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Microbial Control

Laboratory Report

Extended Dry-Film Fungal Optimization Study for Finishes Unlimited Clear and White Coatings

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Summary

Finishes Unlimited submitted preserved and unpreserved samples of a clear and white coating for dry-film fungicide efficacy evaluations by ASTM D-5590. The preserved samples contained DCOIT. The samples were tested under leached and unleached conditions and monitored weekly for four weeks and then again at week 6 and week 8. The ASTM D-5590 is a four week test, which was extended to eight weeks at the request of Finishes Unlimited. The results indicate that a preservative is necessary to protect the clear and white coatings from fungal defacement. Dow Microbial Control evaluated varying levels of BIOBAN 200, containing 20% DCOIT active ingredient during the ASTM D-5590 study. After completion of the standard 4 week test period, all leached and unleached samples were protected at 0.56% BIOBAN 200 with the exception of one leached sample that was protected for three weeks. The use of the EPA label maximum of BIOBAN 200 is recommended for the coatings products:

- BIOBAN 200 at 0.56% (5,600 ppm) for protection of the white coating, and
- BIOBAN 200 at 0.56% (5,600 ppm) for protection of the clear coating.

Introduction

Finishes Unlimited submitted clear and white coating samples for dry-film fungicide evaluation by ASTM D-5590. Finishes Unlimited would like Dow Microbial Control to determine the level of BIOBAN 200 necessary for the protection of both coatings against fungal defacement under leached and unleached conditions.

Experimental Methods

Sample Sterility Test

The samples, as received, were streaked on tryptic soy agar (TSA) and Sabouraud dextrose agar (SDA) to test for bacterial and fungal contamination, respectively. Estimates of microorganism concentration were determined by the number of colonies that grew on the agar plate following an incubation at 30°C for 1 week (refer to Appendix I for microorganism scoring scale).

Dry-Film Fungal Test (ASTM D-5590)

The dry-film fungal test determines the resistance of a dried coating to attack by fungal organisms. The test begins by preparing draw downs of each sample on two sets of Leneta Chart paper and allowing them to air dry. One set of draw downs was leached in one-gallon jars under running distilled water (6 changes of water per day) for 24 hours and then air dried. The other set of draw downs remained at room temperature with no moisture exposure. This latter of draw downs was designated as unleached. Once prepared, the test substrate was placed on a solid agar medium and then contaminated with the appropriate fungal spores. The test substrate was incubated on the agar surface for 28 days at 30°C. Fungal growth inhibition was categorized according to a lack of fungal growth on the test substrate itself. Fungal growth was scored on a weekly basis over a 4 week incubation period. The test was extended, and growth was scored at week 6 and week 8. Ratings of 0 or 1, indicate effective dry-film preservation (refer to Appendix II for procedure details and an explanation of the fungal growth scoring scale).

Results

Sample Sterility Test. The samples were received for dry-film evaluations, and streaked on tryptic soy agar (TSA) and Sabouraud dextrose agar (SDA) to determine the level of bacterial and fungal contamination, respectively (Table 1). Refer to Appendix I for the microorganism scoring scale.

Table 1. Sample Sterility Test

SAMPLE	TSA	SDA	pH
White - Preserved	0	0	-
White - Unpreserved	0	0	8.09
Clear - Preserved	0	0	-
Clear - Unpreserved	0	0	8.08

Dry-Film Fungal Test (ASTM D-5590). The propensity of the clear and white coatings to support fungal growth was evaluated using the ASTM D-5590 method. Growth (or lack thereof) was observed and documented over eight weeks in unleached (Tables 2-3) as well as leached (Tables 4-5) conditions. Growth was rated on a scale from 0 to 4 for the clear coating (Tables 2 and 4) and white coating (Tables 3 and 5). Refer to Appendix II for details of the methodology and organisms used. The preserved samples, unpreserved samples, and samples generated by Dow Microbial Control using the unpreserved sample and BIOBAN 200 were evaluated during the study.

Table 2. Unleached dry-film fungicide efficacy evaluations of the clear coating samples against *A. niger* and *P. funiculosum* as well as *A. pullulans*.

ASTM D-5590 evaluations against <i>A. niger</i> and <i>P. funiculosum</i>						
SAMPLE	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 6	WEEK 8
BIOBAN 200 - 5600 ppm	0	1	1	1	1	2
BIOBAN 200 - 4000 ppm	0	1	1	2	2	2
BIOBAN 200 - 3000 ppm	2	3	3	4	4	4
BIOBAN 200 - 2000 ppm	2	3	3	4	4	4
BIOBAN 200 - 1200 ppm	1	3	3	4	4	4
Preserved	0	0	0	1	1	1
Unpreserved	2	3	4	4	4	4
ASTM D-5590 evaluations against <i>A. pullulans</i>						
SAMPLE	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 6	WEEK 8
BIOBAN 200 - 5600 ppm	1	1	1	1	1	1
BIOBAN 200 - 4000 ppm	1	2	2	2	3	3
BIOBAN 200 - 3000 ppm	1	2	2	4	4	4
BIOBAN 200 - 2000 ppm	3	3	3	4	4	4
BIOBAN 200 - 1200 ppm	1	3	3	4	4	4
Preserved	0	0	0	0	0	0
Unpreserved	2	3	4	4	4	4

Table 3. Unleached dry-film fungicide efficacy evaluations of the white coating samples against *A. niger* and *P. funiculosum* as well as *A. pullulans*.

ASTM D-5590 evaluations against <i>A. niger</i> and <i>P. funiculosum</i>						
SAMPLE	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 6	WEEK 8
BIOBAN 200 - 5600 ppm	0	0	0	0	0	1
BIOBAN 200 - 4000 ppm	1	1	1	1	1	2
BIOBAN 200 - 3000 ppm	1	1	1	2	2	3
BIOBAN 200 - 2000 ppm	1	1	1	2	2	2
BIOBAN 200 - 1200 ppm	3	4	4	4	4	4
Preserved	0	1	1	1	1	1
Unpreserved	3	4	4	4	4	4
ASTM D-5590 evaluations against <i>A. pullulans</i>						
SAMPLE	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 6	WEEK 8
BIOBAN 200 - 5600 ppm	0	1	1	1	1	2
BIOBAN 200 - 4000 ppm	1	1	1	1	2	2
BIOBAN 200 - 3000 ppm	1	2	2	2	3	4
BIOBAN 200 - 2000 ppm	1	2	3	3	3	4
BIOBAN 200 - 1200 ppm	2	3	3	4	4	4
Preserved	0	0	0	0	1	1
Unpreserved	2	4	4	4	4	4

Table 4. Leached dry-film fungicide efficacy evaluations of the clear coating samples against *A. niger* and *P. funiculosus* as well as *A. pullulans*.

ASTM D-5590 evaluations against <i>A. niger</i> and <i>P. funiculosus</i>						
SAMPLE	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 6	WEEK 8
BIOBAN 200 - 5600 ppm	0	1	1	2	2	2
BIOBAN 200 - 4000 ppm	1	2	2	3	3	3
BIOBAN 200 - 3000 ppm	1	3	3	4	4	4
BIOBAN 200 - 2000 ppm	2	3	3	3	4	4
BIOBAN 200 - 1200 ppm	2	2	2	3	4	4
Preserved	0	0	0	1	1	2
Unpreserved	4	4	4	4	4	4
ASTM D-5590 evaluations against <i>A. pullulans</i>						
SAMPLE	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 6	WEEK 8
BIOBAN 200 - 5600 ppm	0	0	0	1	1	2
BIOBAN 200 - 4000 ppm	2	3	3	3	3	4
BIOBAN 200 - 3000 ppm	1	1	2	2	2	3
BIOBAN 200 - 2000 ppm	2	3	3	3	3	4
BIOBAN 200 - 1200 ppm	2	3	3	4	4	4
Preserved	0	0	0	1	1	1
Unpreserved	1	3	4	4	4	4

Table 5. Leached dry-film fungicide efficacy evaluations of the white coating samples against *A. niger* and *P. funiculosus* as well as *A. pullulans*.

ASTM D-5590 evaluations against <i>A. niger</i> and <i>P. funiculosus</i>						
SAMPLE	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 6	WEEK 8
BIOBAN 200 - 5600 ppm	0	0	0	0	1	1
BIOBAN 200 - 4000 ppm	0	0	0	1	1	1
BIOBAN 200 - 3000 ppm	1	1	1	2	2	3
BIOBAN 200 - 2000 ppm	1	1	2	2	3	4
BIOBAN 200 - 1200 ppm	2	2	2	3	4	4
Preserved	0	0	0	1	1	1
Unpreserved	3	4	4	4	4	4
ASTM D-5590 evaluations against <i>A. pullulans</i>						
SAMPLE	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 6	WEEK 8
BIOBAN 200 - 5600 ppm	0	0	0	0	0	1
BIOBAN 200 - 4000 ppm	0	0	0	0	1	1
BIOBAN 200 - 3000 ppm	1	2	2	2	2	3
BIOBAN 200 - 2000 ppm	1	2	2	3	3	3
BIOBAN 200 - 1200 ppm	1	2	3	3	3	3
Preserved	0	0	0	0	1	1
Unpreserved	2	2	3	3	3	4

Recommendations and Conclusions

Sample Sterility Test (Table 1)

The samples as received did not contain detectable levels of bacterial or fungal contamination prior to the start of the biocide evaluations.

Dry-Film Fungal Test – ASTM D-5590 (Tables 2-5)

Unpreserved samples:

The leached condition tested in Tables 4 and 5 is a harsher test environment than the unleached condition shown in Tables 2 and 3. The unpreserved clear and white coatings both showed fungal susceptibility during the study. These results indicate that a dry-film fungicide is necessary to protect the coatings from fungal defacement.

Preserved samples:

All but one of the preserved coating samples supplied by Finishes Unlimited showed passing scores (0 and 1) throughout the 8 week test under both unleached and leached conditions against *A. niger*, *P. funiculosum*, and *A. pullulans*. The preserved sample of the clear coating was given a score of 2 at Week 8 under leached conditions (Table 4).

White coating samples treated with BIOBAN 200:

The white coating was slightly less susceptible to fungal defacement than the clear coating, and the white coating required lower concentrations of BIOBAN 200 to pass the ASTM D-5590 test. The white coating was protected from fungal displacement under leached and unleached conditions with 0.4% (4,000 ppm) and above of BIOBAN 200 for the first 4 weeks of the test. The white coating also passed the 8-week test with 0.4% (4,000 ppm) BIOBAN 200 under leached conditions (Table 5). However, under unleached conditions 0.56% (5,600 ppm) BIOBAN 200 was required to pass the test at Week 8 against *A. niger* and *P. funiculosum*. The sample with BIOBAN 200 at 0.56% (5,600 ppm) was scored with a 2 at Week 8 against *A. pullulans* (Table 3).

Clear coating samples treated with BIOBAN 200:

The clear coating was protected from fungal defacement in the unleached condition by 0.56% (5,600 ppm) of BIOBAN 200, except at Week 8 against *A. niger* and *P. funiculosum* (Table 2). Under leached conditions, 0.56% was not sufficient for protection of the clear coating against fungal defacement by *A. niger* and *P. funiculosum* beginning at the Week 4 time point (Table 4, top portion). The test score remained a 2 for Weeks 4 to 8. Against *A. pullulans* under leached conditions, the clear coating was protected against fungal defacement for six weeks (Table 4, bottom portion).

Conclusions and recommendations:

After completion of the standard 4 week test period, all leached and unleached samples were protected at 0.56% BIOBAN 200 with the exception of one leached sample that was protected for three weeks. Based on the ASTM D-5590 data, the use of the EPA maximum limit of BIOBAN 200 is recommended for the coatings products:

- BIOBAN 200 at 0.56% (5,600 ppm) for protection of the white coating, and
- BIOBAN 200 at 0.56% (5,600 ppm) for protection of the clear coating.

Appendix I

Sample Sterility Test

Procedure:

Test samples are streaked to tryptic soy agar (TSA) and Sabouraud dextrose agar (SDA) plates to test for bacterial and fungal contamination, respectively. Plates are incubated up to 7 days at 30°C. The level of sample sterility/contamination is rated using the following scoring scale:

Plating Results	Score	Approximate CFU (or spores)/mL
No detectable survival	0	$< 1 \times 10^1$
1–9 colonies	1	$1 \times 10^1 - 9 \times 10^1$
10–99 colonies	2	$1 \times 10^2 - 9.9 \times 10^2$
100–300 colonies	3	$1 \times 10^3 - 3 \times 10^3$
> 300 colonies (smear)	4	$> 3 \times 10^3$

Appendix II

Dry-Film Algae Test – ASTM D-5590

Procedure:

Fungal spores were harvested from mature microorganisms grown on Potato Dextrose Agar. Organisms used in this study include: *Aspergillus niger* (ATCC #6275), *Penicillium funiculosum* (ATCC #11797), and *Aureobasidium pullulans* (ATCC #9348). Each spore sample was adjusted to a concentration of $\sim 2 \times 10^6$ spores/mL. Equal volumes of the *Aspergillus niger* (ATCC #6275) and *Penicillium funiculosum* (ATCC #11797) spore suspensions were pooled prior to sample inoculation, whereas *Aureobasidium pullulans* (ATCC #9348) was tested separately.

The samples were prepared by casting 3-mil wet films of the paint samples on drawdown paper and air dried for 24 hours. Two 1-inch strips were cut from each wooden piece. One set of strips was leached for 24 hours with distilled water in one-gallon containers at a flow rate of six changes per day and dried again. The second set of strips remained unleached. The coated strip samples were placed on Potato Dextrose Agar. 0.5mL of the fungal inoculums was deposited directly onto the coated samples and evenly spread over the entire substrate/agar surface. Agar plates were incubated at 30°C, with high humidity, for the courses of the 28 day experiment.

Plates were scored at 7 day intervals over a 28 day period. The readings at week 4 were used to determine sample pass/fail (see Microorganism Scoring Scale below). Samples with scores of 0 or 1 were considered to have passed the test.

Growth Scoring Scale	Score
None	0
Trace (<10% coverage)	1
Light (10 – 30% coverage)	2
Moderate (30 – 60% coverage)	3
Heavy (60 – 100% coverage)	4