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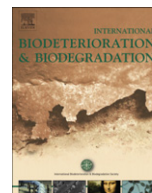

Enzymatic oxalic acid regulation correlated with wood degradation in four brown-rot fungi

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ABSTRACT

Oxalic acid is a key component in the initiation of brown-rot decay and it has been suggested that it plays multiple roles during the degradation process. Oxalic acid is accumulated to varying degrees among brown-rot fungi; however, details on active regulation are scarce. The accumulation of oxalic acid was measured in this study from wood degraded by the four brown-rot fungi – *Gloeophyllum trabeum*, *Meruliporia incrassata*, *Coniophora puteana*, and *Serpula lacrymans* – and found to vary significantly. The amount of oxalic acid present was shown to correlate with the mass loss during wood degradation. However, it did not appear to be a direct or causal relationship as *G. trabeum* produced significantly lower levels of oxalic acid than the other three fungi but generated comparable weight loss. Oxalic acid decarboxylating activity was detected from wood extractions of all four fungi with extractions from *G. trabeum*-inoculated wood showing the highest activity. Formic acid was measured in extractions from decayed wood by the four brown-rot fungi, supporting the presence of the fungal produced oxalic-acid-degrading enzyme oxalate decarboxylase (EC 4.1.1.2). Thus this study indicates that the brown-rot species tested, and in particular *G. trabeum*, are capable of regulating oxalic acid during wood decay by decarboxylation.

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1. Introduction

Brown-rot fungi are some of the principal basidiomycetes responsible for biodegradation of wood. The decay is characterized by a rapid strength loss caused by the marked depolymerization of cellulosic fractions resulting in a limited initial weight loss, marked reduction in cellulose crystallinity, and accumulation of partly degraded sugars (Kleman-Leyer et al., 1992; Zabel and Morrell, 1992; Howell et al., 2007, 2009). Low-molecular-weight (LMW) non-enzymatic compounds including oxalic acid play a crucial role in the initial degradation (Koenigs, 1974; Hammel et al., 2002; Hastrup et al., 2011) before enzymatic hydrolysis.

Oxalic acid is a small organic acid produced by the majority of brown-rot fungi during the primary metabolic phase (Green et al., 1991; Shimada et al., 1997). Oxalic acid accumulation causes a significant lowering of the pH in the wood cell lumen to pH 1.5–2.5 (Green et al., 1991; Hyde and Wood, 1997), which is crucial for mechanisms related to Fenton chemistry as well as metal sequestering and translocation for subsequent reduction by fungal chelators (Goodell et al., 1997; Arantes et al., 2012). Many further functions have been assigned to oxalic acid during brown-rot decay, including calcium precipitation and pectin hydrolysis, causing an increase in wood porosity (Green et al., 1995; Green and Highley, 1997a); copper tolerance (Clausen and Green, 2003; Hastrup et al., 2005); and acid-catalyzed hydrolysis of holocellulose, which increases the permeability of the wood structure for the hydrolytic enzymes or other low-molecular-weight decay agents (Tanaka et al., 1994; Shimada et al., 1997; Green and Highley, 1997b).

Oxalic acid is accumulated in large quantities by most brown-rot fungi (Hastrup et al., 2006; Schilling and Jellison, 2006; Hastrup, 2011), whereas it is only detected in limited amounts in white-rot fungi (Espejo and Agosin, 1991; Dutton et al., 1993). There is a variation that can be attributed to the discrepancies in the structural changes in the wood components observed during degradation by brown- and white-rot fungi including crystalline cellulose (Howell et al., 2011; Arantes et al., 2012; Hastrup et al., in press). It has been speculated that the high levels of accumulated oxalic acid in brown-rot fungi are, at least to some species, such as *Serpula lacrymans*, due to acid hydrolysis of the hyphae (Bech-

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Andersen, 1987). The formation of calcium oxalate crystals by brown-rot fungi may neutralize the oxalic acid at the hyphal surface and substitute the need for oxalate-catabolizing enzymes such as oxalate decarboxylase (Micales, 1995). However, calcium oxalate may result from chelation of calcium in the pit membranes, causing increased porosity of the wood cell (Green et al., 1995). Under the very acidic conditions created in the lumen following oxalic acid accumulation, the acid is able to chelate iron and form soluble oxalate/Fe complexes (Arantes et al., 2012), thus preventing iron reduction and potential damage to the hyphae from Fenton generated •OH (Goodell et al., 1997).

The higher accumulation of oxalic acid observed in brown-rot fungi compared to white-rot fungi is explained by the inability of the former to undertake active regulation of this organic acid (Akamatsu et al., 1992). Oxalic acid regulation in white-rot fungi has been attributed to their oxalate decomposing system (Takao, 1965; Dutton and Evans, 1996). Two different pathways are suggested for oxalic acid conversion: oxalate decarboxylase (ODC; EC 4.1.1.2) converts oxalate to formic acid and carbon dioxide (Mäkelä et al., 2002; Svedruzic et al., 2005) and oxalate dehydrogenase (OXO; EC 1.2.3.4) converts oxalate to carbon dioxide accompanied by the production of H_2O_2 (Aguilar et al., 1999).

Enzymes for oxalic acid regulation in brown-rot fungi have been partially purified from liquid cultures and mycelium extractions of *Postia placenta* (Micales, 1995, 1997). Decarboxylation activity has been detected in *Meruliporia incrassata* (Howell and Jellison, 2006). Despite this, little is known about possible enzymatic regulation of oxalic acid in brown-rot fungi.

This paper describes the accumulation and regulation of oxalic acid in four brown-rot fungi: *M. incrassata*, *Coniophora puteana*, *S. lacrymans*, and *Gloeophyllum trabeum*. The first three species represent brown-rot fungi known to accumulate high concentrations of oxalic acid, whereas the latter accumulates none to little oxalic acid (Espejo and Agosin, 1991; Green and Clausen, 2003; Hastrup, 2011). The fungal growth and metabolism were described by percentage weight loss, oxalic acid accumulation, and pH regulation. The formation of formic acid and ODC activity were measured to determine potential regulation in wood extractions. The presence of calcium oxalate crystals associated with the hyphae was detected and visualized using scanning electron microscopy (SEM).

2. Materials and methods

2.1. Fungi

The following brown-rot species were studied: *G. trabeum* (Mad 617), provided by Forest Products Laboratory, Madison, WI, USA; *M. incrassata* (MFS 3), provided by Dr. J. Jellison, University of Maine, USA; *C. puteana* (ATCC 44393) purchased from the American Type Culture Collection, USA; and *S. lacrymans* (FPRL 12C), provided by Dr. Sarah Watkinson, University of Oxford, United Kingdom.

Isolates were maintained on 2% (w/v) malt extract with 1.5% Bacto agar at 4 °C. Prior to inoculation isolates were grown for 1–2 wk at 24 °C, except for *S. lacrymans*, which was grown at 20 °C.

2.2. Growth conditions

Modified ASTM soil block jars (AWPA, 2003) were prepared with 100 g of a 1:1:1 mixture of potting soil, sphagnum, and vermiculite, wetted with deionized H_2O. Birch feeder strips were placed on top of the soil mix. The soil was inoculated and incubated according to Howell et al. (2009) with a southern yellow pine (SYP, *Pinus* sp.) sapwood block (size: 1 cm × 1 cm × 1 cm) for 6, 12, or 18 wk. Unless otherwise stated, four replicates were set up per fungal isolate of each treatment. Wood blocks placed in uninoculated jars were used as controls.

2.3. Wood block preparation

The pine sapwood blocks were harvested and brushed free of mycelia, weighed, and cut in half longitudinally parallel to the wood grain. Each half was weighed separately. One half was ground immediately and frozen at −80 °C, to be tested later for oxalate decarboxylating activity. The second half was dried at 103 °C for 24 h, reweighed, and ground to a fine powder. The percentage weight loss was determined from the ratio of the dried half of the blocks.

2.4. Oxalic and formic acid analysis

Oxalic acid extractions were performed to liberate both soluble and membrane-bound oxalate, and crystallized calcium oxalate from the incubated wood blocks. Wood powder was mixed with deionized water at a ratio of 1:10 (w/v), extracted for 24 h at 4 °C on a rotary shaker, and then spun at 12,000 rpm for 5 min. Half of the liquid was removed for measuring soluble oxalic acid and pH. Bound, crystallized oxalate was extracted by acidifying the remaining wood:water mixture with equal amounts of concentrated HCl to 0.2 N, buffered with phosphate buffer (pH 1.35), and extracted for 24 h. The pH was adjusted with NaOH to 2.5 and the sample was spun at 10,000 rpm for 5 min (Schilling and Jellison, 2004).

Soluble and total oxalic and formic acid were measured by high performance liquid chromatography (HPLC) according to Hastrup et al. (2006). Data were analyzed using Unicorn 4.0 peak fitting and the concentration was determined based on oxalic and formic acid standards. Oxalate and formate peaks and retention times were verified through comparison to the standard curve and by spiking with known amounts of oxalic acid and formic acid (Hastrup et al., 2006). The total oxalic acid was calculated as the combined amount of soluble and crystallized oxalic acid in the wood. The results were adjusted according to the recorded density loss.

2.5. Extraction of ODC from non-dried wood

Ground, frozen, undried wood was placed in a tube with a 1:1 sand and glass bead mix and 0.1 M sodium citrate buffer (pH 3.75) with 0.004% PMSF (phenylmethanesulphonylfluoride) as protease inhibitor and 25 mM EDTA (ethylenediaminetetraacetic acid). The sample was run for 60 s on the FastPrep (FP 120, Bio 101 Savent). The suspension was further agitated on a rotary shaker for 24 h at 4 °C before being centrifuged for 10 min at 12,000 g. The supernatant was collected.

2.6. Oxalate decarboxylase activity

Decomposition of oxalic acid was measured using an enzymatic microassay for determination of oxalic acid (Boehringer Mannheim, Cat. No. 755 699) as described by Mäkelä et al. (2002). Oxalic acid (2 mM) was added as substrate to the wood extractions from inoculated and control wood samples and analyzed spectrometrically at 590 nm to determine decarboxylation activity. For each sample, a reference without OA was measured to correct for the fungal accumulated oxalic acid in the wood. Controls containing 2 mM OA with and without wood extraction were used as references. The concentration of possible oxalic acid decarboxylating enzymes was determined from a standard curve of oxalate decarboxylase (OxThera Inc.).

2.7. SEM of fungal hyphae

Rapidly frozen pine samples inoculated with *S. lacrymans*, *C. puteana*, and *G. trabeum* for 10 wk were split in the radial plane, mounted on stubs, and gold-coated using a Denton sputter coater Desk-1. The samples were then examined and photographed using a JEOL 840 scanning electron microscope for the identification of calcium oxalate crystals. Identification of calcium in the crystals was done using the Link Analytical system interface.

2.8. Statistics

In the subset of the experiment with blocks, comparison of the weight loss, soluble oxalic acid, total oxalic acid, and pH were based on a means effect general linear model with contrasts to test the two factors, fungi and exposure time, as well as comparisons with the control and all pairwise comparisons (proc Glimmix, SAS version 9.2; SAS Institute Inc., Cary, NC). Multiple pairwise comparisons were adjusted based on the simulation method in SAS, with adjusted p values ≤ 0.05 considered significant. Responses exhibited heterogeneity and either non-constant variance models and/or transformations were used to improve model fits. Formic acid was not detected in all replicate samples and differences were tested using SAS's proc Multtest assuming heterogeneous groups, and using the step-down bootstrap adjustment for multiple comparisons. Unequal sample sizes and heterogeneous models result in some non-constant group difference standard errors. Therefore, mean separations may not be fully represented by simple letter connections, and SAS's conservative representation is used with footnotes giving exceptions (Westfall et al., 2011). The relationships between the responses, ignoring grouping, were studied using a principal component analysis based on the correlation matrix of suitable transformations of the individual responses (S+ version 8.2; Tibco Spotfire, Somerville, MA). Comparisons of ODC results, Student's two-tailed t-tests were used; and standard errors are presented as $\pm$.

3. Results

3.1. Properties of the degraded wood

For weight loss, correlations were detected between the four brown-rot fungi and exposure time (Table 1); significant differences in density loss were notable between the 6-wk and 12-wk exposure periods (p-value = <0.0001), while the 12-wk and 18-wk groups were only marginally different (p-value = 0.089). The concentrations of total oxalic acid increased in wood blocks after 12 and even further at 18 wk compared to 6 wk for all fungi except *G. trabeum*. Wood inoculated with *G. trabeum* accumulated less soluble oxalic acid than *M. incrassata* and *S. lacrymans* at 18 wk and less total oxalic acid than *M. incrassata*, *S. lacrymans*, and *C. puteana* at all three time points. The amount of total oxalic acid was between 1 and 10 times higher than the amount of soluble oxalic acid. The least difference between soluble and total oxalic acid was observed in wood inoculated with *G. trabeum* (1:1.5, soluble vs. total), whereas the highest difference was seen in wood degraded by *S. lacrymans* for 6 wk (1:9, soluble vs. total). In the three species accumulating a high amount of oxalic acid the difference between soluble and total oxalic acid was highest at the early stages of decay.

The pH in the degraded wood from all fungal strains appeared to decrease to a minimum at 12 wk with the exception of *S. lacrymans*, which showed the lowest pH at week 6. *S. lacrymans* degraded wood displayed the lowest pH among the four fungi with a decrease from pH 4.8 ($\pm$0.11) to 3.4 ($\pm$0.26) over the 18 wk of decay. Formic acid was detected in significant amounts (p-value = 0.0018) in extractions from wood degraded by each of the four fungi, although variation was measured among the replicate samples. There was not significant difference in the accumulation of formic acid between the four fungi; however, overall higher accumulation was observed in samples inoculated with *S. lacrymans*. The highest relative conversion of oxalic acid to formic acid was seen in wood degraded by *G. trabeum*. This is in accordance with the fact that we did not measure a correlation between the accumulated oxalic acid and formic acid. Principal component analysis based on the sample correlation matrix of the soil block responses weight loss, pH, soluble oxalic acid, total oxalic acid, and formic acid further illustrated positive correlations between total oxalic acid, soluble oxalic acid, and weight loss. A negative correlation was seen been between pH and formic acid.

3.2. Oxalate decarboxylase activity in degraded wood

Wood extractions from each of the four species were capable of facilitating the reduction of oxalic acid (Fig. 1). *G. trabeum* and *S. lacrymans* samples from wk 12 and 18 showed a significant reduction (wk 12, p $Gt < 0.0001$, p $Sl = 0.0151$; wk18, p $Gt = 0.0008$,

Table 1
Wood inoculated in soil block jars (100 g soil) for 6, 12, or 18 weeks with *G. trabeum* (*Gt*), *M. incrassata* (*Mi*), *C. puteana* (*Cp*), and *S. lacrymans* (*Sl*). Mean estimates for weight loss (%), pH and formic acid are given with standard errors (SE) in the original basis; median estimates for soluble oxalic acid and total oxalic acid are given in their original basis with standard errors (SE) in their transformed basis (see footnotes on constructing confidence intervals).

Fungi	Week	Weight loss (%)	Oxalic acid (mM/g wood)		pH	Formic acid (mM/g wood)
			Soluble	Total		
		(SE)	(SE on log basis)	(SE on log basis)	(SE)	(SE)
Gt	6	29.4 (1.9)	0.2 (0.62)	0.3 (0.63)	4.4 (0.26)	2.1 (0.56)
	12	62.5 (1.6)	0.7 (0.53)	1.1 (0.54)	4.0 (0.28)	1.3[a] (0.82)
	18	66.6 (1.6)	0.4 (0.53)	0.6 (0.54)	4.2 (0.08)	0.5[a] (0.30)
Mi	6	37.3 (2.3)	0.6 (0.47)	4.1 (0.32)	4.0 (0.50)	1.6 (0.46)
	12	52.3 (2.0)	1.9 (0.41)	8.5 (0.27)	3.8 (0.07)	0.6[a] (0.60)
	18	61.2 (2.0)	3.0 (0.41)	9.4 (0.27)	4.1 (0.07)	1.1[a] (0.53)
Cp	6	26.2 (5.2)	0.8 (0.91)	4.6 (0.55)	4.3 (0.09)	2.1 (0.68)
	12	53.3 (6.8)	4.5 (1.21)	6.7 (0.73)	4.0 (0.25)	1.7[a] (0.63)
	18	61.1 (6.8)	1.2 (1.21)	9.2 (0.73)	4.3 (0.16)	1.2 (0.14)
Sl	6	25.3 (5.0)	0.4 (0.43)	4.6 (0.18)	3.4 (0.11)	5.1 (1.10)
	12	55.0 (6.7)	3.7 (0.57)	12.3 (0.24)	3.8 (0.31)	4.6 (1.59)
	18	59.8 (7.7)	4.5 (0.66)	12.7 (0.27)	4.3 (0.26)	0.9 (0.16)
Control		0.0 (0.03)	0.003 (0.19)	0.02 (0.14)	4.8 (0.04)	0.6 (0.09)

[a] Formic acid was not detected in one of four replicate samples (Mi18, Cp12), two of four replicate samples (Gt12, Gt18), and three of four replicate samples (Mi12).

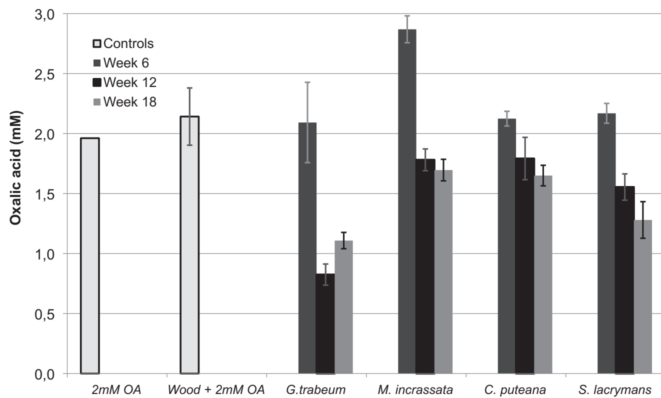

Fig. 1. Amount of oxalic acid remaining after incubation with extractions from wood degraded by *G. trabeum*, *M. incrassata*, *C. puteana* and *S. lacrymans*. Light gray bars with black lining show control samples: 2 mM OA with no wood extraction, 2 mM OA incubated with uninoculated wood.

p Sl = 0.0064). Wood inoculated with *C. puteana* caused significant reduction at wk 18 ($p = 0.0243$), whereas *M. incrassata* from wk 18 showed a marginally significant reduction ($p = 0.0934$).

The extractions of wood inoculated with *G. trabeum* caused a ~50–60% reduction in the amount of oxalic acid at wk 12 and 18 (Fig. 1) corresponding to approximately 0.025 mg/ml ODC at wk 12 and ~0.01 mg/ml ODC at wk 18.

3.3. Precipitation of calcium oxalate crystals

Scanning electron microscopy revealed large quantities of tetrahedral calcium oxalate deposits in wood colonized by *S. lacrymans* and *C. puteana* (Fig. 2). The crystals were observed along the actively growing hyphae and the remains of deceased hyphae (Fig. 2a, c), as well as in and around the degraded pit membrane (Fig. 2b). Hyphal growth was seen in the wood inoculated with *G. trabeum* but no calcium oxalate crystals were detected despite erosion of the pit membrane (Fig. 2d).

4. Discussion

The measured concentration of oxalic acid in the decayed pine blocks is similar to values previously detected in wood degraded by these brown-rot fungi (Green and Clausen, 2003; Schilling and Jellison, 2006; Hastrup et al., 2006, Hastrup, 2011). Oxalic acid appeared to accumulate continuously in all of the fungi except *G. trabeum*, which is in agreement with the reported constitutive synthesis of oxalic acid during growth (Dutton et al., 1993). The measured amount may give an indication of the actual production of oxalic acid taking place in the fungi; however, the variation in the decarboxylation detected among the fungal species may distort the picture. From our study it is difficult to tell anything about the actual amount of oxalic acid synthesized. *G. trabeum*-inoculated wood displayed a reduction in the amount of accumulated soluble and crystalline oxalic acid from wk 12 to wk 18. Although this reduction was not statistically significant it does indicate either active degradation of oxalic acid in the wood and/or possibly a non-constitutive generation of oxalic acid by this fungus (Dutton et al., 1993). Phylogenetic studies have shown *G. trabeum* to be dispersed far from the other brown-rot fungi evolutionarily (Hibbett and Donoghue, 2001; Glaeser and Lindner, 2011), which could be the reason for this fungus also physiologically diverging from the other brown-rot fungi tested. *G. trabeum* displays physiological traits of both brown- and white-rot fungi and may be physiologically closer to the white-rot fungi than to the other brown-rot fungi.

The accumulation of oxalic acid is found to be correlated with the mass loss in the wood, although this is not a direct association as *G. trabeum* has significantly lower oxalic acid accumulation yet causes weight losses parallel to those of the three other species at all three stages of decay measured (6, 12, and 18 wk). The formation of other organic acids including formic acid or other degradative mechanisms may compensate for the lower amount of oxalic acid (Goodell et al., 1997; Green and Highley, 1997a; Hastrup, 2011). The reduction in pH was not correlated with the concentration of oxalic acid measured in the wood blocks, which may be due to the grinding of the samples prior to pH measurements (Dutton et al., 1993). Grinding causes an averaging of the fungus-induced pH level at different locations in the wood cell, thus explaining the absent correlation. Grinding the wood also causes exposure of the oxalic acid to calcium deposits or other charged groups in the wood able to sequester the H^{+}-ions, which can cause neutralization. Although not detectable with the methods used in this study, pH has been found to decrease locally during early stages of decay. Green et al. (1991) found an acidification to pH 1.7 of the environment immediately adjacent to the fungal hyphae in wood inoculated *P. placenta*, whereas wood cells further away from the fungi have a pH of approximately 4.5–5.5 (Hyde and Wood, 1997; Goodell et al., 1997). An increase in pH level from wk 6 and wk 10 in wood decayed by *M. incrassata* was suggested to be related to the switch from non-enzymatic to enzymatic decay (Howell and Jellison, 2006), and may indicate the presence of oxalic acid decarboxylation by ODC. Thus, the increase in pH at later stages of decay seen for all fungi in this study may be related to a regulatory mechanism, e.g., ODC.

The decarboxylation of oxalic acid observed in wood extractions from all four species, in particular extractions from wood inoculated with *G. trabeum*, confirms previous studies detecting rapid conversion of ^{14}C-labeled oxalic acid to CO_2 during cellulose depolymerization (Espejo and Agosin, 1991). In that study, *G. trabeum* showed a higher release rate of $^{14}CO_2$ than did *P. placenta*, although both brown-rot fungi were found capable of degrading oxalic acid (Green and Highley, 1997a). The presence of oxalate decarboxylase was further supported in our study by the detection of formic acid in extractions from all four fungi species. The lack of correlation between the amount of formic acid accumulated and oxalic acid in the samples may be a result of variance in ODC activity and the decomposition of formic acid by formate dehydrogenase (Mäkelä et al., 2010). However, it could also be due to the activity of another oxalic acid decomposing enzyme such as oxalate dehydrogenase (Aguilar et al., 1999). The significantly higher oxalate decarboxylating activity of *G. trabeum* ($p < 0.0001$) compared to the three other brown-rot species may explain the lower accumulation of oxalic acid measured. However, it cannot be ruled out that the initial biosynthesis of oxalic acid in *G. trabeum* could be lower. The oxalic acid is found to be produced from a combination of both mitochondrial (TCA cycle) and glyoxysome (GLOX cycle) isocitrate lyase activity most likely synthesized through the tricarboxylic acid (TCA) cycle or the glyoxylate cycle (Jenkins, 2012).

Tetrahedral (dihydrate) calcium oxalate crystals, the most common crystal form to occur in basidiomycetes, were found to be prevalent in wood inoculated by *S. lacrymans* and *C. puteana* but absent from the interior of wood decayed by *G. trabeum*. In a study of fungal inoculated wood blocks grown in an agar plate microcosm, Schilling and Bissonnette (2008) registered formation of calcium oxalate crystals on the surface of wood inoculated by *G. trabeum*. They found the accumulation of calcium in the wood to be similar to the amount measured from wood inoculated with

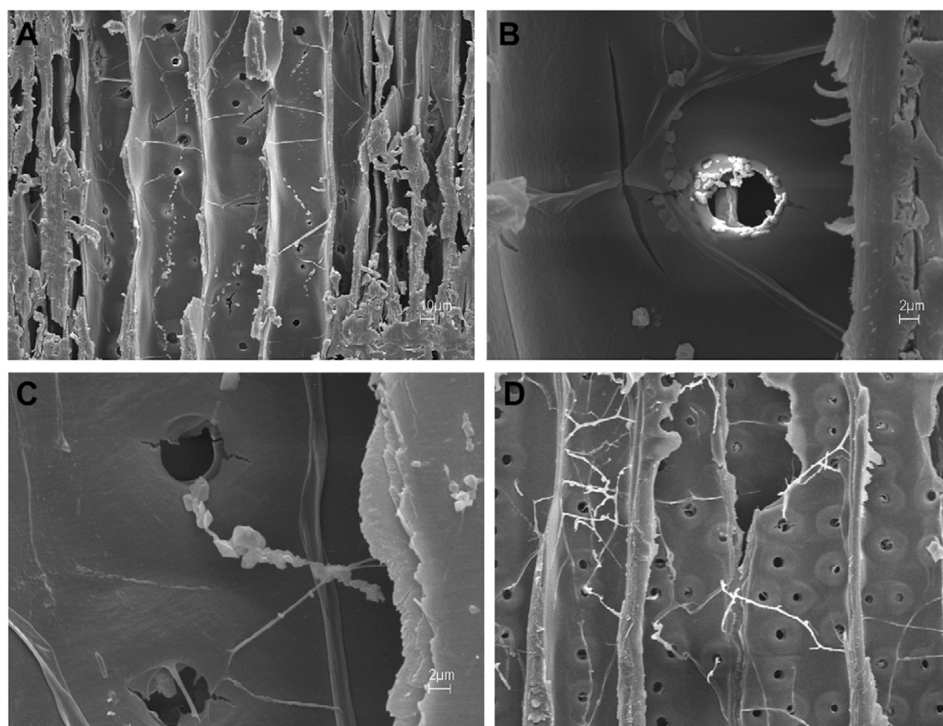

Fig. 2. Southern yellow pine degraded by *S. lacrymans* (a, b), *C. puteana* (c), and *G. trabeum* (d) In soil block jars for 10 weeks. a. Deposits of calcium oxalate crystals following crystallization by *S. lacrymans*. b. Pit membrane lined with calcium oxalate crystals in wood degraded by *S. lacrymans*. c. Tetrahedral calcium oxalate crystals along hyphae of *C. puteana*. d. *G. trabeum* hyphae in degraded wood. No calcium oxalate crystals were observed.

S. lacrymans. Calcium oxalate crystals adhering to the hyphae are produced in both the brown- and white-rot fungi (Connolly and Jellison, 1995). However, as was the case in our study, Schilling and Bissonnette (2008) did not find Ca-oxalate crystals inside the wood matrix. The reason for this lack of calcium oxalate crystal formation inside *G. trabeum*-inoculated wood in spite of the presence of oxalic acid could be the acidic environment. Connolly and Jellison (1994) detected the formation of calcium oxalate crystals by *G. trabeum* in an environment buffered to above pH 6.0 but not at more acidic pH; this supports the lack of calcium oxalate crystals in our study where pH was lower. This conflicts the pronounced presence of crystals in the other two species at equal or lower pH; however, physiological and evolutionary adaptations may explain these differences (Hibbett and Donoghue, 2001; Glaeser and Lindner, 2011). The presence of oxalic acid in wood inoculated with *G. trabeum* is supported by the fully hydrolyzed pit membrane (Fig. 2d) as was seen previously (Green and Clausen, 1999). A major component of the pit membrane is calcium and activity of pectin-degrading enzymes is dependent on Ca^{2+} removal by oxalic acid (Green et al., 1996; Green and Clausen, 1999). Fungal species without the ability to accumulate oxalic acid fail to dissolve the pit membrane (Green et al., 1995). The absence of calcium-oxalate crystal formation in *G. trabeum* inoculated wood still needs to be elucidated.

5. Conclusion

The significantly lower accumulation of total oxalic acid by *G. trabeum* compared to the other brown-rot fungi did not cause a reduction in the wood-degrading ability of this fungus, which suggests the presence of additional degradative mechanisms and/or non-enzymatic and enzymatic compounds. Formic acid was detected in wood extractions from all four species and supports the detected oxalic acid decarboxylating activity in the wood extraction. Oxalic acid decarboxylating activity was highest in extracts from *G. trabeum*, but the detected activity was also significant in extractions from *S. lacrymans* and *C. puteana* degraded wood. This study indicates that the brown-rot species tested, and in particular *G. trabeum*, are capable of conducting oxalic acid regulation during wood decay by decarboxylation. Work is currently being conducted to isolate the ODC protein from liquid medium inoculated by *G. trabeum* as well as to detect the gene coding for oxalate decarboxylase.

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