Alternative Carrier Solvents for Use with Resolubilized Fungal Pigments to Increase Internal Color Coverage in Wood Substrates

by
Lauren Pittis

A THESIS

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Botany and Sustainability
(Honors Scholar)

Presented December 1, 2015
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Abstract approved:

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Sara Robinson

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Key Words: spalting, fungi, dye, solvents, wood science

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Lauren Pittis, Author

Alternative Carrier Solvents for Use with Resolubilized Fungal Pigments to Increase Color Coverage in Wood Substrates

An Honors College thesis by Lauren Pittis

Introduction

Colored wood has been used throughout history for artistic purposes. Modern use of synthetic dyes, such as aniline, can be traced back to the mid 1800s, when dye technology surged (1). Before this time, however, naturally colored wood had to be either found or made.

The most popular type of colored wood for intarsia and marquetry woodworks was wood stained blue-green by the fungi *Chlorociboria aeruginascens* and *Chlorociboria aeruginosa* (1-3). The use of this type of wood, called 'spalted wood', can be traced back to the 15th century, and is still in use today (1, 4-9). The term 'spalting' refers to any coloration of wood caused by fungi, assuming such color penetrates past the wood surface.

Spalted wood is of commercial importance today as a replacement for synthetically dyed wood (10 -12). Unfortunately, methods to quickly and efficiently create spalted wood continue to elude researchers. There have been extensive efforts in the past ten years to find a commercially viable method for large scale spalting of wood (12 -14). Such a method would allow for spalted wood, or even the pigments produced by the spalting fungi alone, to enter into the worldwide market and replace their synthetic counterparts.

Literature Review

1500s-1700s: Historical Use

Since as early as the 15th century, artisans and woodworkers have been using spalted wood in their work. The first historical uses were mostly intarsia from Italy, during the Italian Renaissance. Intarsia is an art form consisting of cutting out shapes of differently colored wood veneer, and inserting them into a carved recess in a solid piece of wood. It comes from the Italian word "*Tarsia*", which means "inlay". It was used to create murals and details in cathedrals, churches and monasteries, and during the first half of the 15th century, began to be incorporated into Italian furniture (1).

The middle of the 15th century saw an increase in the use of blue-green (verdigris) spalted wood throughout Italy (2- 3). *Maestri di legname* (masters of wood) used it to light up details in their intarsia, such as clothing, leaves, and book clasps. Thin shavings of spalted veneer were used to create a marble-like appearance. Fabric and stone were also simulated in the wood using verdigris wood (2). Used sparsely, the spalted wood made details stand out and gave a natural tint to the green part of the landscapes depicted in the art pieces.

By the second half of the 16th century, the use of blue-green spalted wood had spread from Italy to many other European countries. Swiss wood workers began to use stained

and spalted beech. Spanish artists incorporated the colored wood into their art, drawing from the Italian traditions. Augsburg and Southern Germany used spalted green poplar to color landscapes, plants, and colorful accents (2). Soon, spalted wood became an especially distinguishing feature of Bohemia and Augsburg intarsia. Journeyman from Southern Germany then traveled to Sweden, bringing intarsia techniques and the use of spalted wood with them (1).

At this point, artists and wood workers had no idea what caused the wood coloration. Wood was collected in local forests, and spalted wood was a valuable and coveted commodity. Most pieces would be split into many thin veneers, in order to create multiple color variants and to portion out the rare treasure. It reminded people of gems, and was regarded as a *miraculum naturae*- a wondrous creation of nature (1).

However, spalted wood began to fall out of favor in the 17th century. France overtook Italy as the leading center of art in Europe, and the focus was on exotic woods and bright artificial dyes. Occasionally, spalted wood would turn up on middle class wooden furniture, and it was used on instruments such as violins (4).

One exception was Egar, Bohemia. After the 30 Years War, artisans in Egar combined intarsia techniques and relief carving. Almost all the masters in Egar intarsia used spalted wood (both blue-green and blue-gray) in their work, in order to create contrasts in color schemes and to make landscapes appear more natural (1).

1800s: Tunbridge Ware

By the 18th century, spalted wood had gone almost completely out of style, in favor of new dyes and ways to color wood. Southern German masters used green spalted wood for inlays in strap work, and a few middle class furniture pieces had inlays incorporating it. However, after the middle of the 18th century, spalting in marquetry became very hard to find (1).

Starting in the 1830s, Tunbridge Ware entered the scene. The woodworkers in the town of Tunbridge Wells, England, began to create Vandyke parquetry- tessellated wood mosaics on utilitarian objects, such as boxes. The characteristic elongated triangles and cube patterns were created with many different types of wood, and these included spalted oak (referred to as “green oak”) and other spalted hardwoods. Tunbridge Ware is now what Tunbridge Wells is famous for, and spalted wood is still incorporated into the mosaics (5).

1900s: Research

Investigation into fungi that color wood began in earnest in the beginning of the 20th century. Studies were completed on freshly cut wood, left out to be colonized by numerous fungi (6). Surveys were also done on fungi isolated from stained wood found in that condition (7).

The first patent involving spalting was filed in 1913, by Frederick Tom Brooks. It was entitled "Improvements in or Relating to Colouring and/or Preserving Wood", and outlined a process for inoculation and artificial inducement of *Chlorosplenium aeruginosum* and *Peziza aeruginosa* (8).

Now that fungal activity in culture was being studied more often, research began to be conducted on how fungi grew and what nutrients they needed in order to survive (9-10). The interactions between fungi and their ability to color wood gained a research following as well (11-13).

1930-1960: Chlorociboria – the blue-green fungi

The genus *Chlorociboria* was first created in 1936 by Fred J. Seaver, to replace *Chlorosplenium*, the current genus being used at the time (14). Three species were included in the genus: *Chlorociboria aeruginosa, Chlorociboria versiformis*, and *Chlorociboria strobilina*.

However, the genus was not officially recognized as valid until 1957. Though originally thought up in 1936 (14) and published under the name in future publications (15), Seaver had failed to provide a Latin diagnosis for the genus, so it wasn't truly used or correctly published. A revision paper (16) provided a Latin diagnosis, and thus validated the genus and the generic name for future use. A key to the North American species was also included in the official genus listing.

The genus *Chlorosplenium*, from which *Chlorociboria* was created, was discussed in detail in a series of articles on *Chlorosplenium* and its segregate genera (17, 18). Besides *Chlorociboria*, it covered *Chlorosplenium sensu stricto* and suggested a new genus, *Chlorencoleia*. Further consideration went into which species belonged in *Chlorociboria*, and how to tell them apart, focusing especially on look-a-like genera, and the differences between *Chlorociboria aeruginosa* and *Chlorociboria aeruginascens* (18).

1960s: Xylindein - the pigment produced by the *Chlorociboria* genus

The structure of xylindein was proposed in 1965, based on decades of prior research. Blackburn and Todd (19, 20) came up with the first structure, based on NMR spectrum data, absorption spectra, and attempts to restructure the molecule under laboratory conditions. This structure was supported by other research (21), and continues to be expanded on to this day (22, 23, 24).

Work continued slowly on xylindein after this, though it was no longer the main focus of research. In 1979, a model for the synthesis of the pigment was described, in order to attempt to understand the process by which the xylindein molecules form (25).

1970s: Lindquist Revival

In the 1970s, the spark for working with spalted wood was rekindled in the woodworking community by the work of Melvin Lindquist, and later his son, Mark Lindquist. They

brought spalting back into public consciousness after a long span of inactivity, and through the popularity finally moved it into the "art world" where it had long been seen as more of a craft (1). Selling turned spalted pieces, they gained great popularity, and soon began to sell unturned pieces (burls, etc.) as well (1). In 1967, after an encounter with another woodworker in Vermont, they learned of the term "spalted wood", and began using it to refer to their wood, popularizing the term which is used in the same context to this day (1).

The Lindquists focused on the practical applications of working with spalted wood as a woodworker. Mark Lindquist wrote on how to turn spalted wood, how to prepare it, and the beauty of working with such an unpredictable medium. This included the specifics of turning and preparing spalted wood, and what made it different from working with normal wood (26). In a time when wood pieces were extremely polished, working with "imperfect wood" such as the Lindquists did was unique, and also extremely popular. Once Mark had two pieces in *Fine Woodworking*, the frenzy surrounding their spalted pieces moved back to woodturners as well as art show enthusiasts, and spread quickly through the community (1, 26 -27).

1970s-1990s: Fungal and Pigment Research

The interactions, antagonisms, and differences in different decay fungi were studied over this period, in order to determine melanin biosynthesis (28), community formation (29), and antagonistic pairs in the same substrate (30).

In the next ten years, the pigments themselves were the focus of a number of studies. Metabolic products and fungal melanins were isolated from several different fungi (31 - 34), including pigments, both red (35), and yellow (36, 37). A patent for "Red Pigment and Process" was even filed, marking the second patent to make use of spalting (38). Zink and Gengel published a series of papers on the coloring compounds specifically in blue stain fungi (39- 41).

Spalting became a part of many textbooks and collections over the years (42 -46). But regardless of the amount of research done on the subject, many papers and projects still sought to understand the reasons behind discolorations and blue staining in trees and wood around the United States and world (47- 50).

1900s-2000s: Continued Research on Methods

Researchers began to nail down methods for growing the decay fungi in culture, experimenting with different incubation methods and substrates. Fungal colonization via inoculation using agar versus using vermiculite were compared. Though both methods were equally useful for colonization, vermiculite took less time and storage space, and was also easier to prepare (51). Other fungal species continued to be isolated, with decay capacity and pigment production determined (52).

Studies now moved towards synthesizing xylindein in the laboratory, starting with

attempting to recreate precursors in its production pathway (22), though this was difficult to do synthetically while creating a stable molecule. The crystal structure and geometric configuration of xylindein were discovered at the turn of the century, using X-ray crystallography and heavy atom analysis (23).

Chlorociboria aeruginosa pigment was considered for use as an algicide (53) and later a patent was filed for use of the same or similar pigments for plant germination inhibition (54). Work continued on how best to extract xylindein and biological characteristics (55).

The art world was still using spalted wood in pieces. Articles and reviews were created on working with spalting, ranging from turning and working with the wood (56, 57) to enhancing naturally spalted pieces (58). The reviews include overviews of spalting, including types of fungi, basic definitions, temperature and moisture requirements, some suggestions/cautions, and experiences of woodturners who used spalting in their craft. They also included places to go for further information, and were aimed at making spalting more accessible and known to the community. A focus on "DIY Spalting" was seen in these reviews and a few other articles around this time (59).

In order to quantify the effects of spalting on different wood species and in different test trials, a method for color analysis using digital color evaluation was developed (60). This method replaced quantitative earlier methods whose results differed depending on a researcher's color perception and other factors.

Wood veneers and flooring utilizing spalting gained a handful of patent applications (61-63), as well as the general process of "coloring wood" (64).

2010-Current Day: Research

With increased knowledge of the pigmenting fungi came a rush of laboratory experiments focused on pigment production and optimal fungal growth in lab manipulated systems. Different substrates were used, in order to see which could be most effectively spalted. Vermiculite was suggested to be a better substrate for spalting sugar maple, compared to soil, due to a better color contrast and higher levels of external spalting (65). *Chlorociboria* species preferred low-density wood species, such as aspen, as opposed to beech (66). Overall, hard maples were found to be the best for many fungi tested. This includes sugar maple, which is a key wood for spalting and a good control species (67).

A modified decay jar method was created in 2012, using vermiculite and recommending placing all wood blocks above the vermiculite for future tests (68). The effect of moisture content on fungal growth was examined. It was found that spalting could be stimulated by controlling moisture conditions in the substrate, and that each fungal species had different optimal conditions for maximum pigment production, depending on wood species (69).

Work continued on synthesizing xylindein, but once again the precursors weren't stable enough and the experiment had little success (70).

Current Day and Future Research

Spalting is still used as a way to repurpose old wood for pieces by woodturners and artists (71). Creating beauty out of "functional wood" and adding a visual and tactile experience to different pieces was explored in "Destroying Uniformity"(72).

A recent project done by Milo Dool incorporated spalting into different pieces, such as light fixtures, and included basic explanations of how the process worked (73). Zone lines and melanin formation have also been written about recently in terms of adding decoration to turned pieces (74).

Extracting the pigments for use as applied dyes has also gathered a large amount of research. Dichloromethane has been found to work well as an extraction solvent (75) when compared with other solvents in both extraction and resolubilization tests. Comparison research has been done between fungal dyeing and induced fungal pigmentation, to see which is more effective. Overall, fungal dye application had a larger success rate than induced pigmentation, using multiple colors of dye from different fungi (76). Cottonwood seems to work most effectively for this process, compared to other wood species tested (76). More research needs to occur in this area, concentrating on the most effective dye methods and experimenting with different solvents and application processes.

Continuing this idea, artists and researchers have interest in spreading the use of spalted dyes from wood to other mediums, such as textiles. Fungal dyeing has been found to have some antimicrobial effects on fabric, and dye cotton more effectively than leather (77). *Chlorociboria aeruginosa*, *Scytalidium cuboideum*, and *S. Ganodermophthorum* have shown promise as textile dyes, though more trials are needed and in progress (78). There is also thought of using spalted dyes as a more natural replacement for aniline dyes normally used by woodworkers. Pressure treated fungal pigments have been tested for this purpose, and are thought to be a good candidate (79).

Other research that has occurred over the past decade has focused on the implications and potential problems of introducing spalting into industry (80). Spalting is currently performed on a small lab or individual artist basis. The inoculation, growth, and pigment extraction procedures aren't up to handling the large amounts of substrate and pigment that would be needed for industry application. Investigation into inoculation techniques has begun. Thirty-five fungal species were selected for inoculation tests in sugar maple, white birch, and yellow birch, and fourteen were found to be effective in adding coloration. Twenty species of fungi were also tested to decolorize wood, and seventeen of those were found to be promising, with different species able to remove different types of color (81). The first attempt to methodically introduce fungi into non-sterile green logs for color permeation highlighted maple species and *Xylaria polymorpha* (Pers.) Grev as

good candidates for commercial spalting, utilizing zone lines as the most effective pigmentation for mass production (82).

The effect of commercial wood coatings on biological pigments were explored, and *S. cuboideum* has been shown to be well suited for use in wood products due to its ability to resist degradation and increased color permanence when coatings are applied, especially oil-based coatings (83). The pigments were not found to be very effective in blocking UV degradation, though some components did have UV resistant properties. (84). Although the dyes did not prevent UV degradation of the wood, they may be able to mask the color changing effects of weathering by remaining stable after UV exposure.

Though most past research on spalting has focused on North American and European fungi, this is currently changing. Previously, fungi from Australia and New Zealand (85, 86), as well as India (87) have been investigated, though not in great detail. Presently, Southern American fungi are being brought to attention for their spalting potential (88, 89). Many of these fungi have not been genetically analyzed and some have not even been named. This could open the world of spalting to many new colors and properties with the introduction of new fungi.

Alternative Carrier Solvents for Extracted Fungal Pigments

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for submission to *Applied Microbiology and Biotechnology*

Abstract: Five solvents were used to resolubilize the fungal pigments of *Chlorociboria aeruginosa* and *Scytalidium cuboideum*, two common fungi used in the spalting of wood pieces. These solvents were tested as a replacement for dichloromethane in the pigment application process, via analysis of their ability to increase internal color coverage inside wood substrates. Ten of thirteen wood species showed significant results in the comparison of solvent application trials. Acetonitrile was found to produce the highest internal color coverage of the solvents tested. This study shows that acetonitrile is an effective choice for the application of pigments to wood, and offers new opportunities for possible industrial spalting applications.

Introduction

Spalting is the term used for any coloration of wood caused by fungi (1). This coloration can come in multiple forms. White rot fungi produce a bleaching of wood, while pigmenting fungi deposit extracellular pigments inside of wood. The third category contains those decay fungi that create dark lines of melanin dividing sections of wood, known as zone lines (1). Some white rot fungi also produce zone lines.

Adding color to the inside of wood increases its value and improves the marketability of wood turned products. It is a method of decoration that is commonly used throughout the industrial and artistic wood working worlds. The history of spalted wood, as well as artistic use, starts in the 15th century and ranges to the present day. This work has mostly focused on using found spalted wood (1) or inoculating wood with pigmenting fungi (1).

Recently, in the last 10 years, pigments have been extracted from spalting fungi for use as dyes (2-3). These pigments are undergoing research for use in other mediums besides wood, such as fabric (4-5), as well as to increase the color on other wood pieces, with ease of application. Research has been bringing spalting pigments and spalting fungi closer to industry and mass production (6-7). Spalting pigments are an ecological alternative to currently used dyes (8), and create a new market for colored and decorated wood. Mass production of spalted wood products would create a novelty in the current consumer market, but can only be accomplished if application processes can be made efficient. This includes the extraction process, and application of dyes to wood.

In examining solvents to use when extracting fungal dyes, dichloromethane has been

determined to be most effective and is commonly used (2,3). However, in use it has been found to be ineffective in leaving pigment in the interior of wood blocks. It tends to leave pigment on the top and bottom layers of the wood, which is not effective when considering woodworking and industrial uses of the fungal dyes. This paper explores alternative carrier solvents for use with resolubilized fungal dyes from *Chlorociboria aeruginosa* and *Scytalidium cuboideum*, in order to try and find a substitute solvent for dichloromethane that increases the amount of internal pigmentation in wood blocks.

The solvents used in this experiment were acetone, acetonitrile, chloroform, pyridine, and tetrahydrofuran. Acetone is the simplest of all ketones, a colorless liquid commonly used in paints, coatings, sealants, and nail polish remover (9). Acetonitrile is the simplest of all organic nitriles, a colorless liquid with the highest polarity out of the solvents tested. It is commonly used in the production of lithium batteries, in the process of spinning fibers, and as a solvent (10). Chloroform is a volatile colorless liquid, belonging to a group of trihalomethane compounds containing chlorine atoms. The chemical has had many past uses, but is currently only used in the production of the refrigerant Freon and as a lab chemical, due to its moderate toxicity (11).

Pyridine is a basic heterocyclic organic compound, which is structurally similar to benzene, with a substitution of a methane group for a nitrogen atom. The clear or slightly yellow liquid is used as an intermediate in the production of many other chemicals and products, as well as a method of dissolving other substances, and has a high toxicity (12). Tetrahydrofuran is a cyclic ether, a clear liquid with the lowest polarity of the solvents tested. It is used as an adhesive and sealant, as well as a processing aid in petroleum production (13). Ranked from least polar to most polar, the solvents are: tetrahydrofuran, chloroform, acetone, pyridine, acetonitrile (14). Polarity is most likely a contributing factor to the success of the solvent as a pigment deposition medium.

Materials and Methods

Thirteen wood species were selected, and the wood was cut into cubes (14 mm^3) and air dried. Wood species used were: ash (*Fraxinus latifolia* Benth.*),* chinkapin *(Castanea pumila* Mill.*),* Douglas fir *(Pseudotsuga menziesii* (Mirb.) Franco*),* lodgepole pine *(Pinus contorta* Douglas*),* madrone *(Arbutus menziesii* Pursh*)*, mountain hemlock *(Tsuga mertensiana* (Bong.) Carr.*),* Oregon maple *(Acer macrophyllum* Pursh*)*, pacific silver fir (*Abies amabilis* Douglas ex. J Forbes*),* Port Orford cedar (*Chamaecyparis lawsoniana* (A. Murray) Parl.*),* red alder *(Alnus rubra* Bong.*),* sugar maple *(Acer saccharum* Marshall*),* western larch *(Larix occidentalis* Nutt.*)*, and western red cedar *(Thuja plicata* Donn ex D. Don*).*

Two fungal species were selected: *Scytalidium cuboideum* (Sacc. & Ellis) Sigler & Kang UAMH 4802 (isolated from Na-pentachlorophenate-dipped red oak lumber, location unknown) and *Chlorociboria aeruginosa* (Nyl.) Kanouse UAMH 7615 (isolated from a single isolated ascospore, in Lake District, UK).

Fungal cultures were plated and then grown on a mixture of 2% malt agar and white rotted wood chips (*Acer saccharum*) for a month, via the procedure in Robinson, 2012 (15). Pigment was extracted using dichloromethane via the procedure in Robinson (2). The extracted pigments were then color read with a Konica Minolta Chroma Meter CR-5 color reader and the program Spectramagic NX, Color Data Software, in order to meet specification standards determined by conversion from rough pigment weight to L*a*b* values (3).
The L*a*b* standards are as follows (3), and the values of the vials must fall +/- 2.0 of these standards:

C. aeruginosa: L*= 82.28, a*= -11.06, b*= -5.40
S. cuboideum: L*= 82.32, a*= 26.84, b*= 13.19

The vials of extraction solution were then evaporated with a Büchi rotovap, model 461, in a cold water bath, until only dry pigment remained in the vials, and were stored in 8 dram clear glass screw cap vials. Pigments were resolubilized using five different solvents: tetrahydrofuran (THF), pyridine, chloroform, acetonitrile (ACN), and acetone, using the method determined in Robinson, 2014 (2). The vials of pigment solution were placed on a Thermo Scientific LabQuake shaker for 24-48 hours, at room temperature (20 °C). This time was chosen as a uniform length for rotation, in order to give pigments time to solubilize. It was based on the amount of time available to perform research.

Four drip tests were performed with each solvent on the wood blocks (three blocks per wood type per test) using borosilicate glass disposable Pasteur pipettes, with reusable VWR latex pipet bulbs. Drips were on the transverse face. Drips were calibrated to the standard in Robinson, 2014 (3), with the average volume of a drop at 0.0165 mL.

Drip tests performed were: 16 drops (8 drops, wait 24 hours, 8 drops), 30 drops (15, wait 24 hours, 15 drops), 30 drops (applied in one session without waiting), and 60 drops (30 drops, wait 24 hours, 30 drops). These drip tests were performed for all solvents except for pyridine, which only had the first three performed (16, 30 drops straight, and 30 drops with waiting), as it became too viscous to allow more drops to enter the wood after 30 drops.

Blocks were left in a fume hood for 24 hours following the drip testing. This process was performed with both *C. aeruginosa* (green) and *S. cuboideum* (red) pigments.

After drying, each block was cut in half along the radial face with a Grizzly bandsaw, (61.75“ blade). The interior of one half of each block was scanned using an Epson Perfection V370 Photo scanner, and Epson Scan software. Each scan was color evaluated using the protocol for ImageJ by Robinson et al. 3-way ANOVA tests followed by Tukey HSD were run for each wood species, with percent coverage as the dependent and color, solvent, and drops as independent variables.

Results

Results for the 3-way ANOVA were significant for ash (P-value of <.0001), chinkapin (P-value of <.0001), Douglas fir (P-value of <.0001), madrone (P-value of <.0001), mountain hemlock (P-value of <.0001), Oregon maple (<.0001), Port Orford cedar (<.0001), pacific silver fir (P-value of <.0001), red alder (P-value of <.0001), and sugar maple (P-value of <.0001). Color, solvent, and drops were all significant for this group of wood species at P <0.0001.

Western larch had no internal pigment so was not used in statistical analysis. Lodgepole pine and western red cedar had no significant results in 3-way ANOVA, 2-way ANOVA, and 1-way ANOVA tests.

Ash

The two tests that were most effective for ash were *S. cuboideum* with chloroform and 30 straight drops, as well as *S. cuboideum* with acetone and 16 drops. However, these tests were not significantly different from: *S. cuboideum* with acetonitrile and 16 drops, both tests with *C. aeruginosa* with acetone and 30 drops (30 with waiting 24 hours and 30 straight), *S. cuboideum* with acetonitrile and 30 drops with waiting, *S. cuboideum* with tetrahydrofuran and 30 straight drops, *C. aeruginosa* with acetonitrile and 30 straight drops, *C. aeruginosa* with acetonitrile and 16 drops, and *C. aeruginosa* with acetone and 60 drops.

Chinkapin

The most effective test for chinkapin was *S. cuboideum* with acetone and 60 drops. This test had a 100% coverage average. However, it did not differ significantly from: *S. cuboideum* with acetonitrile and 30 straight drops, with acetone and 30 straight drops, with pyridine and 16 drops, with tetrahydrofuran and 60 drops, with acetonitrile and 30 drops with waiting, with pyridine and 30 drops with waiting, with tetrahydrofuran and 30 drops with waiting, with acetonitrile and 60 drops, with chloroform and 60 drops, with tetrahydrofuran and 30 straight drops, with pyridine and 30 straight drops, and with tetrahydrofuran and 16 drops. No tests with *C. aeruginosa* were in the top group.

Douglas Fir

The most effective tests for Douglas fir were *S. cuboideum*, one with pyridine and 16 drops and the other with acetone and 30 straight drops. These two tests were determined significantly different compared to every other test performed on Douglas fir. Douglas fir had 6 other tests that had a percentage besides 0.00 for mean percent coverage: *S. cuboideum* with tetrohydrafuran and 30 straight, *S. cuboideum* with pyridine and 30 straight drops, *C. aeruginosa* with pyridine and 16 drops, *S. cuboideum* with pyridine and 30 drops with waiting, *S. cuboideum* with acetonitrile and 30 drops with waiting, and *C. aeruginosa* with acetone and 16 drops. All other tests had no measurable internal pigment.

Madrone

Madrone had a large group of tests that were the most effective: *S. cuboideum* with acetone and 60 drops, *S. cuboideum* with acetonitrile and 30 drops with waiting, *C. aeruginosa* with acetonitrile and 16 drops, *C. aeruginosa* with acetone and 60 drops, *C. aeruginosa* with acetone and 16 drops, *S. cuboideum* with chloroform and 30 straight drops, *S. cuboideum* with acetone and 30 straight drops, *S. cuboideum* with acetone and 16 drops, *S. cuboideum* with pyridine and 30 drops with waiting, *C. aeruginosa* with acetonitrile and 30 drops with waiting, *S. cuboideum* with pyridine and 30 straight drops, and *S. cuboideum* with acetonitrile and 30 straight drops. All of these tests had a mean percent coverage of 85% or higher, with two highest means being 100% coverage (*S. cuboideum* with acetone and 60 drops, *S. cuboideum* with acetonitrile and 30 drops with waiting). However, these tests could not be determined significantly different from: *C. aeruginosa* with acetonitrile and 30 straight drops, *S. cuboideum* with Pyridine and 16 drops, *S. cuboideum* with acetonitrile and 16 drops, *S. cuboideum* with acetonitrile and 60 drops, *S. cuboideum* with chloroform and 60 drops, *C. aeruginosa* with acetone and 30 drops with waiting, *S. cuboideum with acetonitrile* and 60 drops, and *C. aeruginosa* with acetone and 30 straight drops.

Mountain Hemlock

The most effective test for mountain hemlock was *S. cuboideum* with acetonitrile and 30 straight drops. This test could not be determined to be significantly different from: *S. cuboideum* with acetonitrile and 30 drops with waiting, *S. cuboideum* with acetone and 60 drops, *S. cuboideum* with acetone and 30 drops with waiting, *C. aeruginosa* with pyridine and 30 drops with waiting, *S. cuboideum* with pyridine and 16 drops, *S. cuboideum* with pyridine and 30 drops with waiting, and *C. aeruginosa* with pyridine and 30 straight drops.

Oregon Maple

The test that was most effective for Oregon maple was *S. cuboideum* with tetrahydrofuran and 30 straight drops. This could not be determined significantly different from *S. cuboideum* with acetonitrile and 30 drops with waiting. This was the only wood species where tetrahydrofuran was in the most effective test group.

Port Orford Cedar

The most effective test for Port Orford cedar was *S. cuboideum* with acetonitrile and 30 straight drops. This could not be determined significantly different from: *S. cuboideum* with acetonitrile and 30 drops with waiting and *S. cuboideum* with tetrahydrofuran and 30 straight drops.

Pacific Silver Fir

The most effective test for pacific silver fir was *S. cuboideum* with acetonitrile and 30 drops with waiting. This test was significantly different when compared to every other test performed on pacific silver fir.

Red Alder

The most effective test for red alder was *S. cuboideum* with acetonitrile and 30 drops with waiting. This test was determined to be significantly different compared to every other test performed on red alder. This test had a percent mean coverage of 54.333, and only 5 other tests had a mean percent overage over 0.00: *S. cuboideum* with acetonitrile and 16 drops, *S. cuboideum* with tetrahydrofuran and 30 straight drops, *S. cuboideum* with acetonitrile and 30 straight drops, *C. aeruginosa* with acetone and 16 drops, and *S. cuboideum* with chloroform and 30 straight drops. None of those tests had a mean percent coverage over 10.00.

Sugar Maple

The most effective test for Sugar Maple was *S. cuboideum* with acetonitrile and 30 drops with waiting. This test could not be determined significantly different against two other tests: *S. cuboideum* with pyridine and 16 drops and *S. cuboideum* with pyridine and 30 drops with waiting. No test with *C. aeruginosa* had over 3.667 mean percent coverage.

Western Larch, Lodgepole Pine, and Western Red Cedar

None of these tests had significantly significant results.

Discussion

In the analysis of this experiment's data, acetonitrile combinations worked the most effectively over the other solvents. Seven wood species (Douglas fir, madrone, mountain hemlock, Port Orford cedar, pacific silver fir, red alder, and sugar maple) had an acetonitrile combination in the most statistically significant and effective grouping for all of the tests. Ten tests in the collection of effective combinations used acetonitrile. Acetone was included in the most effective trials of four wood species (ash, chinkapin, Douglas fir, and madrone), with eight tests overall in that group. Chloroform and pyridine had two wood species (ash and madrone for chloroform, Douglas fir and madrone for pyridine), which included those solvents in their most effective trials. Tetrahydrafuran was only included in one wood species' most effective tests (Oregon maple).

Of all the most effective tests with significant statistics, only madrone had tests with a combination involving *C. aeruginosa* in the top group. Including all tests that could not be determined significant from the most effective tests, only five wood species had combinations with *C. aeruginosa* (ash, Douglas fir, madrone, mountain hemlock, red alder). The solvent and *C. aeruginosa* combinations had the highest percent of color coverage with were different from those *S. cuboideum* combinations which had a high percent coverage. Seven of the significant *C. aeruginosa* tests were in combination with acetone, while five were with acetonitrile, and three with pyridine. No combinations containing chloroform or tetrahydrofuran and *C. aeruginosa* were statistically significant and effective.

Dichloromethane was determined to be the most effective solvent for use in pigment extraction and resolubilization by Robinson, 2014 (2). Acetonitrile also demonstrated strong solvency (2). Overall, acetonitrile was not as effective at carrying pigment as dichloromethane, and so dichloromethane has been used for following drip test research (2). Finding a solvent that removes the most pigment per drop of solvent maximizes the amount of color that can be transferred to the wood piece per extraction, which intensifies the possible color and reduces the amount of solute needed to perform the process. Though multiple solvents tested were able to resolubilize pigment, dichloromethane was the most effective at removing color and picking up pigment from the fungus.

Acetonitrile (CH_3CN) is a dipolar aprotic solvent. Though it has a larger dipole moment (3.90 D) than dichloromethane (1.60 D) (16) and is more polar (18.0 δP compared to 7.3 δP) (14), acetonitrile has less hydrogen bonding capacity than dichloromethane (6.1 δH compared to 7.1 δH) (16). As suggested in Robinson, 2014, effective extraction may be more connected to molecular hydrogen bonding than dipole and polarity interactions (2).

Based on this conclusion, dichloromethane is still the best solvent for extracting pigment. Acetonitrile may be a better candidate for resolubilization and application in art and industry. In order to use dyed wood efficiently for industry purposes (such as furniture or flooring), the wood needs to be colored throughout, instead of just on the top and bottom surfaces of the piece. Because acetonitrile holds onto pigment molecules less tightly (due to reduced ability to hydrogen bond) but has a large amount of polarity, it may be able to hold onto pigment molecules (often high-molecular-weight polyaromatics (2)) for a long enough time to enter the wood, but be unable to pull them through entirely. This leads to the dye molecules being deposited inside the piece of wood, rather than on the surfaces.

For this experiment, L*a*b* values were collected for each block, but not analyzed. ΔE* (the total color difference) could have been used for analysis, by determining the difference between each variable (L* (light/dark), a* (red/green), b* (yellow/blue)) between different blocks/tests (12). However, percent coverage was chosen instead, because in order to be useful for application purposes, color change on a small scale needs to be visible. L*a*b* values, though intended to approximate human color vision, pick up many colors that are outside the human vision range (17). Even if color could be measured, it was not counted unless it was easily visible to the human eye. Colors outside the human vision range are not effective for industry use. The comparison of percent coverage method was also used in Robinson, 2014 (3).

Acetonitrile is a considerably less toxic solvent than dichloromethane, and also cheaper. It has been classified as Group D by the EPA (18), not classifiable to human carcinogenicity. This increases the possibility that it can be used in commercial dyes, due to the lower toxicity threat. As consumers will not be extracting their own pigment, instead purchasing it from a company that would complete the extraction process, dichloromethane would still be used for extraction processes in the company or lab facilities. Pigment could then be converted to dry pigment and resolubilized with

acetonitrile for the customer to purchase and use. This would increase the marketability of spalted dyes overall.

Appendices: Figures

Figure Legend

Test

16: 8 drops, wait 24 hours, 8 drops
30w: 15 drops, wait 24 hours, 15 drops
30s: 30 drops without a 24 wait
60: 30 drops, wait 24 hours, 30 drops

Pigment

R: *S. cuboideum*
G: *C. aeruginosa*

Solvent

A: acetone
AC: acetonitrile
C: chloroform
THF: tetrahydrofuran
P: pyridine

Test	Pigment	Solvent	Mean percent coverage	Tukey group	Standard deviation
30s	r	C	61	A	16.81
16	r	A	59.33	A	14.71
16	r	AC	46	AB	12.12
30w	g	A	35	AB	10.29
30s	g	A	33.67	AB	9.11
30w	r	AC	30	AB	7.72
30s	g	AC	20	AB	6.30
30s	r	THF	20	AB	5.64
16	g	AC	19	AB	4.76
60	g	A	16	AB	3.67
60	r	AC	8.67	B	2.53
30w	g	AC	8.33	B	2.13
60	r	C	6	B	1.61
30w	g	C	4	B	1.26
16	r	P	4	B	1.07
16	g	P	3.33	B	0.80
60	g	THF	1.33	B	0.50
30w	r	A	1.33	B	0.45
16	r	C	1.33	B	0.38
30s	g	P	1	B	0.27
30w	r	C	0.67	B	0.17
16	g	C	0.33	B	0.08
16	g	A	0	B	0.00
60	g	AC	0	B	0.00
60	g	C	0	B	0.00
30s	g	C	0	B	0.00
30w	g	P	0	B	0.00
30s	g	THF	0	B	0.00
30w	g	THF	0	B	0.00
16	g	THF	0	B	0.00
30s	r	A	0	B	0.00
60	r	A	0	B	0.00
30s	r	AC	0	B	0.00
30s	r	P	0	B	0.00
30w	r	P	0	B	0.00
30w	r	THF	0	B	0.00
16	r	THF	0	B	0.00
60	r	THF	0	B	------

Figure 1. Statistical analysis results for ash. Different letters under Tukey group represent significant differences at alpha = 0.05.

Test	Pigment	Solvent	Mean percent coverage	Tukey group	Standard deviation
60	r	A	100	A	32.76316848
30s	r	AC	98.67	AB	31.16938859
30s	r	A	92.33	ABC	29.35449505
16	r	P	89.33	ABCD	27.62351037
60	r	THF	85	ABCD	25.748445
30w	r	AC	72.33	ABCDE	23.79886886
30w	r	P	67	ABCDEF	22.42932181
30w	r	THF	65.67	ABCDEFG	21.16264915
60	r	AC	62.67	ABCDEFG	19.70483271
60	r	C	60	ABCDEFG	18.14571742
30s	r	THF	59	ABCDEFG	16.42016798
30s	r	P	46.67	ABCDEFG	14.26177148
16	r	THF	38.33	ABCDEFG	12.88349208
30s	g	P	32.67	BCDEFG	11.97909081
30w	g	P	29.33	BCDEFG	11.35511127
30s	g	THF	28.67	CDEFG	10.84931529
16	r	A	27	CDEFG	10.24984566
30w	r	A	26.67	CDEFG	9.626893531
16	r	AC	26.33	CDEFG	8.822141463
30w	r	C	24.33	CDEFG	7.741064421
30w	g	C	23	CDEFG	6.491915838
16	g	P	13.33	EFG	4.775476493
16	g	C	11.67	EFG	4.214816673
60	g	THF	10	EFG	3.666537227
60	g	A	8.33	EFG	3.143682309
30s	g	C	7.67	EFG	2.666528282
16	g	A	6.67	EFG	2.005850534
60	g	C	3	FG	0.958450454
30s	r	C	1.33	FG	0.497327301
16	r	C	1	FG	0.333333333
30w	g	A	0	G	0
30s	g	A	0	G	0
30s	g	AC	0	G	0
16	g	AC	0	G	0
30w	g	AC	0	G	0
60	g	AC	0	G	0
30w	g	THF	0	G	0
16	g	THF	0	G	------

Figure 2. Statistical analysis results for chinkapin. Different letters under Tukey group represent significant differences at alpha = 0.05.

Test	Pigment	Solvent	Mean percent coverage	Tukey group	Standard Deviation
16	r	P	25	A	17.67766953
30s	r	AC	19.667	A	13.90666907
30s	r	THF	10	B	7.071067812
30s	r	P	8.333	BC	5.892320808
16	g	P	7.667	BC	5.421387691
30w	r	P	5.333	BC	3.771000464
30w	r	AC	4	BC	2.828427125
16	g	A	0.333	C	0.235466558
30w	g	A	0	C	0
30s	g	A	0	C	0
60	g	A	0	C	0
30s	g	AC	0	C	0
16	g	AC	0	C	0
30w	g	AC	0	C	0
60	g	AC	0	C	0
30w	g	C	0	C	0
16	g	C	0	C	0
60	g	C	0	C	0
30s	g	C	0	C	0
30s	g	P	0	C	0
30w	g	P	0	C	0
60	g	THF	0	C	0
30s	g	THF	0	C	0
30w	g	THF	0	C	0
16	g	THF	0	C	0
16	r	A	0	C	0
30w	r	A	0	C	0
30s	r	A	0	C	0
60	r	A	0	C	0
16	r	AC	0	C	0
60	r	AC	0	C	0
30s	r	C	0	C	0
60	r	C	0	C	0
16	r	C	0	C	0
30w	r	C	0	C	0
30w	r	THF	0	C	0
16	r	THF	0	C	0
60	r	THF	0	C	-------

Figure 3. Statistical analysis results for Douglas fir. Different letters under Tukey group represent significant differences at alpha = 0.05.

Test	Pigment	Solvent	Mean percent coverage	Tukey group	Standard deviation
60	r	A	100	A	70.71067812
30w	r	AC	100	A	70.71067812
60	g	A	96.67	A	68.35601254
16	g	AC	96.67	A	68.35601254
16	g	A	93.33	A	65.99427589
30s	r	C	93.33	A	65.99427589
30s	r	A	90	A	63.63961031
16	r	A	88.33	A	62.45874198
30w	g	AC	86.67	A	61.28494473
30w	r	C	86.67	A	61.28494473
30s	r	AC	85	A	60.1040764
30s	r	P	85	A	60.1040764
30s	g	AC	83.33	AB	58.92320808
16	r	P	83.33	AB	58.92320808
16	r	AC	68.33	AB	48.31660636
60	r	AC	60	ABC	42.42640687
30w	g	A	53.33	ABC	37.71000464
60	r	C	53.33	ABC	37.71000464
60	g	AC	43.33	ABC	30.63893683
30s	g	A	40	ABC	28.28427125
30w	r	A	20	BC	14.14213562
30w	g	C	0	C	0
16	g	C	0	C	0
60	g	C	0	C	0
30s	g	C	0	C	0
16	g	P	0	C	0
30s	g	P	0	C	0
30w	g	P	0	C	0
60	g	THF	0	C	0
30s	g	THF	0	C	0
30w	g	THF	0	C	0
16	g	THF	0	C	0
16	r	C	0	C	0
30w	r	P	0	C	0
30s	r	THF	0	C	0
30w	r	THF	0	C	0
16	r	THF	0	C	0
60	r	THF	0	C	--------

Figure 4. Statistical analysis results for madrone. Different letters under Tukey group represent significant differences at alpha = 0.05.

Test	Pigment	Solvent	Mean percent coverage	Tukey group	Standard deviation
30s	r	AC	58.33	A	13.63468346
30w	r	AC	34.67	AB	10.90424041
60	r	A	33.33	AB	9.964252996
30w	r	A	33	AB	8.912968476
30w	g	P	26.67	AB	7.588855072
16	r	AC	25.67	AB	6.577965125
16	r	P	21.33	AB	5.33714531
30w	r	P	14.33	AB	4.21673981
30s	g	P	13.67	AB	3.642180239
30s	r	THF	11.67	B	2.941036101
16	g	P	9	B	2.241555907
60	r	THF	6.67	B	1.664332297
16	g	THF	4.33	B	1.219139924
60	r	AC	4.33	B	0.981007136
16	g	A	2.33	B	0.584760573
60	g	A	1.33	B	0.413416786
30s	g	THF	1.33	B	0.349429963
30w	g	C	0.67	B	0.250425352
60	g	THF	0.67	B	0.227065954
16	r	THF	0.67	B	0.193803119
60	g	AC	0.33	B	0.141171569
30s	r	C	0.33	B	0.129674366
60	r	C	0.33	B	0.112716458
30w	r	THF	0.33	B	0.085205634
30w	g	A	0	B	0
30s	g	A	0	B	0
30s	g	AC	0	B	0
16	g	AC	0	B	0
30w	g	AC	0	B	0
16	g	C	0	B	0
60	g	C	0	B	0
30s	g	C	0	B	0
30w	g	THF	0	B	0
16	r	A	0	B	0
30s	r	A	0	B	0
16	r	C	0	B	0
30w	r	C	0	B	0
30s	r	P	0	B	------

Figure 5. Statistical analysis results for mountain hemlock. Different letters under Tukey group represent significant differences at alpha = 0.05.

Test	Pigment	Solvent	Mean percent coverage	Tukey group	Standard deviation
30s	r	THF	45	A	8.680633677
30w	r	AC	28	AB	5.565636864
30w	g	C	12.333	B	3.782345146
16	g	P	12.333	B	3.46670853
60	g	C	11.667	B	3.070367844
30w	g	AC	8.333	B	2.627743165
30s	g	P	8.333	B	2.409626761
16	r	AC	7.333	B	2.132186867
16	g	THF	6.667	B	1.881885836
16	r	P	5	B	1.629718879
16	g	A	4.667	B	1.488536853
30s	g	C	4	B	1.341221577
16	g	AC	3.333	B	1.22224987
30w	g	P	3.333	B	1.137383793
60	r	THF	3	B	1.026230869
30s	g	THF	2.667	B	0.918033618
30s	r	AC	2.333	B	0.814248142
30w	r	THF	2.333	B	0.717137189
30w	r	A	1.667	B	0.575129301
30s	r	C	1.667	B	0.487023313
60	r	AC	1.333	B	0.345584269
16	r	A	0.667	B	0.176237494
30w	r	C	0.333	B	0.08325
30w	g	A	0	B	0
30s	g	A	0	B	0
60	g	A	0	B	0
30s	g	AC	0	B	0
60	g	AC	0	B	0
16	g	C	0	B	0
60	g	THF	0	B	0
30w	g	THF	0	B	0
30s	r	A	0	B	0
60	r	A	0	B	0
60	r	C	0	B	0
16	r	C	0	B	0
30s	r	P	0	B	0
30w	r	P	0	B	0
16	r	THF	0	B	---------

Figure 6. Statistical analysis results for Oregon maple. Different letters under Tukey group represent significant differences at alpha = 0.05.

Test	Pigment	Solvent	Mean percent coverage	Tukey group	Standard deviation
30s	r	AC	72.333	A	18.93788755
30w	r	AC	66.667	AB	16.55197035
30s	r	THF	64	ABC	14.06366274
16	r	AC	38.333	BCD	10.99263449
60	r	AC	33.667	CDE	9.990523319
30s	r	C	30	DEF	9.161132285
30w	r	P	24	DEF	8.452858684
16	r	A	23.667	DEF	8.061604653
60	r	A	20.333	DEF	7.606668315
16	g	P	20	DEF	7.298779995
60	g	A	19.667	DEF	6.942657127
30w	r	A	19	DEF	6.524199216
16	r	P	18	DEF	6.053828939
30w	r	THF	17.667	DEF	5.547984489
16	r	C	14	DEF	4.91556518
30w	g	C	12.333	DEF	4.515009667
30w	g	P	11.333	DEF	4.171497291
16	g	C	11	DEF	3.82719032
30s	r	P	10.333	DEF	3.396556569
60	g	C	8.667	DEF	2.88259425
16	r	THF	7.333	DEF	2.417890838
30s	r	A	6	EF	1.968097067
60	r	THF	4.667	EF	1.545471588
30w	r	C	4.333	EF	1.173733705
30s	g	C	1.667	F	0.501599403
60	g	THF	1	F	0.277350098
30w	g	A	0	F	0
30s	g	A	0	F	0
16	g	A	0	F	0
30s	g	AC	0	F	0
16	g	AC	0	F	0
30w	g	AC	0	F	0
60	g	AC	0	F	0
30s	g	P	0	F	0
30s	g	THF	0	F	0
30w	g	THF	0	F	0
16	g	THF	0	F	0
60	r	C	0	F	--------

Figure 7. Statistical analysis results for Port Orford cedar**.** Different letters under Tukey group represent significant differences at alpha = 0.05.

Test	Pigment	Solvent	Mean percent coverage	Tukey group	Standard deviation
30w	r	AC	75.333	A	13.67372523
30s	r	AC	34.667	B	7.278108196
30s	r	P	25.667	BC	5.111995012
30w	r	P	13	BC	3.314713859
16	g	P	10.333	BC	2.756421778
60	g	C	8.333	BC	2.337971142
30w	g	C	6.667	BC	2.021618611
16	r	P	6.667	BC	1.795858251
16	g	A	6.333	BC	1.494992594
30w	r	A	3.667	C	1.110482244
30s	r	THF	3.333	C	0.974278572
30w	g	P	3	C	0.833373621
16	r	AC	2.333	C	0.684868474
30s	g	C	2	C	0.576740554
60	r	THF	1.667	C	0.474636947
16	g	C	1.333	C	0.381841388
30w	r	THF	1	C	0.30394715
60	g	THF	0.667	C	0.24887372
16	r	A	0.667	C	0.223642166
16	r	THF	0.667	C	0.187301614
30s	g	P	0.333	C	0.127699671
60	r	AC	0.333	C	0.110591159
30s	r	C	0.333	C	0.08325
30w	g	A	0	C	0
30s	g	A	0	C	0
60	g	A	0	C	0
30s	g	AC	0	C	0
16	g	AC	0	C	0
30w	g	AC	0	C	0
60	g	AC	0	C	0
30s	g	THF	0	C	0
30w	g	THF	0	C	0
16	g	THF	0	C	0
30s	r	A	0	C	0
60	r	A	0	C	0
60	r	C	0	C	0
16	r	C	0	C	0
30w	r	C	0	C	-------

Figure 8. Statistical analysis results for Pacific silver fir. Different letters under Tukey group represent significant differences at alpha = 0.05.

Test	Pigment	Solvent	Mean percentage cover	Tukey group	Standard deviation
30w	r	AC	54.333	A	8.986230639
16	r	AC	10	B	2.286782065
30s	r	THF	9	B	1.686826908
30s	r	AC	4.333	BC	0.848221301
16	g	A	2.667	C	0.459217784
30s	r	C	0.333	C	0.057967859
30w	g	A	0	C	0
30s	g	A	0	C	0
60	g	A	0	C	0
30s	g	AC	0	C	0
16	g	AC	0	C	0
30w	g	AC	0	C	0
60	g	AC	0	C	0
30w	g	C	0	C	0
16	g	C	0	C	0
60	g	C	0	C	0
30s	g	C	0	C	0
16	g	P	0	C	0
30s	g	P	0	C	0
30w	g	P	0	C	0
60	g	THF	0	C	0
30s	g	THF	0	C	0
30w	g	THF	0	C	0
16	g	THF	0	C	0
16	r	A	0	C	0
30w	r	A	0	C	0
30s	r	A	0	C	0
60	r	A	0	C	0
60	r	AC	0	C	0
60	r	C	0	C	0
16	r	C	0	C	0
30w	r	C	0	C	0
16	r	P	0	C	0
30s	r	P	0	C	0
30w	r	P	0	C	0
30w	r	THF	0	C	0
16	r	THF	0	C	0
60	r	THF	0	C	-------

Figure 9. Statistical analysis results for red alder. Different letters under Tukey group represent significant differences at alpha = 0.05.

Test	Pigment	Solvent	Mean percent coverage	Tukey group	Standard deviation
30w	r	AC	24.667	A	4.925198209
16	r	P	12.333	AB	3.346658257
30w	r	P	11	AB	2.909681225
16	r	THF	9	B	2.486661491
30w	r	A	7	B	2.158299221
30s	r	THF	6.333	B	1.945301696
30s	r	P	5.667	B	1.740823621
30w	r	THF	5.333	B	1.547692322
30s	r	AC	4.333	B	1.332417991
30w	g	C	3.667	B	1.167459394
60	r	THF	3.667	B	1.028329906
30s	g	P	3.333	B	0.837305453
60	r	AC	2.333	B	0.608752132
16	r	AC	1.667	B	0.451376506
60	r	A	1	B	0.339309954
60	r	C	1	B	0.291878061
16	r	C	1	B	0.221480013
30w	g	P	0.333	B	0.072666557
30w	g	A	0	B	0
30s	g	A	0	B	0
60	g	A	0	B	0
16	g	A	0	B	0
30s	g	AC	0	B	0
16	g	AC	0	B	0
30w	g	AC	0	B	0
60	g	AC	0	B	0
16	g	C	0	B	0
60	g	C	0	B	0
30s	g	C	0	B	0
16	g	P	0	B	0
60	g	THF	0	B	0
30s	g	THF	0	B	0
30w	g	THF	0	B	0
16	g	THF	0	B	0
16	r	A	0	B	0
30s	r	A	0	B	0
30s	r	C	0	B	0
30w	r	C	0	B	------

Figure 10. Statistical analysis for sugar maple. Different letters under Tukey group represent significant differences at alpha = 0.05.

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