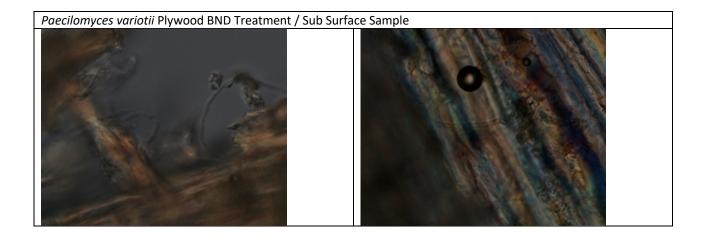




Observations of post-treated sample: Atypical hyphal and conidiophore morphology observed. Atypical spore morphology was also observed. The spores appeared to have collapsed somewhat.

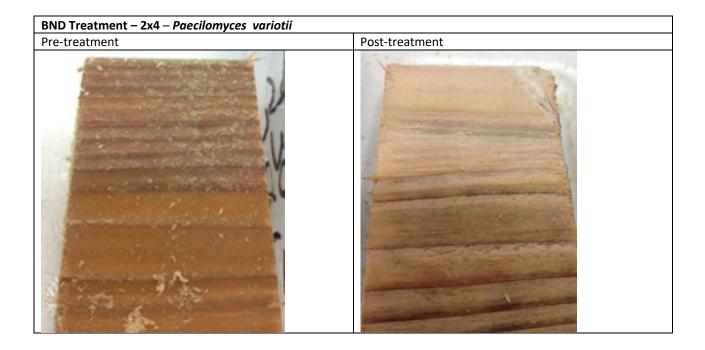




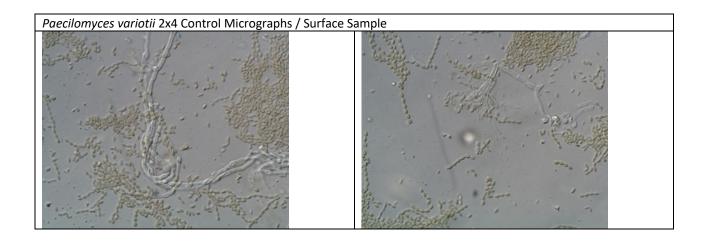


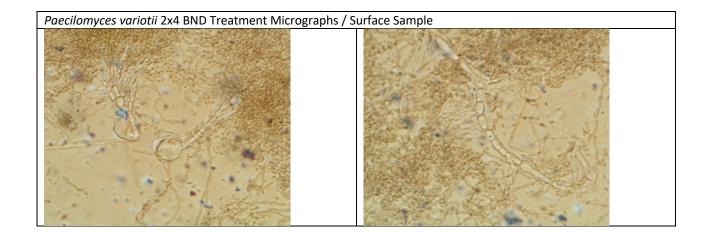
Observations of post-treated sample: Hyphae appeared similar to the control.





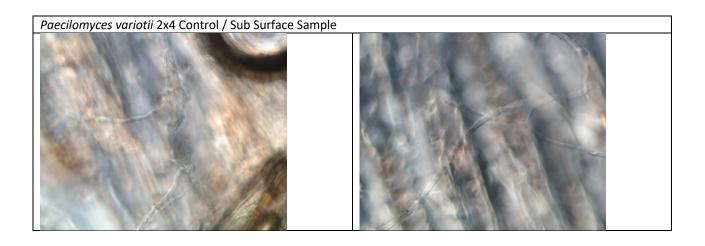


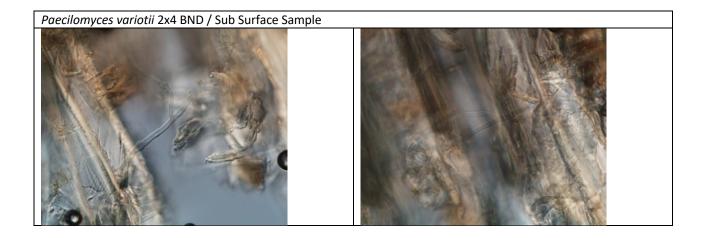




Observations of post-treated sample: Atypical hyphal and conidiophore morphology was observed, including swelling and fragmentation. Atypical spore morphology observed in that the spores seemed to have collapsed somewhat.





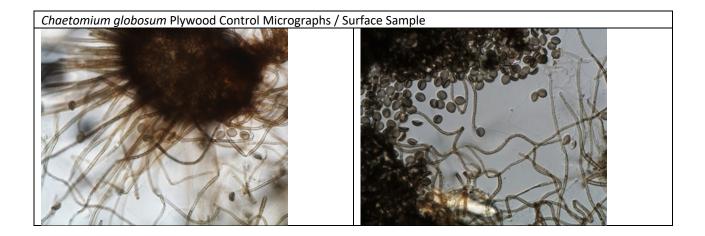


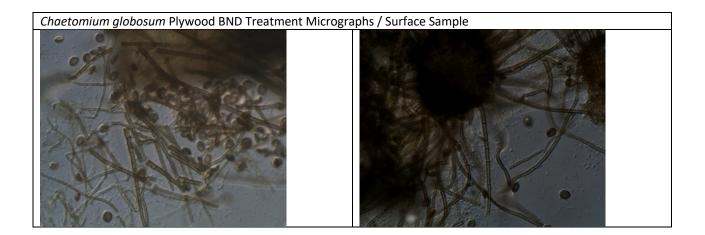
Observations of post-treated sample: Hyphae appeared similar to the control.



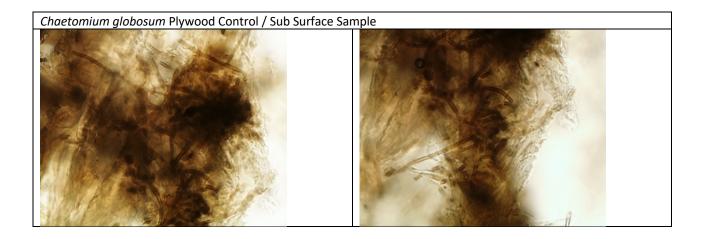


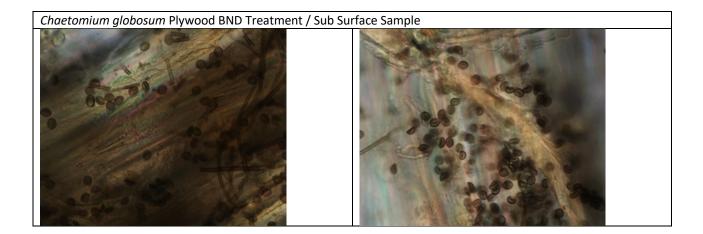








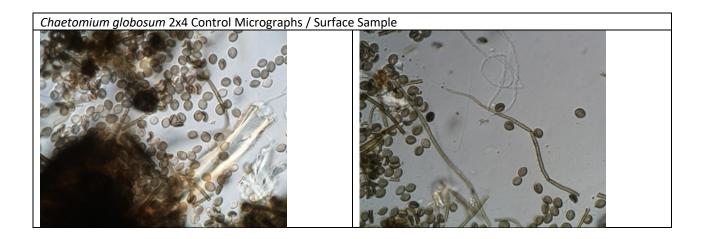


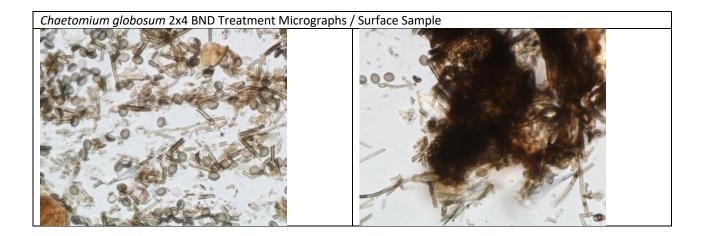




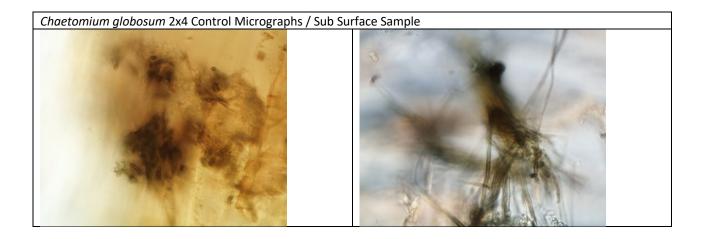


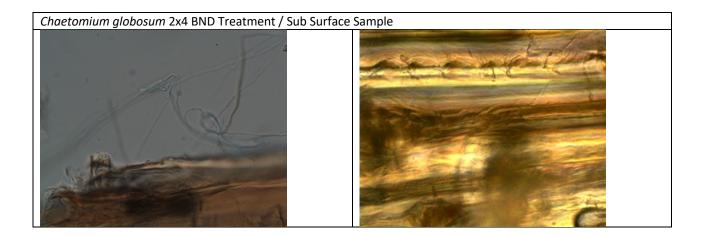








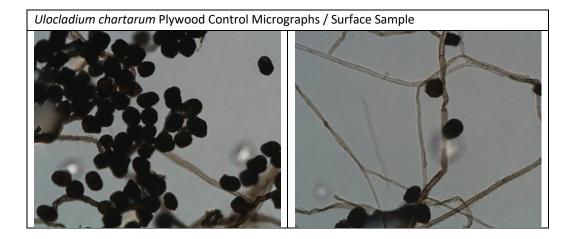


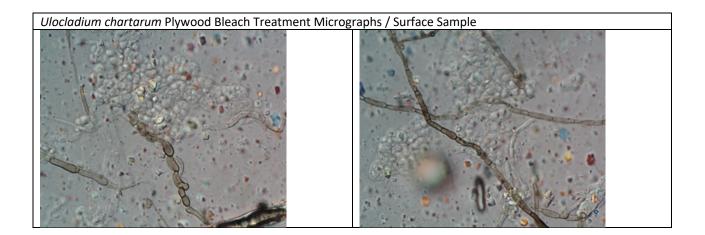






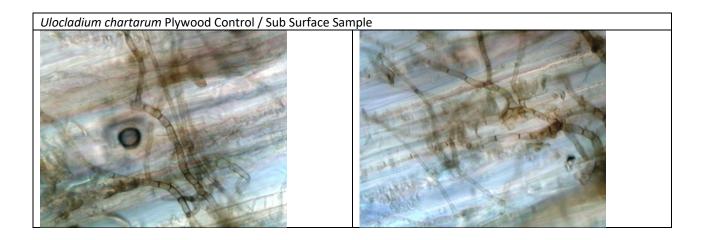


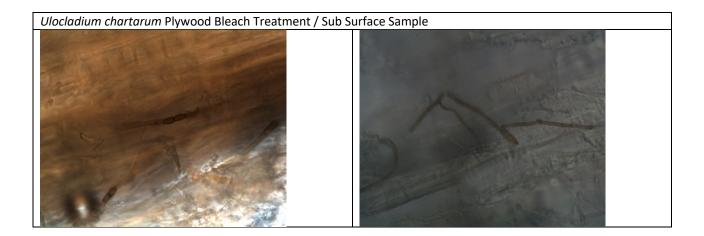




• **Observations of post-treated sample:** Extensive destruction and partial bleaching of the hyphae and conidiophores was observed. Many hyphal elements did however remain intact and unbleached. Spore morphology was distorted and the vast majority of spores were completely bleached out.







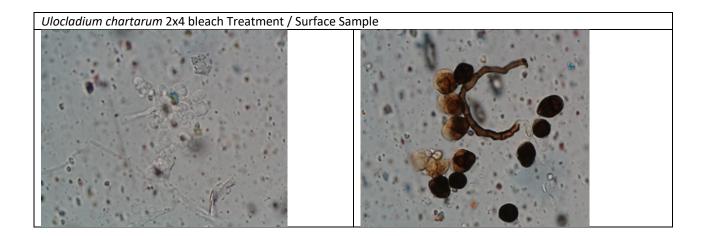
• **Observations of post-treated sample:** Hyphae below the surface growth appeared similar to control samples.





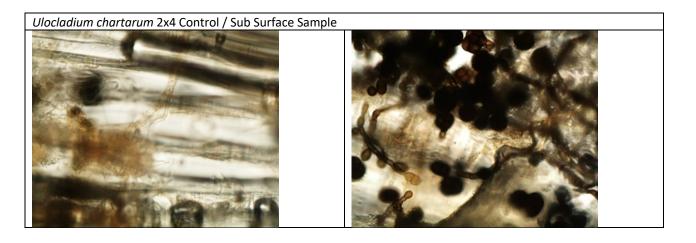


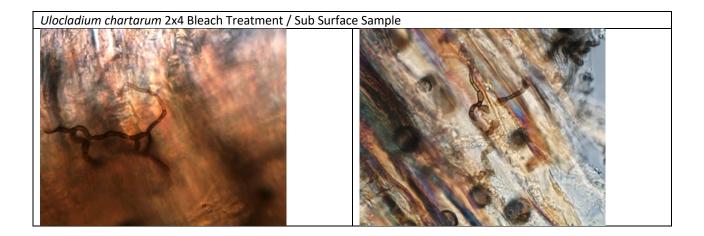




• **Observations of post-treated sample:** Extensive destruction and partial bleaching of the hyphae and conidiophores was observed. Many hyphal elements did however remain intact and unbleached. Spore morphology was distorted and the vast majority of spores were partially to completely bleached out.

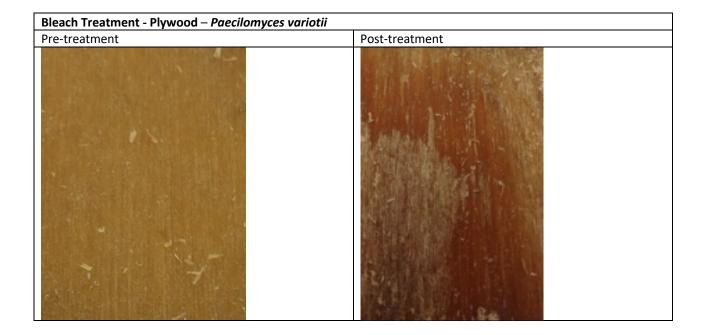




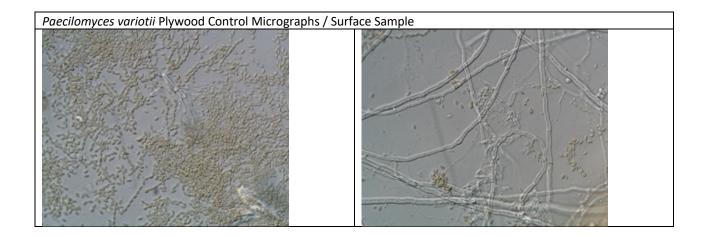


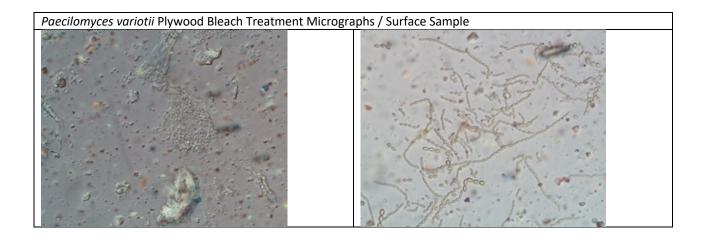
• **Observations of post-treated sample:** Hyphae below the surface growth appeared similar to control samples.





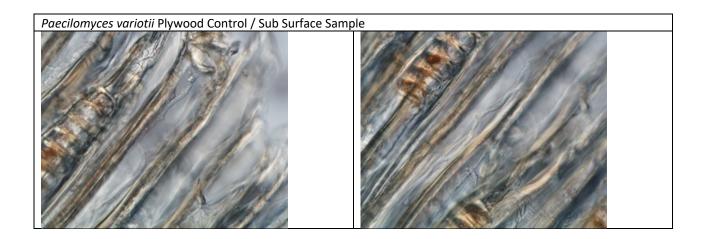


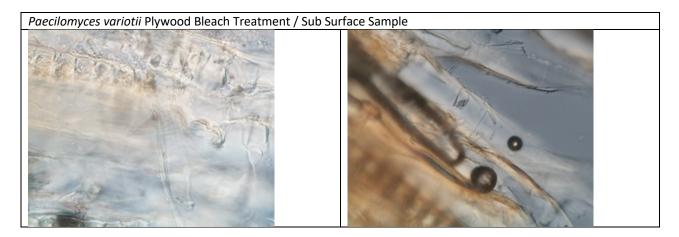




• **Observations of post-treated sample:** Extensive destruction of conidiophores and hyphae was observed. Spore morphology remained consistent with the control.

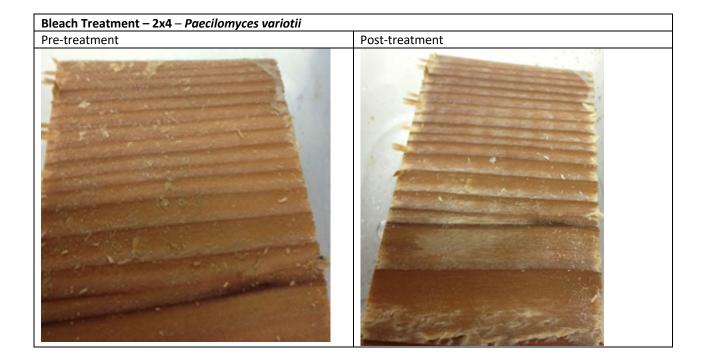




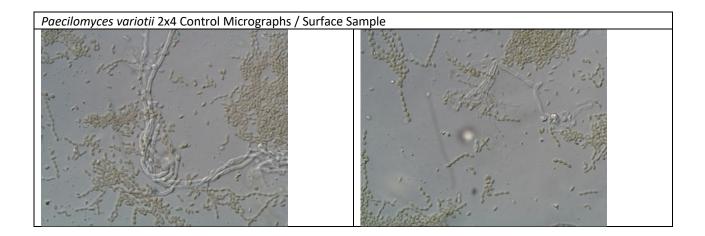


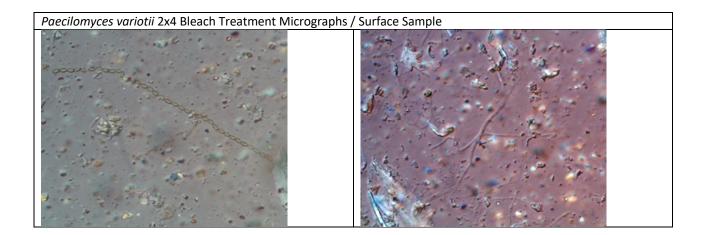
• **Observations of post-treated sample:** Hyphae below the surface growth appeared similar to control samples.











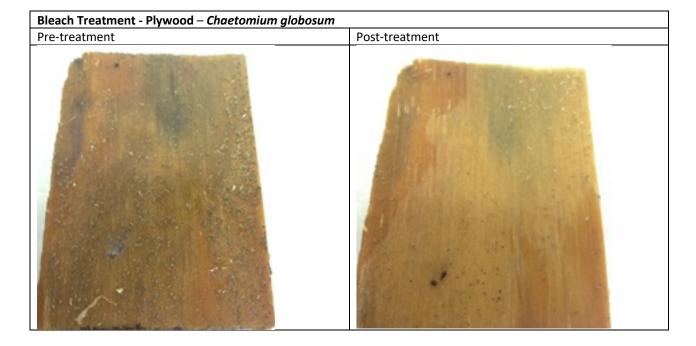
Observations of post-treated sample: Extensive destruction of hyphae and conidiophores was observed. Spores appeared very similar to control sample.



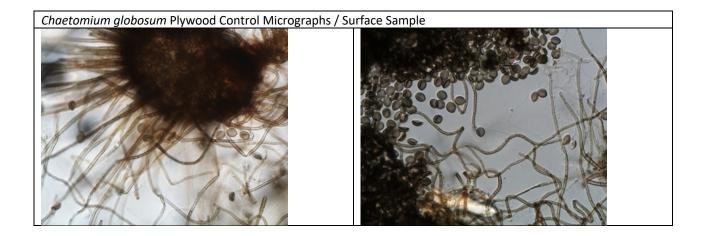


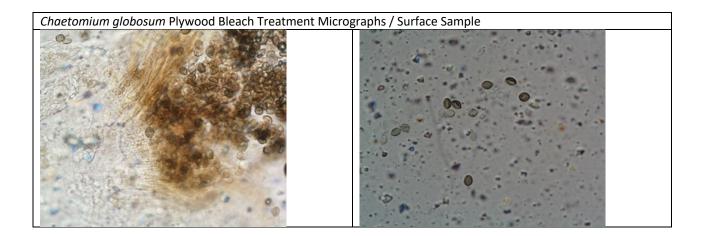
Observations of post-treated sample: Subsurface hyphae were hard to detect. The hyphae that was detected appeared similar to the control.





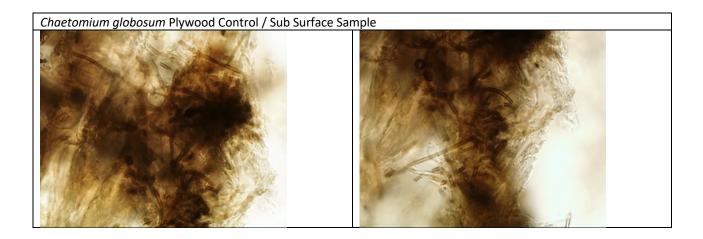


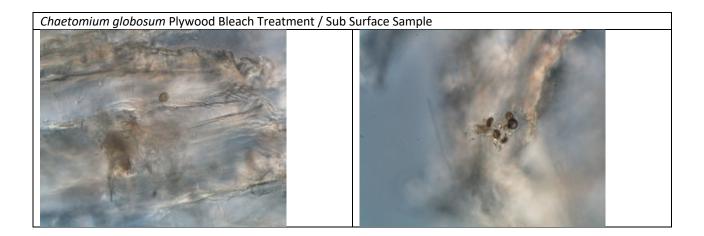




Observations of post-treated sample: Extensive destruction of ascocarps and partial bleaching of ascocarps was observed. Extensive destruction of hyphae was observed. Many spores were bleached out; however many remained very similar to control.





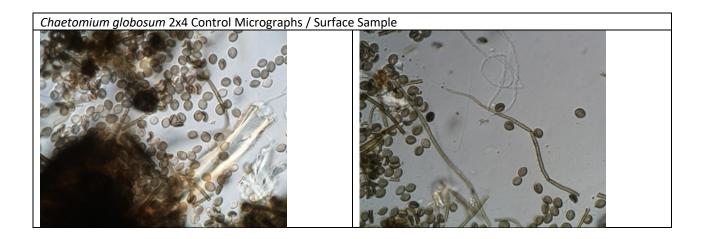


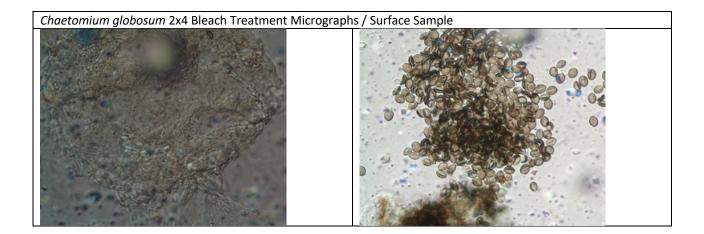
• **Observations of post-treated sample:** Subsurface hyphae were hard to detect. The hyphae that were detected appeared similar to the control.





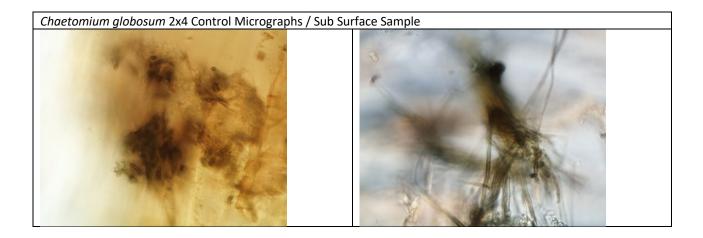


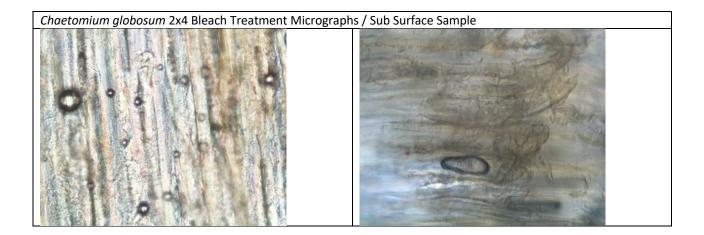




• **Observations of post-treated sample:** Extensive destruction of ascocarps and partial bleaching of ascocarps was observed. Extensive destruction of hyphae was observed. Many spores were bleached out; however many remained very similar to control.







Observations of post-treated sample: Could not detect any subsurface hyphae.



Overall conclusions and discussion of the morphological evaluation:

The post-treatment MMR macroscopic photos show that the wood appeared clean with little or no visible sign of fungal contamination. The micrographs show that the MMR is extremely penetrative and destructive to all fungal elements. MMR also appears to penetrate the wood and destroy subsurface mycelium.

The post-treatment bleach macroscopic photos show that the wood appeared much cleaner than pre-treatment; however evidence of fungal growth was apparent. The micrographs show that the bleach was extremely destructive to the surface growth, but the bleaching of surface growth was not as complete as MMR. The bleach did not appear to penetrate into the subsurface growth with the same penetrative power of the MMR and much of the subsurface growth remained unbleached and very similar to the control samples.

The post-treatment BND macroscopic photos show that the wood appeared just as contaminated post-treatment. The micrographs show little to no visible destruction of the surface growth or subsurface growth.



Glossary:

Moist Chamber: A water tight container used to keep the samples in question at 100% humidity. In the case of this experiment chemically sanitized and UV irradiated food storage containers.

Confluent growth: Growth over the entire surface of the sample

Sporulation: Production of spores by the fungi.

Spores: Reproductive propagules.

Inoculants: Spore suspensions of the fungi used in the testing.

Malt Extract Agar (MEA): A common malt based media used for the cultivation of many common environmental fungi.

Vortexed: Mixed vigorously using a type of laboratory equipment specifically designed to thoroughly mix solutions.

Negative controls: Un-inoculated samples. Samples that were never inoculated with fungi nor treated with any of the testing solutions.

Positive controls: Samples that were inoculated with the fungal suspensions, but never treated with any of the testing solutions.

Serial dilution: A process by which the sample is diluted in repeated steps to ascertain the concentration of an organism or organisms in question. In the case of this experiment the concentration of the original spore suspension.

References:

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<u>G. S. de Hoog</u> (Editor), <u>M. J. Figueras</u> (Editor), <u>Gene J. Cuarro</u> (Editor) Atlas of Clinical Fungi / 2nd Edition, Centraalbureau voor Schimmelcultures, 2000

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