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The influence of pH on pigment formation by lignicolous fungi

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ABSTRACT

Wood-decay patterns are strongly influenced by the conditions of the wood substrate, and the pH of the substrate is one of the most important factors. As a reaction to a stressed environment, some lignicolous fungi respond with pigment formation that helps to isolate and protect their mycelia; other fungi, with high specificity, produce pigmentation regardless of the changes in the conditions in which the fungus grows. These changes result in only minor variation in the color intensity of the pigment. The occasional dark-colored reaction pigment, also known as melanin, is the most common pigment formed by wood-decay fungi. To investigate pigment formation under the influence of pH variation, sugar maple and beech samples adjusted with buffer solutions to different pH values were inoculated with various basidiomycetes and ascomycetes fungi, known to produce pigmentation. Based on the range and increments of the pH treatments tested on the wood substrate, maximum pigmentation and minimum mass loss occur at adjacent values of pH treatments for all wood–fungus combinations, and never coincide. Maximum pigment production occurred at treatment with pH 4.5 for beech and sugar maple inoculated with *Trametes versicolor*, while *Xylaria polymorpha* produced external pigmentation in beech treated with buffer at pH 5 and sugar maple at pH 4.5. Fungi tested in agar substrate produced maximum pigmentation at the pH range 4–5.5, except for *Scytallidium cuboideum*, which produce maximum intensity of red pigment at pH 6 and blue pigment at pH 8.

1. Introduction

Many wood-decay fungi inhabiting living trees, dead trunks, or logs produce certain types of pigmentation as secondary metabolites (Margalith, 1992; Duran et al., 2002).

Fungi produce pigmentation as a response to antagonistic reactions by other fungi. These pigmented substances are secondary metabolites that act as physical and chemical barriers in the wood substrate. *Scytalidium* species are among most common studied stain fungi. They produce extracellular pigmentation, which plays an important role in controlling the growth of other fungi. The diffusible toxic factor that is produced seems to be altered at pH 7, and restored when the pH is readjusted to 5. The toxic factor appears active even after autoclaving the wood (Brian, 1957). Although the studied fungus *Scytalidium cuboideum* (Sacc. & Ellis) Sigler & Kang is strongly cellulolytic (Eriksson et al., 1990), it can colonize and stain many deciduous and coniferous woods without causing significant degradation of the wood structure (Schmidt and Diez, 1985). In addition to the most common red pigmentation that is found, the fungus can also produce a tyrian blue pigment in culture media (Chidester, 1940). As reported also by Golinski et al. (1995), the red pigment of *S. cuboideum*, which is similar to that of naphtoqiunones formed by *Fusarium moniliforme* J. Sheld., can change from red to blue when dissolved in solvents with higher polarity, and in basic solution. Although the fungus was initially classified as *Arthrographis* by Sigler and Carmichael (1976), recent phylogenetic analysis suggests it is more appropriately placed in *Scytalidium* (Kang et al., 2010).

Other pigments produced by *Monascus* species are in the carotenoid group, ranging in color from yellow to orange and red (Duran et al., 2002, 2009). Some functional metabolite compounds are known to have antibacterial and antioxidant activity and to inhibit mutagenesis (Juzlova et al., 1996), while others are of great interest to the food industry (DeCarvalho et al., 2005).

Chlorociboria species produce a green pigment, xylindein. The bright green wood has been used in intarsia works on furniture and wood panels since the 15th century (Blanchette et al., 1992). Although the pigment has been thoroughly studied (Gümbel, 1858; Rommier, 1868; Blackburn et al., 1962; Saikawa et al., 2000), there have been few known properties and functions assigned to it. While Rommier (1868) studied its dyeing properties on silk and wool, most recent studies determined that xylindein inhibits plant

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germination without any hazardous effects on cultivated crops (Shibata et al., 2007).

The black pigment formation, seen as fine demarcation lines in all wood sections, are common between mycelia of white and brown-decay fungi, due to mutual antagonism (Rayner and Todd, 1979). Also known as fungal melanin (Nicolaus et al., 1964; Prota et al., 1989; Jacobson and Tinnell, 1993; Butler and Day, 1998), the black pigment has a protective role for fungal hyphae, and is produced in antagonistic reaction to other fungi that colonize the substrate in the immediate vicinity (Adams and Roth, 1967, 1969; Barrett and Uscuplic, 1971), and as a reaction to extreme conditions such as desiccation or UV radiation (Campbell, 1933, 1934; Pearce, 1991; Butler and Day, 1998; Henson et al., 1999). Melanin formation is produced by fungal pathogens as a reaction to host responses, or for host invasion that requires appressorial turgor pressure (Howard et al., 1991). Melanized pigment production, as a form of fungal survival strategy in wood decay, is often characterized by granule depositions in the lumens of the wood cells to form distinctive zone lines, and is associated with slower fungal growth and delay of wood mass loss (Bell and Wheeler, 1986).

Fungal pigmentation is affected by the availability of nutrients, and by pH and temperature (Lichter and Mills, 1998). However, the conditions responsible for this pigment formation are unclear. It is not known what triggers melanin formation in the absence of antagonistic reactions and extreme environmental conditions. Previous research has shown that fungi such as the ascomycete *Xylaria polymorpha* (Pers.) Grev. and the basidiomycete *Trametes versicolor* (L.) Lloyd are capable of producing black pigment in monoculture (Rayner and Todd, 1977, 1979; Williams et al., 1981; Robinson et al., 2007), by different biosynthesis pathways (Bell and Wheeler, 1986). Many ascomycete fungi, including *X. polymorpha*, synthesize pentaketide melanin from the polyketide monomer 1,8-dihydroxynaphthalene (DHN). Basidiomycete fungi may produce melanin from phenols and catechol precursors, characterized by a dark brown color, in contrast to the true black appearance of DHN melanin (Wheeler, 1983; Butler et al., 2001). Taylor et al. (1988) argue that *T. versicolor* produces melanin by the catechol pathway, and Wheeler and Bell (1988) indicate that the secretion of phenols produces dark pigmentation of the wood substrate, especially at pH levels above 7.

Hydrogen ion concentration of the wood substrate could vary within the tree along and across the stem, and this factor is thought to influence the behavior of wood-degrading fungi by affecting enzyme activity for wood degradation and metabolism (Pearce, 1991; Schmidt, 2006). It is known that basidiomycete fungi prefer a slightly acidic environment, while ascomycetes tolerate a more alkaline substrate. Fungi are also capable of changing the substrate pH value through metabolic regulation activity (Humar et al., 2001). All these aspects of pH variation may play an important role in fungal biodiversity distribution and species succession on wood debris.

The goal of this research was to determine to what extent the pH conditions influence fungal pigment formation, and if the substrate pH variation could be considered a stress factor that could trigger pigmentation by different fungi. Two common North American wood species with contrasting natural spalting prevalence, sugar maple (*Acer saccharum* Marsh), which readily spalts (Robinson et al., 2012), and American beech (*Fagus grandifolia* Ehrh.), which rarely spalts, were tested against *X. polymorpha* and *T. versicolor*. To extend the degree of comparison of fungal behavior for melanin production by different biosynthesis pathways, along with production of other types of pigments, we tested additional ascomycetes and basidiomycetes fungi in malt extract agar at different pH values. The ability to alter fungal pigments through pH changes in wood offers a chance to broaden the color palette currently available with spalted woods. It also offers an avenue to spalt wood that is not easily spalted, a process that adds considerable value to underutilized hardwood species.

2. Materials and methods

2.1. Wood and fungi species selection

Two wood species harvested in southern Ontario were selected for testing: sugar maple (*A. saccharum* Marshall) and beech (*F. grandifolia* Ehrh.) The average oven-dry specific gravity of the wood species used for testing was SG = 0.68 for sugar maple and SG = 0.74 for beech.

Nine fungi were selected based on their spalting ability. Wood testing involved three strains of the ascomycete *X. polymorpha* (Pers.) Grev.: strains UAMH 11518, UAMH 11519, and UAMH 11520; and three strains of the basidiomycete *T. versicolor* (L.) Lloyd: strain MAD 697 from the Forest Products Laboratory in Madison, WI, USA, isolated from a cankered area of *F. grandifolia* in Vermont, USA; strain R105 from the Forest Products Laboratory in Madison, WI, USA, isolated from a dead branch of *Malus* sp. in New York, USA; and strain UAMH 11521, isolated from *A. saccharum* in Houghton, MI, USA.

In-vitro tests on agar media utilized the same strains of *X. polymorpha* and *T. versicolor*, along with an additional seven fungal species, one strain per species, as follows: *Polyporus squamosus* (Huds.) Fr. UAMH 11653, isolated from beech in Toronto, ON, Canada; *Polyporus brumalis* (Pers.) Fr UAMH 11652, isolated from sugar maple in the Toronto area, ON, Canada; *Fomes fomentarius* (L.) J.J. Kickx UAMH 11654, isolated from birch in Haliburton Forest, ON, Canada; and *Inonotus hispidus* (Bull.) P. Karst. F2037, of unknown origin. Staining fungi investigated for pigment formation were *Monascus ruber* Tiegh. UAMH 416, isolated from silage in West Virginia, USA; *Chlorociboria aeruginascens* (Nyl.) Kanouse ex C.S. Ramamurthi, Korf & L.R. Batra UAMH 11655, isolated from sugar maple in Haliburton Forest, ON, Canada; and *Scytalidium cuboideum* (Sacc. & Ellis) Sigler & Kang UAMH 4802, isolated from Na-pentachlorophenate-dipped red oak lumber.

All fungi used for inoculation were grown on 95 × 15 mm petri dishes with 2% malt extract agar at 21 °C for 2 wk prior to inoculation. These conditions are considered the standard optimal growing conditions for most North American fungi (AWPA, 2009, E10-06).

2.2. Test procedure

2.2.1. pH test preparation

To avoid the eventual influence of soil substrates on fungal pigment formation, the experiments were performed using a modified decay jar test with vermiculite instead of soil, as outlined in Robinson et al. (2009b). Sugar maple and beech 14-mm cubes, nine replicates per set, were weighed and treated under vacuum for 1 h with potassium phosphate buffer adjusted to pH 4.5, 5, 5.5, 6, and 6.5. The pH range was selected within the normal values characteristic for wood. After treatment, wood blocks were weighed to determine retention, and kept overnight at 4 °C for conditioning. One additional set was added as controls.

To expand on the fungal behavior in regard to pH variation of the substrate, petri dishes of 2% malt extract agar, five replicates per set, were prepared with distilled water adjusted to a pH range from 2 to 8 in increments of 0.5. One additional set per treatment was added to measure the final pH of the media, and one additional set without any treatment of the distilled water was added as a control.

Table 1
pH variation in beech and sugar maple samples after treatment with phosphate buffer at different pH-values.

Wood sp./Treatment	4.50	5.00	5.50	6.00	6.50	Control samples
Beech	4.85	4.65	4.93	5.26	5.46	4.29
Beech SD	0.22	0.16	0.14	0.20	0.24	0.23
Sugar maple	4.88	4.87	5.00	5.32	5.53	4.72
Sugar maple SD	0.07	0.13	0.05	0.03	0.34	0.05

2.2.2. Inoculation and incubation

Culture jars with vermiculite and distilled water were sterilized in an autoclave for 30 min and cooled overnight in a laminar flow hood. Wood blocks cut into 14-mm cubes were first conditioned at 50 °C to provide an initial dry weight and then surface steam sterilized for 30 min under conditioned pressure at 100 °C, and placed in vermiculite after cooling, with three blocks placed per jar. Wood blocks were not autoclaved. A strip of inoculum of roughly 0.5 × 2 cm was placed on the vermiculite at equal distance from the three wood blocks. Inoculated jars were incubated at 27 °C ± 2 °C and 80% ± 5% relative humidity for 8 wk for *T. versicolor* strains, and 10 wk for *X. polymorpha* strains. At the end of the incubation period, blocks were removed from jars, gently brushed to remove mycelium and any traces of vermiculite, and weighed before and after overnight oven drying at 50 °C to determine moisture content and mass loss.

Petri dishes with 2% MEA (malt extract agar) adjusted to various pH values were inoculated and incubated at 21 °C for 4 wk.

2.2.3. Pigment assessment

Dried wood samples were scanned with an Epson WorkForce 500 scanner at 2400 dpi, first on one external side with the most pigment occurrence, and then on an internal face, after the blocks were cut in half to expose a radial section. Zone demarcation lines that define the fungal colony in wood (zl), and pigment deposition in cluster, or pigmentation (p), were considered for analysis. External and internal pigment evaluation was performed with Scion Image software, following the protocol described in Robinson et al. (2009a). Data were analyzed with a one-way ANOVA followed by Tukey HSD using SAS, version 9.2.

Fungal cultures in petri dishes that produce pigments other than melanin were evaluated for red, blue, yellow, and green pigment formation using a CIE Lab Konica Minolta CM-2002 spectrophotometer, and only values for a* (green/red) and b* (blue/yellow) were utilized. For melanin formation in pure fungal culture on agar, petri dishes were scanned with an Epson WorkForce 500 scanner at 2400 dpi, with the pigment evaluation method utilized the same way as described above.

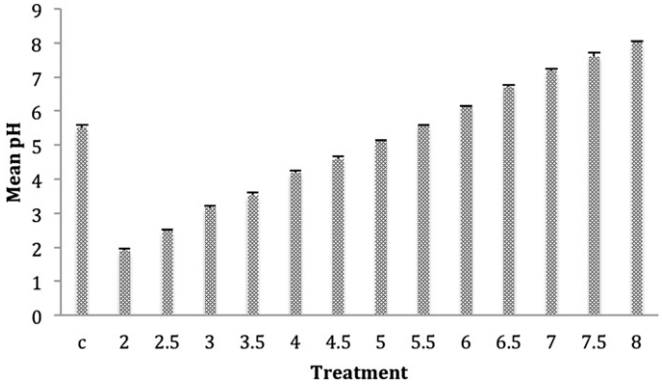

Fig. 1. pH value of MEA measured after settling of media treated with phosphate buffer for pH adjustment.

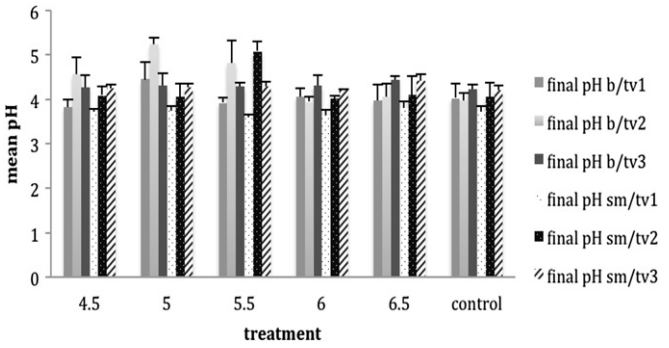

Fig. 2. Final pH value in beech (b) and sugar maple (sm) sample treated with phosphate buffer, decayed by *T. versicolor*, strains: tv1 – Mad 697, tv2 – R105, tv3 – UAMH 11521. Error bars represent one standard deviation.

2.2.4. Estimation of initial conditions and changes of substrate pH

To estimate changes in wood pH after treatment with the phosphate buffer with adjusted pH, three sets of readings and five representative replicates per treatment were measured. Wood samples were treated, conditioned, and steam-sterilized, and pH measurements were recorded before and after complete exposure to decay fungi. The pH value was determined by an extraction method; wood samples dried at 50 °C overnight were ground on a Wiley mill and passed through a 40-mesh screen, and 1 g of wood dust from each sample was placed in a glass vial. Ten milliliters of previously boiled and cooled distilled de-ionized water was added to each vial. The mixture was stirred for 5 min and kept in a closed vial for 25 min. The extract was filtered and the pH of the solution was measured with a glass electrode on a digital Orion SA520 pH meter.

To avoid precipitation of media in acid solution during sterilization, 16.8 g of MEA powder from Difco Laboratories was initially diluted in 150 g distilled water and 350 g of phosphate buffer adjusted to the required pH with hydrochloric acid (HCl), and the two solutions were mixed after sterilization and then poured into petri dishes. The adjusted pH of MEA media was measured from the liquefied substrate in five replicas per treatment and in the control.

3. Results

3.1. Initial conditions

The methodology used for pH determination provides a close estimate of the true value of wood pH (Campbell and Bryant, 1941), and should be adequate for comparison purposes. The purpose of this study was to deduce pH change of treated wood with the phosphate buffer and the pH change after fungal decay.

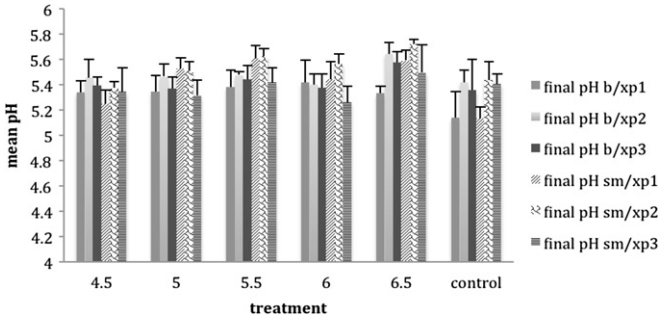

Fig. 3. Final pH value in beech (b) and sugar maple (sm) sample treated with phosphate buffer, decayed by *X. polymorpha*, strains: x1 – UAMH 11518, x2 – UAMH 11519, x3 – UAMH 11520. Error bars represent one standard deviation.

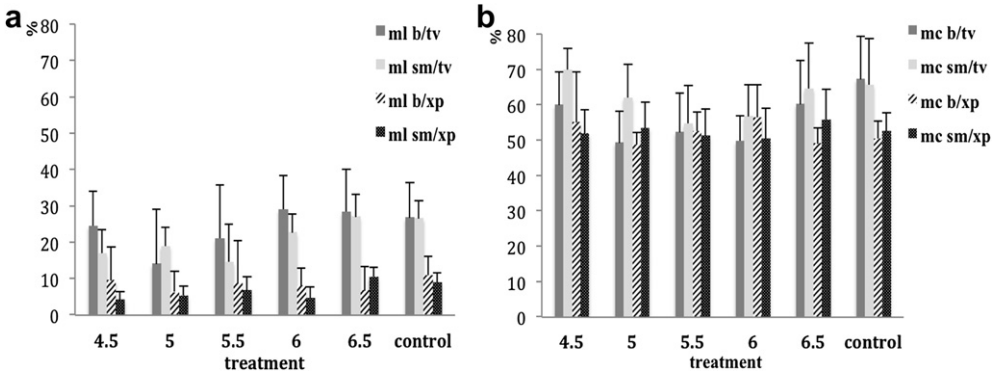

Fig. 4. Mass loss (ml) and moisture content (mc) of decayed sugar maple (sm) and beech (b) samples by *T. versicolor* (tv) and *X. polymorpha* (xp). Error bars represent one standard deviation.

Our results show that after treatment, the pH values of the two wood species were similar within the treatments, and control beech samples had an average pH of 4.54, slightly more acidic than sugar maple samples, which measured on average pH 4.79. As shown in Table 1, the buffer capacity of the wood influenced the final pH values. For the treatment with phosphate buffer adjusted to pH 5 it was observed that wood samples of both wood species recorded on average a minor drop of pH value, for unknown reasons.

Measurements of the MEA after settling in petri dishes indicate that the pH adjustment was consistent and the treatment is reliable. Untreated media had pH = 5.5 on average, while treated media had an average pH close to the desired value (Fig. 1).

3.2. Influence of induced condition on decayed wood

3.2.1. pH changes

At the end of the incubation period, wood samples were measured for pH values. *T. versicolor* demonstrates a high variability of final pH for treatment in the acid range, with little differentiation on the final pH values for treatments of pH 6 and higher. Beech samples seem to have a higher final pH for the acidic treatments compared with maple. Control samples registered a drop of the pH for both wood species after decay to approximately pH 4 (Fig. 2).

X. polymorpha demonstrated more consistency regarding the final pH value measured. The pH of control samples with this fungus actually increased from pH 4.3 up to 5.7 for beech and from approximately pH 4.7 up to 5.6 for sugar maple, on average (Fig. 3).

As expected, both wood species decayed by *T. versicolor* had a pH between 4 and 5, lower than that for wood decayed by *X. polymorpha*, with a pH range from 5 to 5.8.

3.2.2. Moisture content and mass loss

Beech samples inoculated with *T. versicolor* and *X. polymorpha* had the lowest mass loss rates (14% and 6%, respectively) for pH 5 treatment; this was associated with a moisture content of approximately 50% and 48%, respectively (Fig. 4).

Sugar maple samples treated with pH 5.5 buffer inoculated with *T. versicolor* had the lowest mass loss (15%), and also the lowest moisture content of wood samples (about 55%), comparable to the moisture content acquired by wood without fungal activity, suggesting that the fungus could have been partially inhibited by the treatment. Sugar maple inoculated with *X. polymorpha* had a mass loss under 7% for all treatments except for the pH 6.5 treatment, which showed no inhibition, and had a mass loss comparable to that of the control samples (28%; Fig. 4).

3.2.3. Pigment formation

To test the importance of wood pH for fungal pigment formation, a one-way ANOVA was run for each wood species, followed by Tukey's HSD at $\alpha = 0.05$, with fungal inoculation and pH treatments as independent variables. Pigment formation was significantly higher for beech treated with pH 4.5 and inoculated with

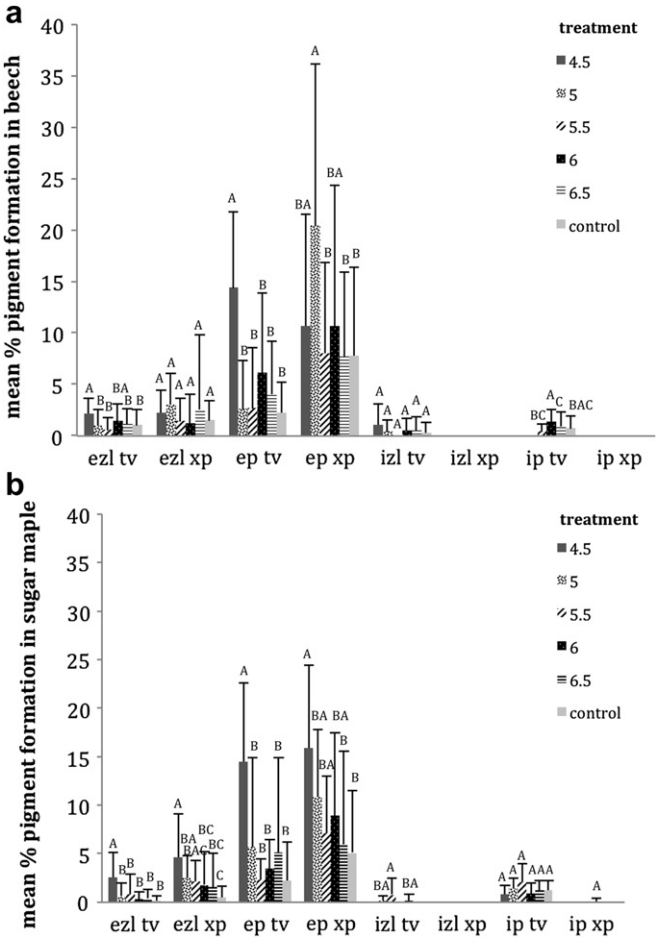

Fig. 5. Pigment formation in beech (a) and sugar maple (b) treated with phosphate buffer at various pH values, inoculated with *T. versicolor* (tv) and *X. polymorpha* (xp). ezl – external zone lines; ep – external pigmentation; izl – internal zone lines; ip – internal pigmentation. The horizontal axis represents the buffer pH used for treatment vs final pH. Error bars represent one standard deviation. Different letters represent significant differences at $\alpha = 0.05$ within each category.

Table 2
Fungal pigment formation in MEA at various pH values. Fungal growth in culture: intermediate = -, maximum = °; minimum values were marked by blank space. Means with the same letter are not significantly different.

treatment / fungal specie	control	pH 2	pH 2.5	pH 3	pH 3.5	pH 4	pH 4.5	pH 5	pH 5.5	pH 6	pH 6.5	pH 7	pH 7.5	pH 8
T. versicolor	o	-	-	-	o	o	o	o	o	-	-	-	-	
Mad697	B	B	B	B	A	B	B	B	B	B	B	B	B	B
T. versicolor	o			-	-	-	-	o	o	o	-			
R105	A	C	C	CB	B	A	C	C	C	C	C	C	C	C
T. versicolor	o		-	o	o	o	o	o	o	o	o	o	-	
UAMH 11521	A	C	C	CB	B	A	C	C	C	C	C	C	C	C
P. squamosus	o				-	-	-	-	o	-	-	-	-	
UAMH 11653	BA	B	B	B	B	BA	BA	A	BA	BA	BA	BA	B	B
P. brumalis	o		-	-	o	o	o	o	o	o	o	-	-	-
UAMH 11652	DE	F	F	FE	BC	BC	BA	BA	A	A	F	F	F	F
F. fomentarius	o			-	-	-	-	o	-	-	-	-	-	
UAMH 11654	DEC	E	E	BDAC	BA	A	BAC	BDAC	BADC	DEC	DE	DE	E	E
I. hispidus	o			-	-	-	-	-	o	-	-	-	-	-
F2037	EGDFC	G	G	BAC	EGDFC	A	BAC	BDAC	BA	EBDAC	EBDFC	EGDF	GF	EGF
X. polymorpha	o			-	-	-	-	-	o	o	-	-	-	-
UAMH 11518	B	D	D	CB	B	A	CBD	CBD	CD	D	D	D	D	D
X. polymorpha	o			-	-	-	-	-	o	o	o	-	-	-
UAMH 11519	A	D	D	D	D	A	B	CB	CD	CD	D	CD	D	D
X. polymorpha	o			-	-	-	-	o	-	-	-	-	-	-
UAMH 11520	BA	E	CD	BC	BCD	BA	BA	A	ECD	E	E	E	E	E
C. aeruginascens	-		-	-	-	-	-	o	-	-	-	-		
UAMH 11655	B	D	A	D	D	B	B	A	D	B	C	D	D	D
M. ruber	-	-	-	-	-	-	-	o	-	-	-	-	-	-
UAMH416	BA	B	B	B	BA	BA	BA	A	BA	BA	BA	BA	BA	BA
(a*-red pigment)														
S. cuboideum	o	-	-	o	o	o	o	o	o	o	-	-	-	-
(a*-red pigment)	A	BC	BA	BAC	BAC	BC	BC	BA	BC	A	DC	ED	EF	F
(b*-blue pigment)	EDF	ED	F	EF	EDF	ED	D	EDF	ED	EDF	C	BC	BA	A
UAMH4802														

T. versicolor for external pigmentation ($P < 0.0001$) and for external zone lines ($P < 0.0001$), and for pH 5 and inoculated with *X. polymorpha* for external pigmentation ($P = 0.0004$) (Fig. 5a). In sugar maple samples, *T. versicolor* and *X. polymorpha* reacted at treatment with pH 4.5 buffer with significant external zone lines and pigment formation ($P < 0.0001$), and *T. versicolor* produced significantly more internal zone lines in samples treated with pH 5.5 ($P < 0.0001$) (Fig. 5b).

3.3. Influence of pH variation in pigment formation on agar

Maximum fungal pigmentation occurred between pH 4 and 5.5 for all fungi tested, values that also comprised the range of the maximum fungal growth rate (Table 2). *T. versicolor* and *X. polymorpha*, tested as multiple strains, exhibit growth variation within strains. In agar, maximum pigmentation was registered at pH 4 and control samples for two strains of *T. versicolor*, while one strain developed no pigmentation. Two of the *X. polymorpha* strains exhibited maximum pigmentation also at pH 4, while one strain enlarged the pH range for topmost pigmentation from 4 to 5.

The green-staining fungus *C. aeruginascens* displayed a change in color spectra with the pH variation. In acidic media the fungus produced a yellow-green pigment that became dark green in the neutral pH, and slowly faded into a light green-brown pigment, with maximum pigmentation at pH 2.5 and 5.

The pigment produced by *M. ruber* evolved from light yellow in the acidic range to a bright red in the basic range, with maximum pigment intensity and growth at pH 5.

For the staining fungus *S. cuboideum*, two types of pigmentation were noted: a red pigment was constantly produced at all pH values of the agar, with maximum intensity registered at pH 6.5, and a dark-blue pigmentation, which was absent in acidic conditions, that reached maximum intensity at pH 8.

4. Discussion

Wood properties and fungal activity interlock in a mutual relationship; wood chemistry and physical properties trigger specific fungal gene expressions according to the circumstances, which enable the fungal activity to modify wood substrates according to their needs. Invariably, the outcome includes wood decomposition, although with variable time required for wood structure collapse and fungal decay strategies involved in the process (Lambert et al., 1980; Holmer et al., 1997). In the case of pH value, wood changes differently based on the wood and fungal species interaction. Wood samples used in our experiments had an initial average pH value of 4.29 for beech and 4.72 for sugar maple, as measured in control samples. After treatment with buffer solution at different pH values, beech samples were more acidic than the sugar maple samples. As a general trend, after 8 wk of decomposition, *T. versicolor* modified the pH of both substrates toward acidic values, while *X. polymorpha* increased the pH relative to the initial conditions.

The pH of the wood substrate affects fungal growth and activity. It has an effect on the fungal cell capacity for nutrient uptake, due to influencing the net charge of the outer membrane surface, with little influence on the cytoplasmic pH, and the degree of dissociation of nutrients by ionization of organic acids or bases in the wood substrate. In exchange, fungi tend to alter the pH or the substrate to create their own favorable condition by selective uptake and exchange of ions (Griffin, 1994; Deacon, 1997). The lowest mass loss for both beech and sugar maple samples inoculated with the ascomycetes *X. polymorpha* occurred for the treatment with pH 4.5 and pH 5, respectively, with mass loss under 7%, while final moisture content was similar in all samples treated with different pH solution. *T. versicolor* showed a low level of decomposing activity in beech treated with pH 5 and sugar maple treated with 5.5, and this was also accompanied by the lowest final moisture content of the respective treatments and a similar final pH value after 8 wk of fungal exposure (4.33 for beech, initially 4.65, compared with 4.32 for sugar maple, initially 5).

Specific fungal enzymes use molecular oxygen to oxidize phenols and polyphenols from the wood substrate for melanin biosynthesis. It was inferred by Duckworth and Coleman (1970) and Nagai et al. (2003) that those types of enzymes appear to be stable over the pH range 4–7 and similarly, most fungi produced optimum growth at the same pH (4–7), all well within the normal range of the wood (Griffin, 1994). Significant pigmentation produced by *T. versicolor* was recorded in beech treated with pH 4.5, and in sugar maple at pH 4.5 for external pigmentation, and pH 5.5 for internal zone lines. Those treatments provide a similar initial pH value after the treatment, close to 5, a condition that provides intermediate fungal activity. *X. polymorpha* reacts mainly to low pH. Significant pigmentation was observed for beech treated with pH 5 (after treatment with 4.65 on average), and for sugar maple treated with 4.5 (after treatment with 4.88 on average). Wood treatments that stimulate pigmentation do not inhibit fungal decomposition activity. However, minimum mass loss occurs at close values of pH treatments.

The results of the experiments set up on malt extract agar were consistent with the tests on wood substrate in regard to the range of pH in which fungal pigmentation occurs. All fungi tested showed pigmentation of the mycelia between pH 4 and 5.5, and both *T. versicolor* and *X. polymorpha* showed distinctive dissimilarity among strains.

An exception from this trend was *S. cuboideum*, which recorded maximum pigmentation close to neutral pH for both types of pigments produced. The red pigment was present at all pH values of the agar, with maximum intensity registered at pH 6. Dark-blue pigmentation was absent in acidic conditions, reaching maximum intensity at pH 8.

Our findings confirmed previous research by Chidester (1940) that indicated the production of tyrian blue pigment in additional to the red pigmentation in culture media of *S. cuboideum*, and research of Golinski et al. (1995) that observed a change in color of the red pigment to blue, when dissolved in solvents of higher polarity (methanol for example) or in basic solutions. The functional role of this pigment might have an important role in fungal virulence. The fungus does not cause significant wood cell wall decay (Schmidt and Dietz, 1985), and its tolerance and adaptability to a wide range of substrate pH values might be reflected in its ability to colonize many types of wood species and to compete with other fungi (Robinson et al., 2011).

5. Conclusions

This research indicates that pH of the substrate potentially plays an important role in fungal melanin formation, which occurs within the pH range from 4.5 to 5.5. Wood-inhabiting fungi demonstrate the capacity to produce melanin at the pH values within the normal range of the wood substrate pH even when stresses such as temperature, moisture content variation, and antagonistic reactions were absent. Fungi *T. versicolor* and *X. polymorpha* tested on wood substrates were mainly pigment-active with the pH 4.5 treatment, and at the initial and final pH values in both wood species tested. *X. polymorpha* can also produce pigmentation in beech treated with pH 5. While it is apparent that the pH of the wood substrate is directly related to melanin formation, pH is not the sole determining factor for melanin formation. A multitude of other factors, both environmental and chemical, could also be responsible and interact within a complex

relationship. We recommend future research investigating gene expression and regulation to identify the process by which information encoded in fungal DNA directs the synthesis of melanin, to further elucidate other highly probable pathways of melanin creation and stimulation.

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