Control of Black Stain Fungi with Biocides in Semitransparent Wood Coatings

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Abstract

One of the frequent signs of early failure in semitransparent wood coatings is colonization by ''black stain'' fungi, such as *Aureobasidium pullulans*. A laboratory method evaluating the ability of various wood coating formulations to inhibit the growth of black stain fungi on artificially weathered, coated wood in petri dishes was developed. Meaningful results were obtained after only 6 weeks of incubation. Selected biocides and biocide combinations formulated in a semitransparent coating including selected combinations of diiodomethyl-*p*-tolylsulfone, 3-iodo-2-propynyl butylcarbamate (IPBC), propiconazole, tebuconazole, thiabendazole, fludioxonil, chlorothalonil, oxine copper, copper metal, and naphthoquinone were evaluated. Combinations of propiconazole with IPBC and propiconazole with IPBC and thiabendazole were most effective in this test. Further work is needed to evaluate the use of other coatings and test fungi with the laboratory test method and to evaluate the performance of the best-performing biocides in field exposures.

One of the limits to expanded use of wood in aboveground exterior applications is the unavailability of a transparent or semitransparent coating that will provide long-lasting protection. Semitransparent coatings are used to limit moisture ingress, protect against degradation by ultraviolet (UV) and visible light, and reduce checking (Williams and Feist 1999, Nejad and Cooper 2011). They also allow the natural wood surface to be seen, which is viewed positively by consumers and is difficult for competing products to imitate. In applications exposed to direct sunlight and precipitation, however, refinishing may be required after 18 months or less (Groves et al. 2002). This is a significant impediment to competing with products that claim to be low maintenance. The performance of semitransparent coatings depends on their ability to resist UV and visible light, moisture, shrinking and swelling, and microbial degradation (de Meijer 2001).

Colonization of the coating or wood–coating interface by ''black stain'' fungi can be one of the most obvious signs of coating failure. Black stain fungi cause an unsightly discoloration and can penetrate coatings and weaken the adhesion to wood (Sharpe and Dickinson 1992, Bardage and Bjurman 1998). Moreover, they may detoxify naturally occurring phenolic compounds and lead to earlier colonization by decay fungi (Bjurman 1988).

Black stain is often referred to as blue stain in service, gray stain, black yeasts, or mold/mildew. The stain sometimes appears as slimy black streaks when wet or as black dots or small streaks when dry. Typical black stain genera that occur on wood in service or wood finishes include *Aureobasidium, Hormonema,* and *Epicoccum* (Ray et al. 2004, Gobakken and Westin 2008, Pfeffer et al. 2010). In contrast, blue stain fungi, typically from genera *Ophiostoma, Ceratocystis, Grosmannia,* and *Sphaeropsis* (*Diploida*), have brown-pigmented hyphae and cause bluish stains on sapwood of logs and lumber (Byrne and Uzunovic 2005). Molds occur as surface fuzzy growth in various colors and on various substrates, including wood in service. While the spores of black stain are generally dispersed by water and occasionally by air or insects, mold spores are mostly dispersed by wind. Mildew is to an extent a synonym for mold in the coatings industry; however, this term in the strict sense describes mold-like pathogens on plants.

Coating type also plays an important role in black stain resistance. In the absence of biocides, polyurethane

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semitransparent water-based coatings are reported to be more resistant to black stain fungi than acrylic and alkyd acrylic formulations (Van den Bulcke et al. 2007), and acrylics are reported to be more resistant to black stain than alkyds (Viitanen 2002). The type of binder may also be more important than the solvent. Both waterborne and solvent-borne alkyd formulations have been shown to be more resistant to *Aureobasidium pullulans* than waterborne and solvent-borne acrylic formulations (Bjurman and Herder 1992). A waterborne stain with an acrylic top coat was associated with significantly better performance against black stain in a field test than a solvent-borne semitransparent alkyd paint and a white paint system with a solvent-borne primer and a waterborne acrylic top coat (Gobakken and Lebow 2010).

Transparent and semitransparent coatings provide additional challenges in controlling the growth of black stain fungi. Opaque systems are generally more resistant to black stain fungi (Van den Bulcke et al. 2007), and pigments present in opaque coatings may have activity against fungi (Salvin 1944, Chen et al. 2009). The generally poorer resistance of transparent and semitransparent coatings to UV and visible light can lead to weakened or degraded coatings that provide pathways for colonization and poor wood–coating adhesion, providing ideal habitat for black stain fungi. Moreover, some black stain fungi, such as *A. pullulans,* can utilize lignin photodegradation products as carbon sources (Sharpe and Dickinson 1993). This ability may explain the success of black stain fungi in the wood–coating interface niche (Schoeman and Dickinson 1996).

One way to extend the life of transparent and semitransparent finishes is to include biocides to control black stain fungi. Biocides are often included in coatings as in-can preservatives, which can provide some protection to the dry film (Schoeman and Lloyd 1999). Generally, however, in-can preservatives are only intended to protect coatings from spoilage before application, not to control fungal growth in service. Biocides may also be included to provide dry-film protection against black stain fungi and other microorganisms. Carbendazim, diuron, and 2-*n*-octyl-4-isothiazolin-3-one were reported to provide effective dry-film protection against a range of fungi (Gillatt 1996). On painted surfaces, combinations of 3-iodo-2-propynyl butylcarbamate (IPBC) in the top coat and IPBC plus triazoles in the primer were best able to resist mold growth at two Norwegian sites (Gobakken and Jensen 2007). In laboratory tests, IPBC and an isothiazolone were most effective in controlling the growth of mold on paint films (Viitanen and Ahola 1997); propiconazole did not perform well in that study. In follow-up work on weathered and unweathered alkyd and acrylic systems, combinations of propiconazole and IPBC and propiconazole and isothiazolone in both primer and top coat were most effective against mold fungi, including *A. pullulans* (Viitanen 2002). Schauwecker et al. (2009) also observed that the isothiazolone 4,5-dichloro-2-*n*-octyl-3-isothiazolone prevented significant biological discoloration. One of the aims of the present research was to identify combinations of biocides best able to control the growth of black stain fungi.

Standardized methods have been developed for evaluating the ability of coatings to resist black stain colonization (Nordtest 1988, European Committee for Standardization [CEN] 2007, ASTM International 2010). The American Society for Testing Materials (ASTM) standard and the CEN standard specify the use of filter paper as a substrate. The potential effects of wood nutrients, moisture, and coating adhesion therefore are not considered in these tests. The Nordtest method uses a wood substrate but does not include any weathering or contact with liquid water. This method has been used to evaluate naturally weathered material (Viitanen and Ahola 1998), but the approach is difficult to standardize due to the variations inherent in field exposures. One of the aims of the present research was to evaluate a modified laboratory test method that included artificial weathering of coatings applied to wood samples.

The present article describes a laboratory screening method that uses solid wood samples and includes artificial weathering for evaluating the performance of biocides in dry-film coatings. Using this method, selected biocide combinations were evaluated for their ability to control black stain on wood coated with a semitransparent stain.

Materials and Methods

Testing procedures were derived in part from ASTM D5590 (ASTM International 2010) and Nordtest NT Build 338 (Nordtest 1988). Stain-free, kiln-dried ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) was obtained from Kalesnikoff Lumber (Thrums, British Columbia, Canada). Sapwood was identified, marked, planed, and cut into 57 by 57 by 6-mm, edge-grained samples. These samples were lightly sanded using 60-grit sandpaper before being separately coated with different formulations of SuperNatural (Napier Environmental Technologies), a two-step, semitransparent, waterborne polyurethane coating (Table 1). The same amount of biocide was added to both Step 1 and Step 2 of each formulation. The samples were first coated by brush on the back and sides with Step 1 of each coating type and then dried for 1 day and coated on the front side. This process was repeated to apply Step 2 of each coating. Each coating was applied to 60 samples. From each coating set, 30 samples were randomly selected for exposure in a 65-W Atlas Weather-Ometer. Samples were exposed for 200 hours to 24-hour cycles consisting of 8 hours of UV light exposure, 8 hours of water spray and UV light exposure, and 8 hours of darkness and water spray. From each coating set, six weathered and six unweathered samples were randomly selected for testing against four black stain fungal isolates and a mixed inoculum.

Two isolates of *Epicoccum nigrum* Link (AU311-16 and AU311-22), two isolates of *A. pullulans* (de Bary) G. Arnaud (AU311-18 and AU311-27), and a mixed inoculum containing spores from these four isolates were used to evaluate the coating's black stain resistance. These fungi were isolated in 2004 from weather-exposed and failed

Table 1.—Coating formulations evaluated in initial laboratory black stain test.[a]

Coating	Added biocides	Average (SD) concentration of biocides in coatings (μg/cm^2)
A	None	0 (0)
B	0.25% IPBC	28 (4)
C	0.5% propiconazole	56 (7)
D	0.25% IPBC + 0.5% propiconazole	87 (7)

[a] $n = 60$.

softwood test material that had been pretreated with a range of fungicides, coated with a clear finish, and exposed in Vancouver, British Columbia, Canada. The four isolates were selected as representative isolates of two species based on their ''spotty'' and ''streaky'' appearance on the test material. Test fungi were grown for a minimum of 2 weeks on cellophane (gel dry grade, catalog no. 1650922; Bio-Rad) that lay atop 1 percent malt extract agar. A few milliliters of a 0.01 percent solution of Tween 80 was added to each of two plates, and a blunt scalpel was used to scrape the aerial portions of the fungus off the cellophane into the Tween solution. The suspension from each plate was poured into a sterile blender jar, topped up with the remaining Tween solution, blended for 15 seconds, and then filtered through a sieve lined with sterile glass wool to remove large particles and media from the suspension.

Before inoculation, petri dish test chambers were set up to house each sample individually. The test chambers were prepared by placing two pieces of pulp blotter in the bottom of each 100-mm-diameter, 25-mm-high, sterile petri dish. A glass, U-shaped, bent rod was then placed on top of the pulp blotter. A separate strip of pulp blotter was positioned between two arms of each glass rod such that one side of the pulp blotter was below the rod and the other side was above. This allowed moisture to be wicked to the bottom surface of the test sample to keep the sample wet. Immediately before inoculation, 5 mL of sterile water was added to the bottom of each test chamber to soak the pulp blotter.

The test materials were placed on sterile aluminum foil and inoculated in batches of six. Four milliliters of inoculum suspension was sprayed over each sample set using a sterile airbrush (Li 2005). When half of the inoculum in the reservoir remained, the test samples were turned over, and the rest of the inoculum was sprayed on the other face of the samples. Inoculated test pieces were each put into individual test chambers, which were stacked in sets of six, covered loosely with a polyethylene bag, and incubated in the dark at 22.5°C. Sterile water was added to the chambers periodically during the incubation period to maintain humidity inside the chambers. Samples were inspected after 6 weeks and rated using a subjective visual rating system adopted from the US Department of Agriculture Forest Products Laboratory based on ASTM D3274-95 (ASTM 1995). The scale ranged from 1 (complete failure) to 10 (perfect); a rating of 7 or greater was considered to be a pass (Morris and McFarling 2006).

In a second test, coatings were formulated with a wider range of selected biocides and biocide combinations to evaluate their efficacy against black stain fungi (Table 2). Coatings were applied to six ponderosa pine samples as described above. To remove water-soluble carbon sources, two sets of samples were Soxhlet extracted with water for 48 hours and air dried before application of the coatings (Formulations 21 and 22). In addition, two sets of samples were pressure treated with disodium octaborate tetrahydrate to an average retention of 0.88 percent boric acid equivalent (BAE) before being coated (Formulations 23 and 24). All samples were weathered and inoculated with a mixed-spore suspension containing *A. pullulans* AU311-18 and AU311-27 and *E. nigrum* AU311-16 and AU311-22 as described above. The concentration of viable spores in the inoculum was determined by counting the colonies formed on malt extract agar plates inoculated with inoculum diluted 10^3 and 10^4 times. The inoculum was found to contain 9.9×10^4 CFU/mL. Test pieces were incubated at 20°C and evaluated as described above.

Results and Discussion

After 6 weeks of incubation, samples colonized by *E. nigrum* had a discrete, dotty appearance on the surface. The black stain associated with *A. pullulans* most often had a streaky appearance and colonized the rays, but it was also observed to form discrete patches in some of the samples containing biocides. These patterns were similar to those seen on the field test material from which these fungi were isolated.

In the unweathered samples, all of the biocides tested were able to control one of the *E. nigrum* isolates (AU311-16) and both of the *A. pullulans* isolates (Fig. 1). Against *E. nigrum* AU311-22, IPBC was effective, but coatings containing only propiconazole had levels of stain similar to those of untreated controls.

In the weathered samples, ratings were generally lower than those in unweathered samples (Fig. 1). This is consistent with weathering stimulating the growth of black stain fungi (Viitanen and Ahola 1998, Van den Bulcke et al. 2007). All of the biocides tested were able to control one of the *E. nigrum* strains (AU311-16). However, only the combination of IPBC and propiconazole was able to control the other strain (AU311-22); this combination of propiconazole and IPBC has previously been shown to be effective against black stain fungi (Viitanen 2002, Gobakken and Jensen 2007). In this test, propiconazole was slightly better at controlling *E. nigrum* than IPBC. The formulations containing IPBC were able to control one of the *A. pullulans* strains (AU311-18), while propiconazole alone was not. None of the biocides tested were able to control the other *A. pullulans* isolate (AU311-27). The mixed inoculum was not fully controlled by any of the biocides tested.

The black stain ratings highlight the importance of weathering. Unweathered samples performed better than weathered samples in almost all groups. Weathering is a critical component in black stain colonization, because it degrades the underlying wood, forming lignin breakdown products that serve as a nutrient source for *A. pullulans* (Sharpe and Dickinson 1993). Weathering also creates cracks in the coating, which provide openings for germinating spores. Unlike brown-rot fungi, *A. pullulans* is capable of penetrating very small pores with thin hyphae (Bardage and Daniel 1997). Black stain fungi such as *A. pullulans* colonize coated wood either by hyphae entering cracks in the coating or by penetration of the coating (Sharpe and Dickinson 1992). Exposure of weathered samples with mixed inoculum was the toughest condition of this test and was therefore selected to evaluate further biocide combinations.

The laboratory test method was able to screen biocides for their ability to inhibit black stain fungi in coatings. The use of artificially weathered wood accelerated the rate of black stain colonization and more closely represented field conditions. With this test, failure of controls and partial colonization of test samples occurred within 6 weeks. This could represent about a fourfold acceleration over the Nordtest method (Nordtest 1988), though the two methods were not directly compared.

In the second test, after 6 weeks of incubation, only three coatings had mean ratings of 7 or greater (Table 2). One of these coatings contained elevated concentrations of IPBC

Table 2.—Additional coating formulations evaluated in a laboratory black stain test.

Formulation	Added biocides	Average (SD) concentration of biocides in coatings (μg/cm^2)	Average (SD) discoloration rating[a]
1	None	0 (0)	4.8 (0.4)
2	0.25% IPBC + 0.5% propiconazole	128 (16)	6.8 (0.8)*
3	0.42% IPBC + 0.83% propiconazole	221 (25)	8.7 (1.0)*
4	0.5% diiodomethyl-*p*-tolylsulfone	97 (12)	5.0 (1.1)
5	0.25% IPBC + 0.5% propiconazole + 0.5% diiodomethyl-*p*-tolylsulfone	204 (26)	4.0 (0.6)*
6	0.5% powdered copper metal	118 (37)	3.8 (0.4)*
7	0.25% IPBC + 0.5% propiconazole + 0.5% copper powder	250 (54)	3.7 (1.2)*
8	0.5% naphthoquinone	83 (61)	4.3 (0.5)
9	0.25% IPBC + 0.5% propiconazole + 0.5% naphthoquinone	195 (15)	5.5 (1.2)
10	0.5% fludioxonil	80 (13)	5.7 (0.5)*
11	0.25% IPBC + 0.5% propiconazole + 0.5% fludioxonil	183 (46)	5.0 (0.9)
12	0.5% tebuconazole	96 (12)	5.0 (1.5)
13	0.25% IPBC + 0.5% propiconazole + 0.25% tebuconazole	191 (36)	6.8 (0.8)*
14	0.25% IPBC + 0.5% propiconazole + 0.5% tebuconazole	174 (34)	6.7 (0.5)*
15	0.25% IPBC + 0.5% propiconazole + 0.75% tebuconazole	213 (201)	6.5 (1.4)*
16	0.25% IPBC + 0.5% propiconazole + 0.25% thiabendazole	198 (30)	7.2 (1.0)*
17	0.25% IPBC + 0.5% propiconazole + 0.5% thiabendazole	201 (35)	6.8 (0.8)*
18	0.25% IPBC + 0.5% propiconazole + 0.75% thiabendazole	315 (42)	9.2 (0.8)*
19	0.25% IPBC + 0.5% propiconazole + 0.5% oxine copper	208 (19)	6.5 (0.8)*
20	0.25% IPBC + 0.5% propiconazole + 0.5% chlorothalonil	224 (28)	5.8 (1.0)*
21	None (extracted wood)	0 (0)	5.3 (0.5)
22	0.25% IPBC + 0.5% propiconazole (extracted wood)	136 (20)	6.0 (0.6)*
23	None (borate-treated wood)	0 (0)	3.7 (0.8)*
24	0.25% IPBC + 0.5% propiconazole (borate-treated wood)	151 (6)	3.2 (1.2)*

[a] An asterisk (*) indicates significantly different ($P < 0.05$) from control (Formulation 1).

and propiconazole, while the others contained IPBC, propiconazole, and thiabendazole. These data provide limited information, because not all combinations of biocides used in the present study were tested in the second experiment. Moreover, only one coating system, one wood species, and four fungal strains were evaluated. Further work is needed to more fully understand the performance of these biocides in laboratory and field tests.

Varying the concentration of tebuconazole in formulations with IPBC and propiconazole produced no observed effect (all were rated as 7 after 6 weeks). A small effect was noted from varying the concentration of thiabendazole in

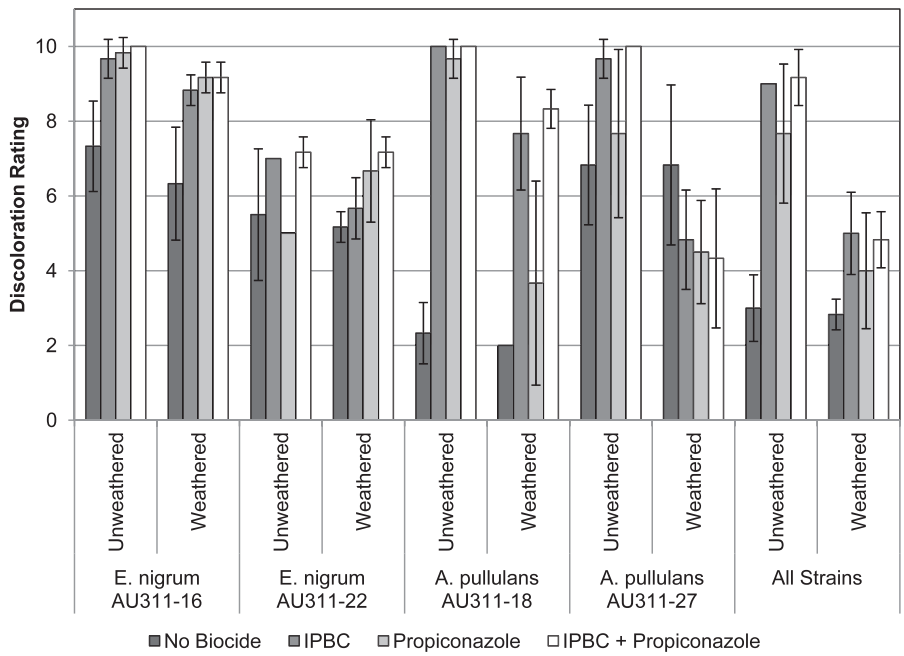

Figure 1.—Black stain discoloration ratings on weathered and unweathered coatings after 6 weeks of incubation (error bars represent the standard deviations).

formulations with IPBC and propiconazole, because the formulation with the highest concentration had a mean rating of 9 after 6 weeks while the others had mean ratings of around 7.

At the loadings tested here, diiodomethyl-*p*-tolylsulfone, copper metal, naphthoquinone, fludioxonil, and tebuconazole on their own did not give good performance; all had ratings under 7 after weathering. Furthermore, the addition of diiodomethyl-*p*-tolylsulfone, copper metal, naphthoquinone, fludioxonil, and oxine copper to the basic combination of IPBC and propiconazole did not improve ratings sufficiently to reach a mean rating of 7.

Samples that were pre-extracted to remove potential fungal food sources (Formulations 21 and 22) did not show any improved resistance to the black stain fungi. Similarly, samples pretreated with borates (Formulations 23 and 24) did not show any improved black stain resistance. The loading of 0.88 percent BAE may have been too low, because previous research has found borates to be effective against *A. pullulans* at higher concentrations (Laks et al. 1993).

The *E. nigrum* AU311-16 isolate has been associated with the degradation of propiconazole and tebuconazole at concentrations up to 15 $\mu g/cm^2$ (Stirling and Morris 2010). Although the concentrations used in the present work were 3 to 10 times greater, this cautions against using a triazole-only formulation, because biocides may be depleted in field samples and become vulnerable to biodegradation.

In the present study, the fungi evaluated were difficult to control with the biocides in the coatings tested. Other techniques may be needed for fully effective black stain control. These might include pretreatments to protect or stabilize the wood surface itself, methods of restricting nutrients on the wood surface, or use of thicker, more weather-resistant films that would resist the penetration of hyphae.

Conclusions

The laboratory method used was able to screen biocides for their ability to prevent black stain disfigurement of a semitransparent wood coating. The formulations best able to control the growth of *A. pullulans* and *E. nigrum* contained combinations of IPBC with propiconazole and IPBC with propiconazole and thiabendazole. Future studies are needed to validate the test method with other coating systems and test fungi. Further testing of biocide combinations, particularly in field tests, is needed to optimize performance against black stain fungi.

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