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Influence of Chaga (*Inonotus obliquus*) treatment of wood in decay tests

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Abstract

An aqueous suspension made with ground sterile sclerotium of the fungus *Inonotus obliquus*, commonly called chaga, was used as a pressure treatment to pine and birch wood blocks. Pine blocks were exposed to three brown rot fungi and birch blocks were exposed to three white rot fungi using a standard soil block decay test. In most cases chaga treatment significantly stimulated decay of wood blocks (measured as percent weight loss) compared to untreated blocks. This was mainly true for white rot fungi, however chaga-treated blocks also significantly inhibited decay by two brown rot fungi. Different metabolic or nutritional needs of decay fungi may explain why the chaga treatment had varying effects. Although many biologically active natural compounds isolated from *I. obliquus* have been studied, this report marks the first to our knowledge regarding potential antifungal properties in wood decay tests.

Key words – antifungal – betulin – soil block test – wood preservatives – natural products

Introduction

Chaga is the common name of the black mass of the tree disease fungus *Inonotus obliquus* (Fr.) Pilát. The fungus is the pathogenic agent of canker rot of birch, causing a heart rot that eventually kills the tree; infection is characterized by black sterile sclerotium, or conks, on trunks of infected trees (Fig. 1) (Sinclair & Lyon 2005). Decay is of the white-rot type, consuming both cellulose and lignin and spores are not produced by the fungus until after the tree dies and has fallen (Sinclair & Lyon 2005). The disease is found across the northern hemisphere wherever birch trees occur; paper birch (*Betula papyrifera* Marsh.) and yellow birch (*B. alleghaniensis* Britton) are most commonly affected in North America; *I. obliquus* is also known to infect alder (*Alnus* spp.), beech (*Fagus* spp.), cottonwood (*Populus* spp.) and ironwood (*Ostrya virginiana* (Mill.) Koch) (Sinclair & Lyon 2005).

Chaga and other polypore fungi have been used medicinally for many years due to the presence of a variety of biologically active compounds that occur in their fruiting bodies (Zjawiony 2004, De Silva et al. 2013). Birch bark contains up to 24% betulin (Budavari et al. 1989). It is presumed that betulin and related compounds in the bark of birch trees confers resistance to pathogens and to decay fungi (Kratusky 2006, Borchardt et al. 2008, Shorohova & Kapitsa 2014). Betulin and its water-soluble derivative, betulinic acid, have numerous biological properties including antiviral, antibacterial, anti-inflammatory, antihelmintic and antimalarial (Yogeeswari &

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Sriram 2005). There has been increased interest in investigating additional benefits and uses of 'medicinal mushrooms' or traditionally used fungi in recent decades (Balandaykin & Zmitrovich 2015, Goldhor 2017). One of the potential applications of fungi and fungal extracts is as a wood preservative (Yang 2009).

Fig. 1 – *Inonotus obliquus* sterile basidiome, or conk, naturally occurring on *Betula papyrifera* tree in a northern hardwood forest.

Chaga is also reported to contain betulin and betulinic acid (Kahlos 1994). The sterile conk of *I. obliquus* (sometimes referred to as a sclerotium, as it is a solid mass of sterile mycelium) contains other compounds that are active against animal cells and viruses (Kim et al. 2011). Cultures of *I. obliquus* are also known to produce biologically active metabolites such as sterols and related compounds (Kahlos 1994). Antifungal compounds are known to be produced by pathogenic wood decay fungi. For example, *Sparassis crispa* Wulf. ex. Fr. which causes a root and butt rot in conifers, and *Peniophora polygonia* (Pers.) Bourdot & Galzin which causes wood decay among *Populus* species, have both been reported to produce compounds significantly inhibiting decay of other fungi (Woodward et al. 1993, Trifonov et al. 1992). However, little is known of the antifungal properties of compounds found in the sterile conk of *I. obliquus*. In this study, a finely ground suspension and infusion of *I. obliquus* was used to pressure-treat wood blocks. Treated wood blocks were then exposed to several white rot and brown rot fungi in a standard soil block decay test to determine the effect of *I. obliquus* on weight loss caused by the decay fungi.

Materials & Methods

Sterile basidiomes (conks) of *Inonotus obliquus* (Fr.) Pilát ("chaga") were collected from several living but diseased trees of paper birch (*Betula papyrifera* Marsh.) and yellow birch (*B. alleghaniensis* Britton) in Houghton County, Michigan (47.10248° N, 88.51702° W). Several pieces of *I. obliquus* (approximately 5 - 10 cm diameter) of the hardened hyphae protruding from the wood (Fig. 1) were air dried prior to use. The pieces were ground in a Wiley mill using a 1 mm mesh screen resulting in particles ranging in size from powder to 1 mm diameter. The *I. obliquus* treatment mixture was prepared by placing 500 g of ground *I. obliquus* into 1.5 L distilled water.

Air dried, pre-weighed loblolly pine (*Pinus taeda* L.) and paper birch blocks (14 mm^3 or 19 mm^3) were submerged in the *I. obliquus* treatment mixture and left to sit at room temperature for 24 hrs before pressure treatment. Submerged blocks in *I. obliquus* solution were exposed to 110 psi for 1 hr in a pressure cylinder to thoroughly saturate blocks with chaga solution. Following pressure

treatment, blocks were removed from *I. obliquus* solution, blotted on paper toweling and a representative sample weighed to determine uptake of *I. obliquus* liquid for 19mm^3 blocks. Blocks were then dried to constant weight at 40ºC for 24 h, and individual weights were recorded prior to fungal exposure in the decay test.

Wood decay tests were conducted using American Wood Protection Association (AWPA) E-10, *Standard Method of Testing Wood Preservatives by Laboratory Soil-Block Cultures* (AWPA Technical Subcommittee P6 2008). Briefly, 100 ± 1 g dried (50 ºC for 72 hours) forest topsoil (pH 5.8) was added to a square flint jar (5 x 5 x 13.5 cm); 30 ± 1 ml of distilled water was added to bring soil to approximately 90-100% water holding capacity; a plastic lid with a 5 mm diameter hole covered by a strip of adhesive first-aid tape was placed on the jar to allow for air exchange. For brown rot fungi a pine feeder strip (0.5 x 3.2 x 2.0 cm) was placed on top of the soil prior to autoclaving. For white rot fungi a birch feeder strip was used and added at the time of inoculation. Jars were autoclaved (15 psi, 120ºC) for 30 min. The AWPA decay test method allows for either 19 mm^3 blocks incubated for 12 weeks or 14 mm^3 blocks incubated for eight weeks, with all other conditions being the same.

Pure cultures of basidiomycete fungi were exposed to individual wood blocks under sterile conditions. Brown rot fungi were *Gloeophyllum trabeum* (Pers.) Murr. ATCC 11539 (19 mm^3 and 14 mm^3 blocks), *Rhodonia placenta* (Fr.) Niemelä, K.H. Larss. & Schigel (syn. *Postia placenta)* MAD 698 (19 mm^3 and 14 mm^3 blocks), and *Neolentinus lepideus* (Fr.) Redhead & Ginns ATCC 12653 (14 mm^3 blocks). White rot fungi were *Trametes versicolor* (L.) Lloyd MAD 697 (19 mm^3 and 14 mm^3 blocks), *Bjerkandera adusta* (Willd.) P. Karst. DR 447 (19 mm^3 and 14 mm^3 blocks), and *Hericium ramosum* (Bull.) Letell. JR 01 (14 mm^3 blocks).

Fungus inocula were grown on 2% malt extract agar in 100 mm petri dishes for 1-2 weeks at 22-24ºC, at which time mycelium covered the surface of the agar. After autoclaving and cooling jars, for brown rot fungi, a piece of colonized agar (approx. 1 x 2 x 0.5 cm) was placed on the pine feeder strip and an oven-dried (40ºC), pre-weighed, pine block that was sterilized by steam for 30 min, was placed firmly on the agar on top of the feeder strip (Fig. 2). For white rot fungi the pre-weighed and sterilized blocks were placed in the sterilized and cooled jars in the soil with one face level with the surface; then a piece of colonized agar as above was placed on the block and a birch feeder strip placed firmly on top of the inoculum. Lids were replaced tightly and jars were incubated at 27º ± 1ºC, 80 ± 4% RH for 8 weeks for 14 mm^3 blocks or 12 weeks for 19 mm^3 blocks. Six replicate 14 mm^3 blocks and ten replicate 19 mm^3 were set up for each chaga treatment or untreated control per fungus. Five replicates are the suggested standard minimum (AWPA Technical Subcommittee P6 2008), but additional blocks were added to strengthen results. After the respective incubation time, blocks were removed from jars, brushed to remove soil or mycelium, and re-dried at 40ºC for 24 hr and weighed to determine weight loss due to decay. Mean percent weight loss of replicate sets of blocks by treatment and fungus exposure was determined. Statistical analysis to compare treated and untreated blocks by fungus was conducted by performing a paired *t*-test (α = 0.05) using Statistix 9.0 (Statistix 9.0, Analytical Software, 2008).

Results

Mean uptake of the *I. obliquus* suspension by 19 mm^3 blocks following pressure treating was approximately 2.0 g (range 1.5-3.0 g), being slightly higher for birch blocks likely due to differences in porosity between the wood species. Decay results were mixed among fungi, however in most cases treatment with *I. obliquus* suspension caused an increase in the amount of decay in wood blocks, although the increase was not always significant.

For the white rot fungus *T. versicolor*, *I. obliquus* treatment significantly increased decay of both the 14 mm blocks (P = 0.02) and 19 mm blocks (P = 0.01) (Figure 3). For the white rot fungus *B. adusta*, chaga treatment also increased decay however the increase was slightly less than that seen in *T. versicolor,* so that the amount was not significant (14 mm blocks P = 0.06, 19 mm blocks P = 0.06). There was no difference in the amount of decay between *I. obliquus*-treated and untreated blocks exposed to *H. ramosum*.

Fig. 2 – Experimental set up for wood decay test of sterile untreated blocks on top of fungi inoculum and feeder strip in soils jars for brown rot fungi.

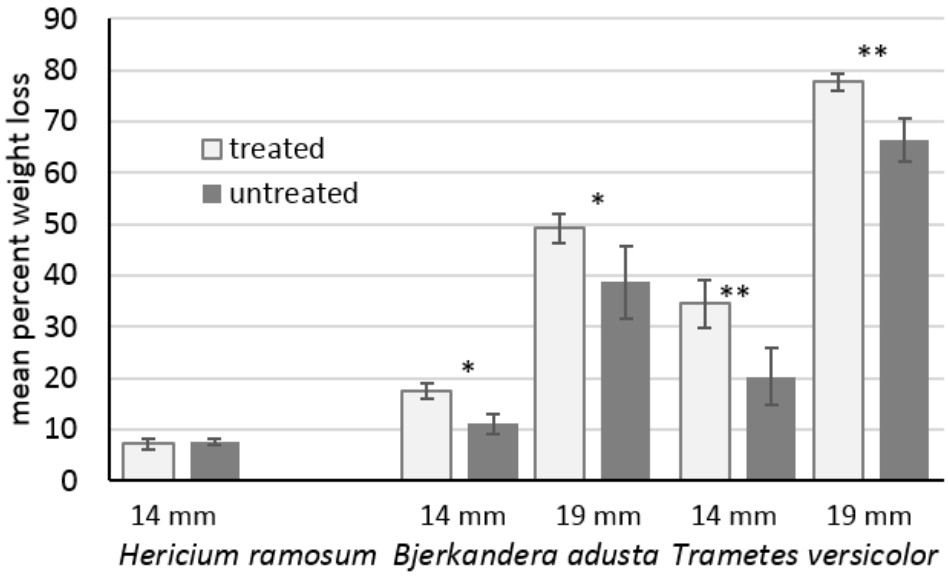

Fig. 3 – Mean percent weight loss by white rot decay fungi of birch (*B. papyrifera*) blocks untreated or treated with *I. obliquus* (chaga) solution. Bars represent standard deviation. Significant differences between paired t test for treated and untreated blocks of the same size for each fungus are indicated at $\alpha \leq 0.05$ level (**) and $\alpha < 0.1$ level (*).

For the brown rot fungus *G. trabeum, I. obliquus* treatment significantly increased decay of both the 14 mm blocks (P = 0.01) and 19 mm blocks (P = 0.001) (Fig. 4). For the brown rot fungus *R. placenta*, there was no significant difference (P = 0.37) in weight loss between *I. obliquus*-treated and untreated 14 mm blocks, though the mean percent weight loss was slightly higher for *I. obliquus*-treated blocks (28.5%) than untreated blocks (26.5%). However, 19 mm blocks treated with *I. obliquus* that were exposed to *R. placenta* were significantly less decayed (P = 0.01) than untreated blocks, indicating that *I. obliquus* suppressed decay by this fungus possibly due to the longer incubation time. A significant decrease in decay caused by *I. obliquus* treatment was also seen in 14 mm blocks exposed to the brown rot fungus *N. lepideus* (P= 0.02).

Discussion

The significant increase in decay of *I. obliquus*-treated blocks by most fungi suggests that the aqueous solution of ground chaga pressure treated into the wood imparted metabolites or nutrients to which the decay fungi responded positively. White rot fungi showed a more consistent positive response than brown rot fungi, in that only the white rot *H. ramosum* was neither stimulated or

inhibited by the *I. obliquus* treatment. The white rots *T. versicolor* and *B. adusta* both decayed the *I. obliquus*-treated blocks more in the four tests conducted with them. These two white rot fungi are often used in wood decay testing of new materials and wood preservatives due to their capability of causing severe weight loss to wood (AWPA Technical Subcommittee P6 2008). White rot fungi decay both the cellulose and lignin components of wood and the three white rot fungi used in this test are common saprotrophs of birch and other hardwoods in natural ecosystems (Rayner & Boddy 1988). It may be that that *T. versicolor* and *B. adusta* are adapted to attacking birch wood that has been pre-colonized by the heart rot *I. obliquus*, as suggested by these results. *I. obliquus* is considered a primary decayer on live birch, and is likely able to use or change compounds to facilitate secondary decay species (Holmer et al. 1997)

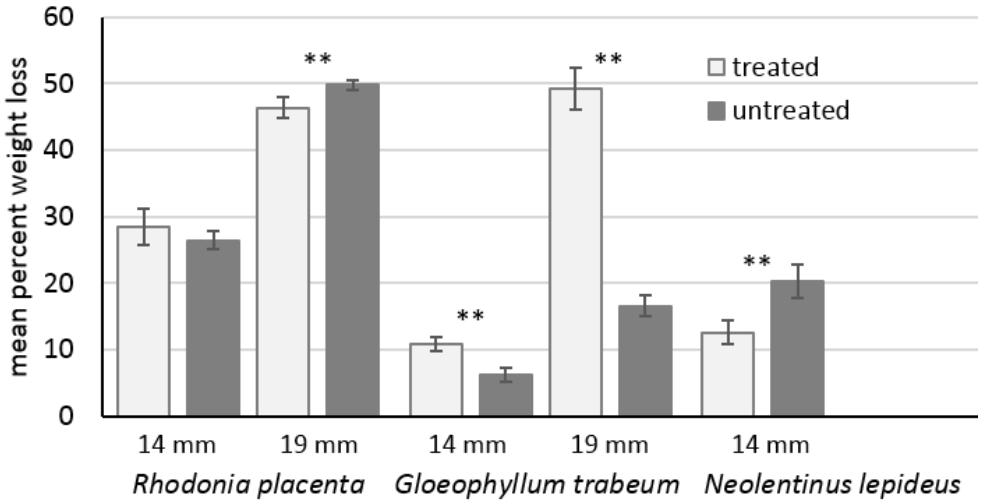

Fig. 4 – Mean percent weight loss by brown rot decay fungi of pine (*P. taeda*) blocks untreated or treated with *I. obliquus* (chaga) solution. Bars represent standard deviation. Significant differences between paired t test for treated and untreated blocks of the same size for each fungus are indicated at $\alpha \leq 0.05$ level (**).

Brown rot fungi, on the other hand, decay only the cellulose components of wood, being much less common on hardwoods such as birch. The three brown rot fungi used in this test are also often used in wood decay testing of new materials and wood preservatives due to their capability of causing severe weight loss to wood (AWPA Technical Subcommittee P6 2008). These fungi are all found primarily as saprotrophs on softwoods, although *N. lepideus* is also known to attack hardwoods including birch (Vlasenko et al. 2017). No clear pattern emerges with respect to rot type and *I. obliquus* treatment among the brown rot fungi used. One of the brown rot fungi, *G. trabeum*, responded to the *I. obliquus* treatment as did white rots that were stimulated by its presence and decayed *I. obliquus*-treated blocks more than untreated wood. For the other two brown rot fungi, *N. lepideus* and *R. placenta*, the presence of *I. obliquus* compounds in the wood inhibited decay, possibly acting as a suppressant to cellulolytic enzymes. Differences between metabolic or nutritional needs of decay fungi may explain why the chaga treatment had varying effects on wood decay ability among the fungi. Overall, it appears that *I. obliquus* as a wood treatment to prevent fungal decay is ineffective against white rot fungi, however further research into the inhibitory effects on brown rot fungi may be warranted.

Much of the antifungal, antimicrobial, or immuno-stimulating properties of *I. obliquus* (chaga) that could have further practical applications are still not completely understood (Balandaykin & Zmitrovich 2015, Glamočlija et al. 2015, Goldhor 2017). This study attempted to demonstrate inhibitory or stimulatory impacts on wood decay by an aqueous suspension of *I. obliquus* on sample wood blocks. An ethanolic or methanolic solvent may be more effective extracting the betulin and related bioactive compounds (Borchardt et al. 2008). Further

experimentation with other extraction methods of *I. obliquus* would provide a clearer picture of the potential antifungal compounds, as the results in this particular study primarily increased wood decay in *I. obliquus* -treated blocks.

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