

Article



Potential Use of the Pigments from *Scytalidium cuboideum* and *Chlorociboria aeruginosa* to Prevent 'Greying' Decking and Other Outdoor Wood Products

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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Abstract: UV-light degradation of wood is one of the top reasons for consumer replacement of outdoor wooden structures. This type of degradation is seldom mechanical, and is instead often motivated by loss of aesthetics (graving). There are numerous commercial products available on the market that deal with this loss of color, many of which contain added pigments to 'rejuvenate' or 'revitalize' greyed wood. These pigments are almost uniformly synthetic. In contrast, pigments from wood decay fungi (spalting), which have been used in woodworking since the 1400s (intarsia), have remarkable optical (UV-light resistance) properties due to their naphthoquinonic configuration. In recent years the pigments made from these fungi have been extracted and tested across numerous substrates, from solar cells to textile dyes. In this work, researchers extracted pigments from Scytalidium cuboideum (red pigmentation) and Chlorociboria aeruginosa (blue-green pigmentation), solubilized the pigments in raw linseed oil, and tested the resulting solution on samples of Douglas-fir (Pseudotsuga menziesii) and western white pine (Pinus monticola). These mixtures were compared against a 'stain and coat' treatment (utilizing an aniline stain and coated with raw linseed oil), raw linseed oil, and untreated wood. The wood samples were then placed in an accelerated weathering machine (Q-UV) following the ASTM G154 standard, for 500 and 1000 h. The results showed that while no visible color change occurred to the wood when the pigmented oil was applied, the red pigment oil significantly lowered the coating degradation for both wood types at an exposure of 500 h. The results show the potential applications for fungal pigments in the wood coating industry, as it offers an increased coating service life. As there is a shift to renewable products, the pigments from wood decay fungi show potential as additives for wood coatings.

Keywords: coatings; fungal pigments; oil coatings; *Scytalidium cuboideum; Chlorociboria aeruginosa;* accelerated weathering; QUV; UV-light; UV resistant; wood weathering

1. Introduction

Wood weathering begins with UV-light degradation of the lignin and water erosion. These two factors create spaces that allow entrance of fungi, bacteria, and insects [1] into the wood and accelerate its degradation. More importantly for consumers, UV-degradation causes 'greying' of wood, which decreases its aesthetic value [2].

Many products are available on the market to extend the 'natural' color of wood components. The most common are coatings [3], which are especially popular for outdoor decking and furniture. Most of the coatings currently available in the market offer UV-light protection and water resistance and often a pigment additive that helps to cover the

greying. These coatings are usually non-renewable chemical formulations (with moderate content of volatile organic compounds) that use titanium dioxide as an active ingredient [4]. Most of the coatings containing titanium dioxide (or other inorganic UV-light blockers) require two layers of coating as well as yearly reapplications [5]. There is also evidence of leaching of components like titanium dioxide and silica from paints and coatings [6]. With raising awareness about chemicals like titanium dioxide impact in water life (coral reefs bleaching, etc.) [7], researchers are looking for possible replacements, with a special focus on renewable compounds. One of these potential alternatives corresponds to the pigments produced by *Scytalidium cuboideum* (Sacc. and Ellis) Singler and Kang, and *Chlorociboria aeruginosa* (Oeder) Seaver ex C.S. Ramamurthi, Korf, and L.R. Batra. Both of these fungi fall under the classification of 'spalting fungi'.

Spalting fungi are wood decay organisms that cause an internal color change. These color changes can occur due to the degradation of lignin (caused by white rot fungi), which results in wood having a lighter color or 'bleached' appearance [8]. A second type, zone-lines, are produced by somatic incompatibility [9] or dehydration (these lines are mostly produced by white rot fungi and mostly composed of melanin) [10,11]. The third and last type corresponds to pigmenting fungi, which are mostly soft rot Ascomycetes. This group is of special interest, as the pigments are produced by the secondary metabolism that can create a wide array of pigmentation in wood [8,12,13]. One of these fungi is *Chlorociboria* spp., which generates a blue-green color (produced by the metabolite xylindein) in infected wood [14,15]. Other wood pigmenting fungi are *Scytalidium ganodermophthorum* Kang, Sigler, Lee and Yun [16], and *Scytalidium cuboideum* [17]. The first one produces an unidentified yellow pigment, whereas the second one produces red pigmentation.

For this particular study, the pigments from *C. aeruginosa* and *S. cuboideum* were of special interest as they are known to have long-lasting color stability. This can be seen well in wood art pieces from the 1400s to 1700s that still show their vibrant blue-green color (corresponding to *C. aeruginosa*) [14,18,19]. Research on the pigments produced by these fungi has shown that they have potential use as a replacements for anilines, and have been previously tested for UV-light resistance with positive results (in wood, textiles, and opto-electronic devices) [20–22]. The pigment from *S. cuboideum* was recently fully identified as an organic crystal named 'dramada' [23]. Previous to the final description of this compound, the pigment was used under the name draconin red, and its potential applications involved its use as a wood [24], and textile colorant [25]. UV-light resistance tests performed by Hinsch [26] on textiles dyed with this pigment, showed that it maintained its red coloration, even after machine washing and crocking. These pigments have also showed low to no toxicity in their purified forms [27] which compared to other pigments can allow for their utilization in a wide array of applications.

For centuries, the use of oils from flax (*Linum usitatissimum* L.), tung (*Vernicia fordii* (Hemsl.) Airy Shaw), and others, have been part of the formulations of varnishes and other types of coatings [28,29]. Flax oil (linseed oil) was used by the ancient Egyptians as an embalming element due to its hardening properties, and there are historical records that point its use as a coating by the ancient Romans and Greeks [30]. For many centuries coating formulations have contained various pigments and linseed oil, but advances within the polymer field and the broaden use of latex eventually replaced linseed oil in the coatings industry [31]. Linseed oil is making a comeback, however, with the growing in renewable and/or natural coatings [32].

Recent studies have been performed into the potential use of linseed oil as a fungal pigment carrier, focusing on testing renewable and non-toxic carriers for spalting pigments, which are traditionally caried in solvents such as dichloromethane (DCM), chloroform, and acetone. These solvents can be hazardous and deeply limit the use of spalting pigments outside a laboratory [33]. Previous research determined that linseed oil could be used as a carrier for spalting pigments, and was tested with *S. cuboideum* as a potential waterproofing coatings for textiles [34]. The results for these studies determined that the pigments remained stable in raw linseed oil [33].

Accelerated weathering is widely used in the material coating sciences, as it allows a rapid and representative evaluation of a material against UV-light, moisture, temperature change, and water exposure. This method is used specially in the formulation stage of coatings, as it allows modifications in the coatings ingredients (by showing potential defects) in a relatively short span of time [35,36]. Although this method is reliable, it does not replace long-term evaluations under natural conditions, as it does not includer biological factors (fungi, insects, etc.) [1].

The purpose of this research was to determine the suitability of two spalting pigments xylindein from *Chlorociboria* species and dramada from *Scytalidium cuboideum*—as UV degradation protectors when carried in raw linseed oil, specifically targeting the outdoor decking market. Results from this study will determine if these two common spalting pigments offer long-lasting UV protection to wood in-service, not just wood indoors and in protected museum works. If successful, these pigments would offer a natural, renewable coating additive option for decking preservative companies who wish to move away from synthetic compounds.

2. Materials and Methods

As a general overview, the pigment xylindein from *Chlorociboria aeruginosa* and dramada from *Scytalidium cuboideum* were solubilized in raw linseed oil and then applied to western white pine (*Pinus monticola*) and Douglas-fir (*Pseudotsuga menziesii*). The treated wood was placed in a Q-UV accelerated weathering machine for two set times—500 and 1000 h. A second set of samples were treated with raw linseed oil and aniline dyes, and also exposed to weathering. Untreated controls were also included. The specifics of the testing can be found below.

2.1. Wood

Western white pine (*Pinus monticola* (Douglas ex D. Don) Rydberg) and Douglasfir (*Pseudotsuga menziesii* (Mirb.) Franco) wood were cut to 4.5 cm \times 4.5 cm \times 15 cm. The samples were conditioned at 12% moisture content before the start of the test. Four repetitions were done per treatment.

2.2. Fungal Pigments

Fungal strains of *Scytalidium cuboideum* UAMH 11,517 and *Chlorociboria aeruginosa* UAMH 11,656 were grown in wood mended malt agar plates (2% MEA combined with white rotted wood chips from sugar maple (*Acer saccharum* Marshall), following the procedure set by Robinson et al. (2009) [37].

After 8 weeks of growth for *S cuboideum* and 16 weeks for *C. aeruginosa*, the plates were placed in a fume hood at room temperature (21 °C) to dehydrate. Once the plates were dry, they were manually ground to obtain small clusters of wood chips. Then, the wood chips were placed in an Erlenmeyer flask with a ratio of 30 mL of dichloromethane (DCM) per ground plate. The flasks were placed on a stir plate for 30 min, and then the content was filtered using a VWR 415 filter paper. The wood chips were then placed again in the flask, to repeat this process two more times. Once that the all the pigments were collected in DCM, each pigment was standardized to the CIELab values set by Robinson et at. (2014). These values were L* = 82.28, a* = -11.06, b* = -5.40 for *C. aeruginosa*, and L* = 82.32, a* = 26.84, b* = 13.19 for *S. cuboideum*. Further studies on the crystal (dramada) produced by *S. cuboideum* [38] determined that the L*a*b* values correspond to a concentration of 0.73 mM.

2.3. Coating Preparation

2.3.1. Linseed oil with Fungal Pigments

The mixture of Sunnyside raw linseed oil with the fungal pigments made considering a 1:1 ratio previously set by Robinson et al. (2017) [33]. For this, one liter of each pigment was mixed with one liter of raw linseed oils separately. The beakers containing the mixtures

were then placed on a stir plate and covered with a glass square in a fume hood. The mixtures were left to stir until all the DCM was evaporated. The evaporation process took between five to eight days.

2.3.2. Aniline

J. E. Moser's peacock blue and cherry red anilines were prepared as instructed by the manufacturer, by diluting 56 g of aniline powder in 950 mL of distilled water. The mixture was made in a beaker, and then placed on a stir plate for 48 h to reach a complete dilution of the pigment.

2.4. Coating Application

2.4.1. Linseed oil with Fungal Pigments

The pigment mixture was placed on a stir plate for 30 min before applying it to the wood. This step was done to reach coating homogeneity. After this step, the coating was applied using a foam brush of 2.54 cm of width. Each wood piece had three coats, with a 24 h wait between each layer. After the final layer as applied, the samples were let dry for 48 h.

2.4.2. Aniline Application

Two layers of aniline solution were applied on each piece of wood, with an interval of 24 h between them. After the last layer of aniline was applied, the wood was left to dry for 48 h. After that, three layers of raw linseed oil were applied, with a 24 h interval between layers. After the final oil coating, the wood was left to dry for 48 h.

2.4.3. Linseed Oil

Each oil control set was coated three times with Sunnyside raw linseed oil with a 24 h interval between each layer. After the final coating as applied the wood was left to dry for 48 h.

2.5. Weathering

Each set of samples were placed in a QUV Accelerated Weathering Tester (Q-Lab Corp, Westlake, OH, USA). The program chosen was the ASTM G154 CYCLE 7 standard for non-metallic surfaces, as it tested for UV-light and moisture. This standard consist of a cycle consisting in four steps. The first step is UV exposure with a radiance of 1.55 W/m^2 at 60 °C for eight hours, the second step is the spraying of the samples with water at room temperature for 15 min, the third step consists on water condensation at 50 °C for three hours and 45 min. The final step is the repetition of the cycle from the first step. The samples were divided into two exposure treatments: 500 and 1000 h cycles.

Each treatment set consisted on four control samples, four with linseed oil, four with red aniline, four with raw linseed oil with red fungal pigment, four with green aniline, and four with raw linseed oil with green pigment.

2.6. Color and Area Evaluation

Two different evaluations were performed on the samples. The first one consisted on color reading the samples with a Konica Minolta Chroma Meter CR-5, before and after the weathering treatment. With both values, the color difference (ΔE) was calculated with the CIE2000 equation:

$$\Delta E * = \sqrt{\left(\frac{\Delta L'}{kLSL}\right)^2 + \left(\frac{\Delta C'}{kCSC}\right)^2 + \left(\frac{\Delta H'}{kHSH}\right)^2 + RT\frac{\Delta C'}{kCSC}\frac{\Delta H'}{kHSH}}$$
(1)

where ΔE is the overall color change, ΔL is the change in the *L* value, ΔC is a combination of the change in Δa and Δb (without *L*), and ΔH is the change in hue. R_T is a hue rotation term,

and the various K terms are dependent upon the application. S_L accounts for lightness, S_C accounts for chroma, and S_H accounts for hue.

The second evaluation consisted on scanning each sample with the use of an Epson V370 Photo scanner with a resolution of 1200 dpi. After scanning the samples, each of them were analyzed with Image J (Ver. 1.47), following the protocol set by Robinson et al. [39] to quantify the percentage area of coating remaining after the weathering treatment.

2.7. Statistical Evaluation

A four-way ANOVA was performed on the color difference data, being color difference (ΔE) the dependent variable, and wood type, coating (no coating, linseed oil, linseed oil with fungal pigments, or linseed oil with anilines), color (no colorant, red, or blue), and exposure time (500 and 1000 h) the independent variables, followed by a Tukey HSD.

A three-way ANOVA was performed on the coated samples only, to evaluate the remaining coating left on the samples. For this test, the remaining area percentage (a%) was the dependent variable, while coating, color, and exposure times were the independent variables. The ANOVA was followed by a Tukey HSD.

3. Results and Discussion

Generally, the untreated wood (control) greyed after both the 500 and 1000 h exposure, as expected. Cracks and checking also occurred. The samples treated with oil and aniline also degraded quickly and most of the 500 h exposure pieces lost most of their color. The aniline stain also leaked from the samples (see discussion below). Defects such as cracks and checking occurred regularly. Samples treated with the spalting pigments showed fewer defects on the surface.

The statistical analysis of the color change of the samples from the colorimeter proved problematic. This number does not adequately convey the color *remaining* on wood, nor what that color is. The follow-up testing which used percent coverage of color, was more effective and found that the dramada (red) pigment significantly extended the life of the wood finish up to 500 h of exposure (an approximate equivalent to four to five years in service). Further details of the testing and results, along with the discussion, are broken out below.

3.1. Color Change

The ANOVA showed that there was a significant interaction (p < 0.0001) between treatment and color change (ΔE). The type of wood, colorants, and weathering exposure time showed no significant effect on the color difference. For Table 1, the Tukey HSD groups were generated based solely on the treatment interaction. All the aniline treatments were statistically different to the controls and all other treatments, meaning the aniline samples lost more color than all the other tests. This is likely because the anilines were water soluble and as the samples were placed in a vertical setting for the weathering test, the water sprayers removed most of the color. Although they were coated with linseed oil, is possible that the oil layer was not effective enough to provide water resistance to the stain (aniline) coat. Xylindein and dramada, however, which are not water soluble, were able to persist longer on the wood. It is interesting that the aniline samples showed more color loss than the raw wood; however, this is likely an artifact of how delta E is calculated, and that the aniline samples started very bright, so had, overall, more color to lose.

Wood	Treatment (Coating Type)	Color	Weathering (h)	Mean (ΔE)
White pine	Control	No color	500	8.878 (EF)
			1000	12.123 (DEF)
	Linseed oil	No color	500	5.260 (F)
			1000	8.685 (EF)
	Linseed oil + aniline	Red	500	36.608 (A)
			1000	19.340 (BCDE)
		Green	500	28.705 (ABC)
			1000	28.910 (AB)
	Linseed oil + fungal pigment	Red	500	9.783 (EF)
			1000	4.293 (F)
		Green	500	8.603 (EF)
			1000	8.598 (EF)
Douglas-fir	Control	No color	500	11.584 (EF)
			1000	11.294 (EF)
	Linseed oil	No color	500	9.408 (EF)
			1000	7.330 (EF)
	Linseed oil + aniline	Red	500	24.598 (ABCD)
			1000	24.610 (ABCD)
		Green	500	32.188 (A)
			1000	29.418 (AB)
	Linseed oil + fungal pigment	Red	500	16.085 (CDEF)
			1000	12.890 (CDEF)
		Green	500	8.363 (EF)
			1000	7.313 (EF)

Table 1. Results for color difference after weathering (p < 0.0001). The table Tukey groups were generated based on treatment only, as it was the only variable that showed statistical significance.

Due to the issues note above, the delta E test did not produce data that was entirely accurate in terms of meaningful UV-protection. Previous experiments focused on xylindein have shown that it can protect color of the wood, but not the chemical composition [20]. It is possible that a similar behavior happened with the oil-pigment mixture, but it requires further testing

Although the controls and oils (with and without fungal pigments) showed similar Tukey groups for color difference, the mean color difference was similar but the color hues were not. This is a common drawback of using the delta E equation, which can be easily rectified with visual examples. Figure 1 shows control samples tended to a gray hue, while all the oil samples tended to brown and dark brown hues. It is important to not discount the visual differences here, as it is visual differences that cause consumers to replace their decking due to greying.



Figure 1. Samples of white pine and Douglas-fir with four different treatments after a weathering exposure of 500 and 1000 h.

3.2. Color Coverage

A more reliable analysis turned out to be the color coverage analysis. For this test, wood type and non-coated samples were not considered for the statistical evaluation as the test relies entirely on using a computer to determine how much surface area is covered by a given color. The rest showed a statistical significance (p < 0.0001) related to the remaining percentage area covered (Table 2).

Table 2. Results for color coverage after weathering 500 h and 1000 h (p < 0.0001). The table Tukey groups were generated based on treatment only, as it was the only variable that showed statistical significance.

Treatment (Coating Type)	Color	Weathering (h)	Mean (a%)
Lincord oil	No color	500	51.916 (BC)
Linseed oil	INO COLOR	1000	43.557 (C)
	Pad	500	15.145 (D)
Linsood oil Lapiling	Rea	1000	22.578 (D)
Linseed on + animite	Green -	500	6.208 (D)
		1000	7.710 (D)
	Ded	500	82.130 (A)
Linseed oil + fungal nigment	Ked	1000	59.804 (BC)
Eniseed on a rungar pignient	Crear	500	63.421 (AB)
	Green	1000	48.221(BC)

The coating on the dramada oil samples remained at 82% (average) coverage, which was significantly more than any other treatment at 500 h (Figure 2). The oil-pigment with *C. aeruginosa* had similar results but it was not significantly different than the colorless

linseed oil. As most decks have an in-service life of two to three years due to greying or coating deterioration (most coating cans recommend one to two years before being reapplied) the use of a fungal pigment coating that can effectively extend the finish and wood service life for up to five years is attractive. It is also possible that a higher percentage of the red pigment in solution might yield better color and coating protection. Figure 2 compares just the controls and red treatments (aniline and dramada), where the suppression of greying is clearly visible.



Figure 2. Weathered samples. (**Left**) column: controls; (**Center**) column: red aniline stain and finish treatment; (**Right**) column: red fugal pigmented oil.

The persistence of dramada pigment may be due to its crystalline nature, which is often extended when carried in oils [34,38]. Previous research on textiles utilizing *S. cuboideum* and raw linseed oil showed that the pigment crystals developed under the surface of the oil coating. Formation under the oil coating would protect the crystals from the direct elements and allow their protective effects to remain longer. It may have also increased the bonding of the pigments, which was seen in a previous textile crocking test [26,34]. Having stable colorant compounds that attach to surfaces is critical in paints and finishes of all kinds, [40], and thus the dramada pigment may be ideal for this type of use.

An important factor relies in previous studies on potential solvent carriers for the pigment from spalting fungi such as [41] as it is possible to use other solvents such as acetonitrile that can replace DCM. In the specific case of the pigment from *S. cuboideum* it is possible to obtain the dramada crystals in high purity with the use of acetone [38]. Theses crystals are the ones that have a higher potential for industrial applications as it can allow a better control in the resulting color as well as having a lesser impact compared to DCM as the crystal can be dissolved in the oils while excluding a solvent carrier. Further research should look at the effect of pigment concentrations on UV resistance and grey weathering, as well as microscopic surface evaluations to visualize the interactions of the oil-pigments with the wood.

Although further research is needed, the feasibility of using fungal pigments in the coatings industry is highly likely, as its use can range from aesthetics protection to coating stabilization, as well as to provide the industry with a renewable pigment source.

4. Conclusions

The dramada pigment from the spalting fungus *S. cuboideum* persisted beyond all other coating treatments and had the statistically highest cover coverage after 500 h of weathering, indicating its potential use as an additive in decking finishes to help delay 'greying'. As dramada is produced readily in nature and in the lab by the fungus *Scytalid*-

ium cuboideum and has shown low to no toxicity in toxicological testing, it makes for an attractive alternative additive to current decking products that is also highly renewable.

5. Patents

Robinson, S.C., Vega Gutierrez, S.M. Use of Fungal Pigments from Wood-Staining Fungi as Colorants in Wood Finishes and Paints. U.S. Patent Application No. 15/266,865 Filed on 18th of September, 2015. Approved in 2016.

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