

**Evaluation of New PureSpace® Formulations Developed for  
Global Prevention Services for Mold Control Applications**

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## INTRODUCTION

Global Prevention Services, Inc. ('GPS'), headquartered in Scottsdale, Arizona, is a company actively involved in developing products and services for controlling microbial growth in residential, institutional and commercial buildings. In their efforts to improve the spectrum of microbial growth mitigation products, the company recently developed new formulations for interior remediation and prevention use. A study was performed to evaluate these new formulations against three fungi (*Aureobasidium pullulan*, *Aspergillus niger* and *Penicillium*) recommended in the ASTM method D3273-standard test method for resistance to growth of mold on the surface of interior coatings in an environment. The microbial challenge studies were conducted at the National Science Foundation Water Quality Center at Arizona State University. The method is used for the accelerated evaluation of interior coatings to mold growth on construction material.

Research was conducted in controlled environmental chambers constructed according to ASTM standard, and temperature and relative humidity were monitored regularly. Experiments were designed to determine the ability of the new formulations to deflect fungal growth and colonization. The objective of this study was to verify the proof of concept for microbial inactivation capabilities of the new formulations on pinewood.

## MATERIAL AND METHODS

### Experimental Details

In their effort to develop microbial growth mitigation products for building/construction application, GPS provided multiple formulations in three batches. Independent experiments were performed to test the formulations in each batch. All the experiments were performed according to ASTM standard method D3273 as described below and results are presented in the results section of this report.

### Test Microorganisms

Pure cultures of *Aureobasidium pullulan* (ATCC 9348), *Aspergillus niger* (ATCC 6275) and *Penicillium* (ATCC 12667) were obtained from American Type Culture Collection (ATCC, Manassas, VA). Standard aseptic procedures as described in the Standard Methods for the Examination of Water and Wastewater, 19<sup>th</sup> Edition were followed for culture maintenance, and propagation. The fungal cultures were maintained on culture media recommended by ATCC using standards methods (Table1). All the cultures were propagated using potato dextrose agar medium as recommended by ATCC.

Table 1. Fungal isolates, culture media and growth conditions used in the study

<b>Fungal Isolates</b>	<b>ID</b>	<b>Growth Medium</b>	<b>Growth conditions (°C)</b>
<i>Aureobasidium pullulan</i>	ATCC 9348	Potato dextrose agar	25
<i>Aspergillus niger</i>	ATCC 6275	Potato dextrose agar	25
<i>Penicillium</i>	ATCC 12667	Potato dextrose agar	25

## Test Material

Ponderosa pine sapwood panels (3 x 4 inch), free of resin and knots were prepared as described in the ASTM D 3273. The panel surface was smooth on all sides. Coupons were dried till they weighed between 6 to 7 grams per square inch.

The new formulations were received at GPS and were handled by their field staff according their standard operating procedure. The coupons were taken to the GPS office and were treated with the new formulations and under the control of laboratory staff at all times (Table 2). After treatment, coupons were air dried. The dried coupons were packed in separate envelopes by laboratory staff and transported to test chamber. In this study, non-treated coupons of pinewood were included as control samples.

Table 2. Description of antifungal formulations applied on pinewood coupons used in the study

Experiment	Treatment #	Formulation Description *	Number of pinewood coupons treated
1 (using first batch of formulations)	1	Interior Coating	3
	2	New Construction Coating 1	3
	3	New Construction Coating 2	3
	Control	No treatment	3
2 (using second batch of formulations)	1	Interior Coating v.2	3
	2	Interior Coating v.3	3
	3	Interior Coating v.4	3
	Control	No treatment	3
3 (using third batch of formulations)	1	Interior Coating v.5	3
	2	Interior Coating v.6	3
	3	Interior Coating v.7	3
	Control	No treatment	3

\* Additional details on these formulations can be obtained directly from GPS

## **Environmental Chamber**

A small environmental chamber was built according to the ASTM standard method D3273. The environmental chamber was built using a polypropylene tank, which contained water at the bottom (Figure1). A pitched top (cover) with straight sides was constructed using acrylic plastic sheet (Figure2). The cover was designed so that moisture condensation will run down the sides and would not drip on the samples. A plastic tray containing potting soil was positioned 25 mm above the water level. Soil was inoculated with the spores and vegetative propagules (source of reproduction) of all three fungal isolates. Water was heated using a thermostatically controlled heater. Inside the chamber, temperature and relative humidity was maintained at 90°F ( $\pm 2^\circ\text{F}$ ) and 96% ( $\pm 2\%$ ), respectively. A digital thermometer/hygrometer was used to monitor these parameters. Before placement of sample, chamber was operated for one month (acclimatization period) to ensure that fungal isolates have fully colonized the potting soil bed. At the end of acclimatization period, samples (treated and on treated wood panels) were vertically hung with the bottom three inches above the inoculated soil.

The environmental chamber provides optimum conditions for continuous inoculation of the surfaces of test coupons with mold spores, which emulate the real world conditions expected in an indoor environment.

## **Test Conditions**

In order to test the ability of new formulations to deflect fungal growth, pine wood coupons treated with the new formulations were placed in the environmental chamber.

The chamber represents the conditions highly favorable for the translocation of fungal isolates from soil bed to wood coupons. During testing, the conditions in the environmental chamber were such that are ideal for fungal growth. The treated and non-treated coupons were incubated in the environmental chambers for four weeks (Figure 3). The fungal growth on treated and control coupons was recorded according to ASTM method 3274 on weekly basis.

## **Assessment of Fungal Growth**

Fungal growth on test and control coupons was rated based on a set of photographic standards published in ASTM D-3274, Test Method for Evaluating the Degree of Surface Disfigurement of Paint Films by Microbial (Fungal or Algal) Growth or Soil and Dirt Accumulation. Under properly operating conditions, control samples should develop a 4 to 6 mold growth rating within 2 to 3 weeks.

On ASTM D3274, a score of 10 means no fungal growth, while zero means maximum fungal growth. A rating of 5 or less on ASTM D 3274 is defined as a failure. Performance at a certain rating (in accordance with ASTM D3273 and D3274) does not imply any specific time period of fungal free coating. However, a better rated coating nearly always performs better in actual end use.

## RESULTS

The temperature and humidity were monitored daily and fungal growth was recorded on a weekly bases. All sides of treated and non treated wood panels were visually inspected for fungal growth and ranked on a 0-10 scale according to the ASTM D 3274. Table 3 shows the progression of fungal growth on treated and non-treated (control) coupons of pine wood. For weekly observations, samples were removed from the environmental chamber and each sample was visually examined for traces of mold and subsequently rated based on the percent area of the coupon then covered by mold growth. If no growth or traces of fungal growth was observed, samples were then further examined under microscope. After visual observation each sample was returned to the environmental chamber.

### Experiment-1

The pine wood coupons treated with formulations Interior Coating, New Construction Coating 1, and New Construction Coating 2 received an ASTM D3274 rating of 0, 8 and 10, indicating a passing rating for New Construction Coating 1 and no visible mold growth on the sample treated with Construction Coating 2, also a passing rating. The non-treated coupons (controls), all each received a rating of zero, which indicates excessive fungal growth on all the coupons' surface as expected. The Interior Coating failed this experiment. (Table 3).

Table 3. Description of fungal growth on pinewood coupons in the first experiment of this study

	Treatment	Reading (0-10)
1 <sup>st</sup> week	Interior Coating	10
	New Construction Coating 1	10
	New Construction Coating 2	10
	Control	8
2 <sup>nd</sup> week	Interior Coating	9
	New Construction Coating 1	10
	New Construction Coating 2	10
	Control	6
3 <sup>rd</sup> week	Interior Coating	4
	New Construction Coating 1	9
	New Construction Coating 2	10
	Control	2
4 <sup>th</sup> week	Interior Coating	0
	New Construction Coating 1	8
	New Construction Coating 2	10
	Control	0

Note: Red color indicates mold growth at unacceptable levels as specified in ASTM D 3274

## Experiment-2

The pine wood coupons treated with Interior Coating versions 2, 3 and 4 received an ASTM D3274 rating of 0, 0, 0 and 0, indicating excessive fungal growth on all over the coupons surface. The non-treated coupons (controls) also received a rating of zero, which indicates excessive fungal growth on all over the coupons surface (Table 4). All the formulation tested in this experiment failed to provide protection against fungal growth under the condition specified in ASTM method 3273.

Table 4. Description of fungal growth on pinewood coupons in the second experiment of this study

	Treatment	Reading (0-10)
1 <sup>st</sup> week	Interior Coating v.2	10
	Interior Coating v.3	10
	Interior Coating v.4	10
	Control	9
2 <sup>nd</sup> week	Interior Coating v.2	8
	Interior Coating v.3	9
	Interior Coating v.4	8
	Control	7
3 <sup>rd</sup> week	Interior Coating v.2	4
	Interior Coating v.3	5
	Interior Coating v.4	4
	Control	3
4 <sup>th</sup> week	Interior Coating v.2	0
	Interior Coating v.3	0
	Interior Coating v.4	0
	Control	0

Note: Red color indicates mold growth at unacceptable levels as specified in ASTM D 3274

## Experiment-3

The temperature and humidity was monitored daily and fungal growth was recorded on a weekly bases. All sides of treated and non treated wood panels were visually inspected for fungal growth and ranked on a 0-10 scale according to the ASTM D 3274. Table 5 shows the progression of fungal growth on treated and non-treated (control) coupons of pine wood. For weekly observations, samples were removed from

the environmental chamber and each sample was visually examined for traces of mold and subsequently rated based on the percent area of the coupon then covered by mold growth. If no growth or traces of fungal growth was observed, samples were then further examined under microscope. After visual observation each sample was returned to the environmental chamber.

The pine wood coupons treated with Interior Coating versions 5, 6 and 7 received an ASTM D3274 rating of 1, 9 and 0, indicating no visible mold growth on sample treated with Interior Coating v.6. The non-treated coupons (controls), and coupons treated with formulation versions # 5 and 7 received a rating of zero, one and zero, respectively, which indicate heavy mold growth on these coupons. A rating of 5 or less on ASTM D 3274 is defined as a failure.

Based on the results of this study, pine wood panels treated with formulation Interior Coating v.6 provided excellent resistant to the growth of mold and mildews under the testing conditions. Construction material with different organic mater contents, such as paper, and dry wall, may show different mold growth potential under the test conditions.

Table 5. Description of fungal growth on pinewood coupons used in the study

	Treatment	Reading (0-10)
1 <sup>st</sup> week	Interior Coating v.5	10
	Interior Coating v.6	10
	Interior Coating v.7	9
	Control	8
2 <sup>nd</sup> week	Interior Coating v.5	9
	Interior Coating v.6	10
	Interior Coating v.7	8
	Control	5
3 <sup>rd</sup> week	Interior Coating v.5	5
	Interior Coating v.6	9
	Interior Coating v.7	4
	Control	2
4 <sup>th</sup> week	Interior Coating v.5	1
	Interior Coating v.6	9
	Interior Coating v.7	0
	Control	0

Note: Red color indicates mold growth at unacceptable levels as specified in ASTM D 3274

## CONCLUSIONS

When tested as described in ASTM method D3273, formulations: **Interior Coating v.6**, **New Construction Coating 1** and **New Construction Coating 2** (at concentration and application technique known to GPS) positively controlled the fungal growth on pine wood coupons during the one month prescribed testing period and passed the ASTM rating criteria. All of the controls for this experiment also met the strict criteria established for a valid test. These conclusions are based on observed data.

Table 6. Summary of final ratings for successful coating used in the study

Experiment	Formulation Description	Reading (0-10)
1	New Construction Coating 1	8
	New Construction Coating 2	10
3	Interior Coating v.6	9

A rating above 5 on ASTM D 3274 is defined as a success or pass.



Figure 1. Front view of environmental chamber



Figure 2. Side view of environmental chamber



Figure 3. Test and control coupons incubated in environmental chamber

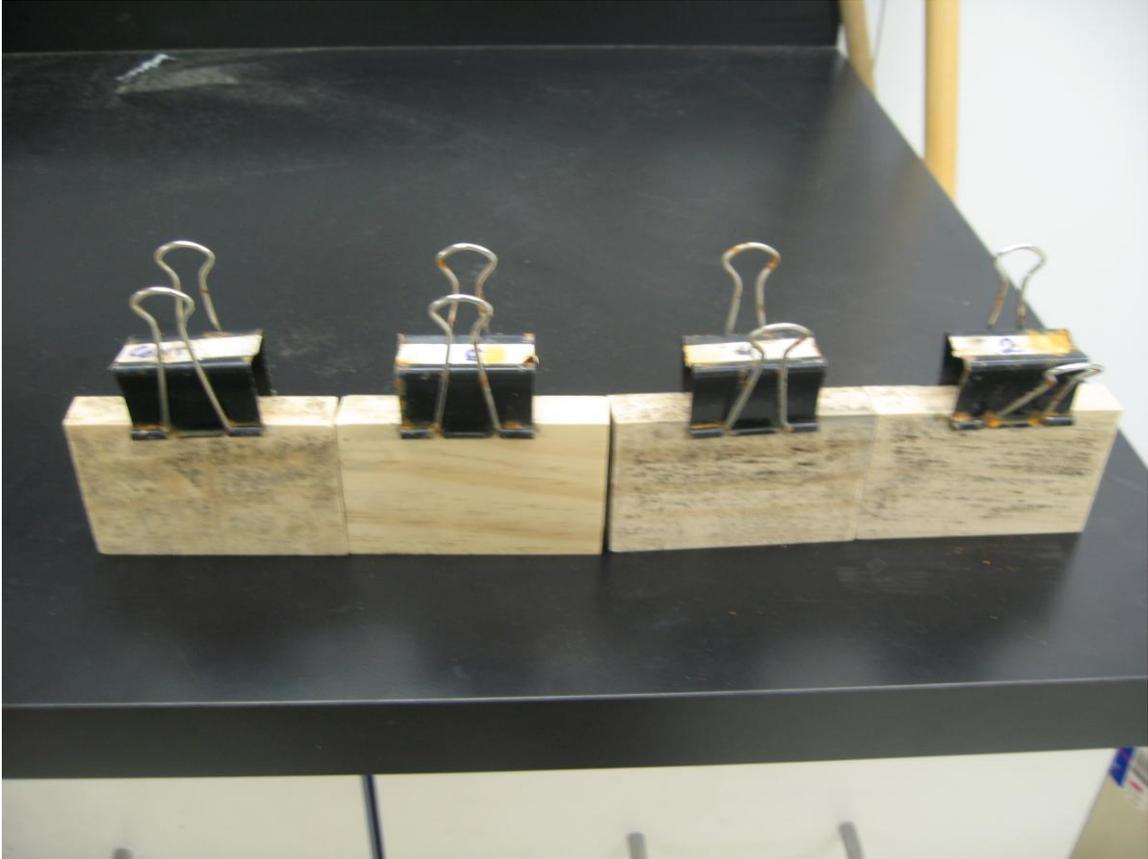


Figure 4. Fungal growth on non-treated (control) and treated coupons after four weeks of incubation in environmental chamber

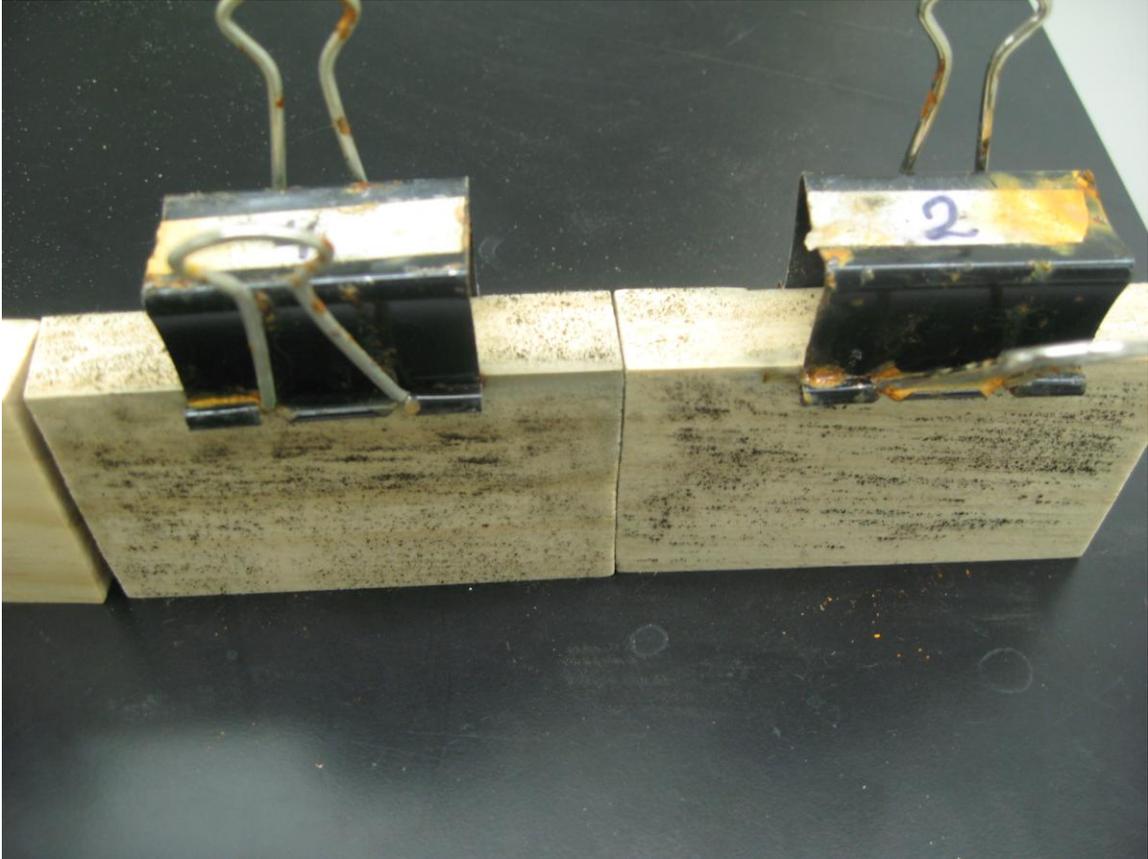


Figure 5. Fungal growth on coupons after four weeks of incubation in environmental chamber