Lodgepole pine decay when inoculated with white rot and brown rot fungi in a competing modified decay jar test

by

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Abstract

Wood preservation experts often perform monoculture fungal decay tests to determine the decay resistance of wood-based materials and the effectiveness of preservative treatments under controlled laboratory and field conditions. In nature, however, wood is rarely colonized by a single fungal species. In a single cubic cm of forest soil, there may be hundreds of species of fungi that interact with each other and can compete for the same space and resources. Similarly, living, dying or dead trees often contain a variety of decay fungi. Factors affecting fungal colonization of wood and the rate of decay include the presence of other interacting fungi, the heterogeneity of the wood, and environmental conditions. In this study, we analyze fungal growth and wood decay rates for single fungi and for pairs of fungi in controlled lab conditions. We selected fungi that cause the two major types of decay: the white rot fungus \textit{Trichaptum abietinum} that degrades carbohydrates and lignin, and the brown rot fungus \textit{Fomitopsis pinicola} that degrades only carbohydrates but not lignin. The decay substrate was lodgepole pine (\textit{Pinus contorta}) wood, a non-durable species native to western North America. In this work we report antagonistic reactions between pairs of fungi in artificial media, as well as the decay ability of single fungi and pairs of fungi by using a modified soil block decay test, to gain insight into how fungi may behave in natural environments.

Keywords: decay fungi, ecology, white rot, brown rot, \textit{Trichaptum abietinum}, \textit{Fomiptosis pinicola}, \textit{Pinus contorta}, intermingling, antagonism, soil block decay test, modified decay jar test
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Introduction

The forest sector of British Columbia is one of the largest manufacturing industries of its kind in the province and in the world. It accounts for about 4% of the total provincial gross domestic product (GDP) and over 30 percent of total exports for the province of British Columbia (British Columbia. Ministry of Forests, 2011). In Canada, the forestry industry accounts for about 1.9% of the national GDP (Natural Resources Canada, n.d.). Commodity products produced from BC forests include dimensional lumber, plywood, chips, and hog fuel and traditional value-added products include goods like cabinets, furniture, and engineered wood products like glulam beams and laminated veneer lumber (Forestry Innovation Investment, n.d.). While there are more than 40 different species of native trees in the province, over 90 percent of its forests are comprised of coniferous species, like pine, spruce, fir, hemlock, and western red cedar, with the most common species being lodgepole pine and spruce (Ministry of Forests, Lands and Natural Resource Operations - Province of British Columbia, n.d.). Lodgepole pine trees themselves account for almost 25 percent of BC’s forestland (“British Columbia’s Mountain Pine Beetle,” n.d.).

Lodgepole pine (Pinus contorta) is a coniferous tree in the family Pinaceae. It grows in a nearly continuous forest throughout western North America (Figure 1) extending from the southern portion of Canada’s Yukon Territory latitudinally to Baja California and longitudinally to the Black Hills of South Dakota (Koch, 1996). However, lodgepole pine has faced a massive epidemic over the last 10 years that has devastated 18 million hectares of forest (CBC News, n.d.). The mountain pine beetle (MPB), a native insect and a natural part of the B.C. forest life cycle, and who is responsible for the current outbreak that started to develop since the late 1990s, has extended beyond its historic geographical ranges (Canadian Forest Service, n.d.) and caused the largest bark beetle infestation in North American recorded history (Ministry of Forests, Lands and Natural Resource Operations - Province of British Columbia, n.d.). It is forecasted that, by 2015, over 75 percent of the pine volume in the B.C. interior will have died as a result of a recent mountain pine beetle epidemic (B.C. Ministry of Forests, Lands and Natural Resource Operations, n.d.). In particular, this represents tremendous fire risk as well as large economic losses derived from reduced fiber yield as decay advances in the dead standing trees. This problem is exacerbated as lodgepole pine wood is considered a non-durable species in terms of resistance to fungal decay (Richardson, 1993), and beetle-killed timber has been estimated to only have a 5-18 year shelf life before losing its economic value.
Figure 1 - Geographical Range of Lodgepole Pine Forests in North America (Elbert Little, 1971).

*Pinus contorta*
Wood decay is a biological deterioration of wood caused by activities of living microorganisms, like bacteria and fungi. It involves changes in the chemical and physical properties of wood, caused primarily by the enzymatic activities of microorganisms, as well as chemical reactions resulting from microbial activity (Zabel & Morrell, 1992). The importance of wood decay is such that, in nature, it acts as a process of nutrient recycling and, without it, the forest ground would be covered with the corpses of dead trees (Richardson, 1993). In addition, the understanding of wood decay can lead to better design and maintenance of wood products to ensure greater performance, longevity, and reduced risk to human harm. The extent to which wood decay occurs can vary greatly through permutations of host species, fungal species and conditions within the wood (Schwarze, 2007). In addition, studies from interactions between different saprotrophic fungi in wood indicate that other factors, such as colonization sequence and combative mechanisms of different fungi, can greatly affect decay rate (Boddy, 2000).

Fungi responsible for the death and decay of lodgepole pine trees in British Columbia have been isolated and studied (Jae-Jin, A, M, & Colette, 2005; Son, Kim, Lim, & Au-Yeung, 2011). These encompass the major types of fungi in wood: saptainers, white-rotters, brown-rotters, and soft-rotters. In early stages of lodgepole pine tree death, ophiostamoid sap-staining fungi have been reported more frequent, whereas decay basidiomycetes were usually found in the later stages of tree decay (Jae-Jin et al., 2005). Decay fungi are categorized by their ability to decay different components of wood, and the general consensus is that there are three main types: white rots, brown rots, and soft rots. White rot fungi are able to degrade cellulose, hemicellulose, and lignin, (which are) all the main components of wood (Zabel & Morrell, 1992). In general, white rots that decay through selective delignification are also more effective at decay in wood species with high parenchyma content (Schwarze, 2007), such as balsa. Brown rot fungi generally decay cellulose and hemicellulose in wood, leaving the lignin mostly intact and resulting in the brownish color (Richardson, 1993). After brown rot decay, wood generally has a brittle and porous appearance, particularly at the end grain. Soft rot fungi have a large variation of in terms of wood decay. Generally, their pattern of decay in involves a process of hyphal tunneling, and are more often active only on the outer layers of wood (Schwarze, 2007). For some soft-rotters, carbohydrates are principally attacked, while others selectively remove more lignin than carbohydrates (Zabel & Morrell, 1992).

The performance of wood products in service is often strongly correlated to the density of the species of wood selected. Sometimes, however, decay or deterioration of wood in service can lower this density, and hence, its structural performance. The susceptibility of wood or preservative-treated wood to decay, however, can be estimated by a controlled decay test of which change in wood density or percent mass loss is the indicator. Decay rate is often represented by a percentage of mass loss of wood following incubation with a decay fungus under controlled conditions. A common test utilized is the E10 Standard Method of Testing Wood Preservatives by Laboratory Soil-Block Cultures (American Wood Protection Association, 2012). In this test, sterile wood blocks are placed over strips of wood covered with growing mycelium inside a jar to encourage decay on the wood blocks by a specific fungus isolate. By measuring the initial oven-dry mass of the wood blocks and final oven-dry mass after incubation, a
percentage mass loss from the original weight can be calculated, and hence susceptibility to decay fungi can be estimated. This test is often used in the preservative wood treatments industry, where chemically impregnated wood blocks are tested for decay under axenic conditions to determine the effectiveness of preservatives.

Objectives of the study

Previous studies from fungi interactions in wood have shown that there are a wide range of interactions of fungi in wood, including mutualistic, to neutral for each other, and to competitive interactions where fungi may exert a chemical or physical pressure over other fungi to control access to a resource (Boddy, 2000). In this study, white and brown rot decay fungi isolated from lodgepole pine trees in British Columbia are tested for their decay ability on lodgepole pine heartwood blocks in a competitive study. Fungi inoculum from two major decay groups, white or brown rot, was paired with inoculum of the same isolate, a different isolate of the same species, and two different isolates of a different species to measure decay rate of the competing fungi on wood blocks. Results are then related to macroscopic and microscopic observations of fungal growth rates and competitive interactions in artificial media to gain some insight on how wood decay fungi interact in their natural substrate, wood.
Materials and Methods

Fungal species and isolates
Fungal isolates collected from different sites in British Columbia were used in this study (Breuil, 2008; Son et al., 2011). The two *Fomitopsis pinicola* brown rot fungi, isolates 850 and 912 (named in this work BR1 and BR2), were collected at Burns Lake and Prince George, respectively. The two *Trichaptum abietinum* white rot fungi, isolates 5530 and “A” (named WR1 and WR2), were collected at Little Fort and Manning Park, respectively. The fungi were maintained on 1% malt extract agar (MEA) plugs, stored in distilled water at 4°C. To study their growth rate and their competitive interactions in artificial media, we used MEA (Oxoid; OMEA). To assess the decay abilities of the different isolates alone or in competition, we used lodgepole pine wood cubes that were sterilized for 40 minutes at 121°C. In total, we used six replicates for each fungus to determine the growth rate, and four replicates of each combination of fungi (Table 1) to observe their interactions in competition on artificial media. For the wood decay test, four replicates were used for each combination, with two wood cubes each. The fungi were grown at least two consecutive times from the leading edge of an actively growing colony, prior to inoculating them onto fresh 1% OMEA plates for their usages in the different experiments.

Table 1 - Fungal combinations inoculated on 1% malt extract agar and on soil block tests

<table>
<thead>
<tr>
<th>Combination No.</th>
<th>Fungus #1</th>
<th>Fungus #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BR1</td>
<td>BR1</td>
</tr>
<tr>
<td>2</td>
<td>BR1</td>
<td>BR2</td>
</tr>
<tr>
<td>3</td>
<td>BR1</td>
<td>WR1</td>
</tr>
<tr>
<td>4</td>
<td>BR1</td>
<td>WR2</td>
</tr>
<tr>
<td>5</td>
<td>BR2</td>
<td>BR2</td>
</tr>
<tr>
<td>6</td>
<td>BR2</td>
<td>WR1</td>
</tr>
<tr>
<td>7</td>
<td>BR2</td>
<td>WR2</td>
</tr>
<tr>
<td>8</td>
<td>WR1</td>
<td>WR1</td>
</tr>
<tr>
<td>9</td>
<td>WR1</td>
<td>WR2</td>
</tr>
<tr>
<td>10</td>
<td>WR2</td>
<td>WR2</td>
</tr>
</tbody>
</table>

***BR1 and BR2: brown rot strains of *Fomitopsis pinicola* (strains 850 and 912, respectively).
***WR1 & WR2: white rot strains of *Trichaptum abietinum* (strains 5530 and “A”, respectively).

Determination of fungal growth rate in 1% malt extract agar (OMEA)
Fresh fungal mycelium was grown in 1% OMEA (7.5g Oxoid technical agar, 8.33 Oxoid malt extract agar, and 500mL water were mixed and sterilized at 121°C for 40 minutes) at room temperature (~22°C) for a period of at least 10 days. To observe and measure fungal growth rate, 6mm plugs were then taken from the leading edge of one of the aforementioned growing fungal colonies and re-grown in reduced agar malt extract (6g Oxoid technical agar instead of 7.5g). Four radial measurements were then taken from each colony every 2 days, starting at day 2 until day 20, or earlier if the fungal colonies covered all
the radiuses of the plates earlier. The 24 radial measurements (4 measurements for each of the 6 plates) for each day were then averaged to obtain the average colony radius at each day. Linear regression for the days corresponding to the exponential and linear growth phase of all fungi (days 4-12) was then used to estimate the fungal growth rate in mm/day.

**Observations of fungal interactions in 1% malt extract agar (OMEA)**

Forty 1% OMEA plates with reduced agar concentration were used to observe the fungal interactions. The medium contained a high concentration of sugars and peptone and as well as a high concentration of nitrogen (*DIFCO Manual, Dehydrated Culture Media and Reagents for Microbiology*, 1984). To establish the interaction, we placed two colony inoculums (plugs) per plate, about 40mm from each other, and about 20mm from the edge of the plate. We took these inoculums (plugs) from the same or different isolates or species from freshly growing mycelium. A total of 10 combinations were done for the four fungi (Table 1). Four replicates of each combination were grown at room temperature in a dark cabinet for 20 days. During the incubation time, photographs were taken every 2 days, starting from day 6. Macroscopic and microscopic observations at low magnification were performed to gain insight into the possible interactions for each of the fungal combinations. In addition, growth rate measurements were taken on the radius of the edge of the colony that grew towards the competing colony as well as the edge of the colony that grew in the opposite direction.

![Figure 2 - Schematic design for the fungal competition experiment in malt extract agar media](image)

Mycelium plugs were placed face down about 40mm apart from each other to enable macroscopic observations of fungal interactions in 1% malt extract agar (OMEA).

**Modified soil block decay test**

**Jar and wood specimen preparation**

We set up our decay tests using a modified version of the E10-08 Standard Method for Testing Wood Preservatives by Laboratory Soil-Block Cultures (*American Wood Protection Association*, 2012). The
modified test consisted of 500mL mason jars with vegetable garden soil at about 50% volume. Each jar was loaded with about 160g of pre-weighed soil at 40% moisture content. The soil surface was then lightly compacted on the top to achieve a flat surface. Then, a plastic mesh was laid above the soil upon which 2 small 19mm lodgepole pine wood cubes were placed with their radial grains in contact and tangential face down (See Figure 3 below).

![Figure 3 - Illustration of the modified soil block decay test with two wood blocks inoculated with fungi on the end grain (other side not shown) and resting tangential face down above plastic mesh above soil.](image)

Wood cubes utilized in this experiment were cut from a single 2x4 kiln-dried lodgepole pine board that was sourced from a local lumber supplier in Vancouver, BC. In order to avoid high weight variability, the wood cubes used were selected with weights of ~ 3.30g to 3.50g at moisture contents stabilized at ambient temperature. Wood cubes with visible defects, such as pitch pockets, knots, grain deflection from knots, or bark inclusions, were removed. The wood cubes were oven-dried (at 121°C for 24 hours) to record the dry wood mass. Subsequently, the wood cubes were submerged under water for about 4 days to ensure fiber saturation point (FSP), so that the fungi could colonize the wood.

In total, the jars were sterilized twice for 40 minutes at 121°C each time, once after the soil was loaded, and the second time after the mesh and wood cubes were loaded on the top of the soil. Because the original soil samples in the jars had a moisture content of only 40%, we added about 20mL of sterilized water to increase the moisture content to ~ 60%.

**Fungal inoculations of lodgepole pine wood blocks in soil jar tests**

In total, we prepared 44 jars. Each soil block culture jar was prepared with two lodgepole pine wood cubes. The wood cubes in the jars were inoculated either with a single fungal isolate or with a combination of two different isolates from the same species, or with two different fungal species (Table 1). Four jars were not inoculated with fungi and were used as controls. Each jar with its two wood cubes represented one replicate, and for each fungal combination we had four jars (Table 1). The wood cubes
were inoculated with two 8mm mycelium plugs, one on each side of the cube’s end grain. The mycelia from the plugs were in direct contact with the wood. In this test, we did not use a wood feeder strip to allow the establishment of the fungal species as described in the Standard Method of Testing Wood Preservatives By Laboratory Soil Block Cultures (American Wood Protection Association, 2012). Although this would normally speed up fungal colonization in the wood cubes, our new approach was necessary because we needed a method to standardize colonization opportunities for dual fungal colonies. When the fungal combinations were different, the two mycelium plugs with the same fungus were attached to both wood cubes on the same side of the jar, while the other isolates or species were placed on the opposite side of the jar. When the fungal combination was the same, this was not necessary as all the plugs were from the same parent colony. After inoculation, the jars were incubated inside a dark cabinet at room temperature for a period of 12 weeks. Throughout the incubation period, sterilized water was added to the jars to maintain a good humidity inside the jars.

**Measuring fungal decay**
After the incubation period of 12 weeks, the agar plugs and the mycelia on the surface of the wood cubes were removed. The wood cubes were then oven-dried for 24 hours at 105°C to obtain the final dry wood mass. To calculate the amount of decay, the oven dry weight after the decay test was subtracted from the wood weight of the post-decay test, and the percent weight loss was then determined.
Results

Growth and morphology of two isolates of *Fomitopsis pinicola* (BR1 and BR2) and two isolates of *Trichaptum abietinum* (WR1 and WR2)

Overall, all replicates of each fungus growing in MEA grew consistently within the replicates of the isolate. Initially, all of the fungal isolates grew very slowly on 1% malt extract agar plates at room temperature in the dark. This growth phase is reported as the lag phase, a phase during which the fungi adapt to a new environment. However, after four days, all of the fungal isolates grew faster and entered the exponential growth phase (Figure 4). Between 12 to 20 days, the growth of all the strains, except BR2, slowed down and reached a plateau. At this point, the fungi did not grow radially anymore, because either their mycelia had already reached the edge of the plates or because the environmental conditions (e.g. presence of metabolites, pH) in the plates negatively affected the growth of the fungi.

![Figure 4 - Growth of two isolates of *Fomitopsis pinicola* (BR1 and BR2) and two isolates of *Trichaptum abietinum* (WR1 and WR2) on 1% malt extract agar.](image)

***White rot fungi *Trichaptum abietinum* strains 5530 (WR1) and “A” (WR2) were the first to fully colonize the media plates, followed by *Fomitopsis pinicola* strain 850 (BR1). Strain 912 (BR2) of *Fomitopsis pinicola* had a much slower growth rate, and thus was unable to fully colonize the media plates even after 20 days.***
Trichaptum abietinum isolates WR1 and WR2 showed the fastest growth, reaching the edge of MEA plates after about 14 days. The isolates of Fomitopsis pinicola, however, grew more slowly, and did not reach the edge of the plates even after 14 days (Figure 5). These results are in agreement with the observed growth rates per day, where the white rot isolates had growth rates of 8.5mm (WR1) to 7.9mm (WR2) per day and the brown rot isolates only 5.6mm (BR1) and 3.7mm (BR2) per day (Figure 5). The growth rates of the brown rot isolates were significantly lower than those of the white rot isolates. Finally BR1 covered the entire plate surface after 16 days, whereas BR2 covered only about 80% of the plate at day 20. We noted that, macroscopically, BR2 produced denser mycelium coverage (Figure 5).

Figure 5 - Colony morphologies of the two isolates of Fomitopsis pinicola (BR1 and BR2) and two isolates of Trichaptum abietinum (WR1 and WR2) grown on 1% malt extract agar for 14 days.

***From left to right: Brown-rot fungi Fomitopsis pinicola isolates BR1 and BR2 respectively, and white-rot fungi Trichaptum abietinum isolates 5530 and “A” (WR1 and WR2, respectively).
Fungal interactions on 1% malt extract agar

Intraspecific interactions of the same isolate of either *F. pinicola* (brown rot) or *T. abietinum* (white rot)

We assessed the interaction between two inoculums of the same isolate taken from an actively growing isolate. Figure 7 and Figure 8 below show the growth and interaction from 6 to 22 days of paired inoculums of the same isolate. Each pair was replicated four times. These paired isolates from either the white rot or the brown rot fungi showed no unusual growth patterns (Figure 7 and Figure 8). Morphologically, both colonies in a pair had a similar appearance and grew at a similar rate; the morphology and growth rates of these paired colonies were similar to those obtained when only one inoculum was placed per MEA plate (Figure 5 and Table 2). After about 14 days, however, the growth of each colony was limited in the diameter axis because of the lack of space. The colonies were only able to grow in the other direction, resulting in a colony shape with a more elliptical than circular geometry.
Figure 7 - Fungal interactions of the same isolate of *F. pinicola* on 1% Malt Extract Agar.

Figure 8 - Fungal interactions of the same isolate of *T. abietinum* on 1% Malt Extract Agar.

Table 2 - Average Fungal Colony Radius Comparison of Single Colony & Dual Colony Per 1% MEA Plate at Day 7.

<table>
<thead>
<tr>
<th></th>
<th>Single Colonies</th>
<th>Paired Colonies</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR1</td>
<td>15.5</td>
<td>12.9</td>
<td>17%</td>
</tr>
<tr>
<td>BR2</td>
<td>14.3</td>
<td>12.9</td>
<td>9%</td>
</tr>
<tr>
<td>WR1</td>
<td>15.7</td>
<td>13.4</td>
<td>15%</td>
</tr>
<tr>
<td>WR2</td>
<td>13.1</td>
<td>12.9</td>
<td>2%</td>
</tr>
</tbody>
</table>

Intraspecific interactions of two different isolates of either *F. pinicola* (brown rot) or *T. abietinum* (white rot)

We assessed the intraspecific interactions of either two isolates of *F. pinicola* (BR1 and BR2) or of *T. abietinum* (Figure 9). Initially, the two isolates of *F. pinicola* grew normally until day 10 (Figure 9). However, after fourteen days we observed that the two isolates produced an antagonistic interaction zone. This phenomenon was observed macroscopically and microscopically. We noticed a light brown
zone of separation between the two colonies (Figure 10). This was not the case, however, with the two 
*T. abietinum* isolates which appeared first to form a light coloured line of contact, but eventually the 
hyphae of both colonies intermingled (Figure 11). Furthermore, there was no line of discoloration in *T. 
abietinum*’s zone of interaction, in contrast to the lines observed in *F. pinicola*.

Figure 9 - Intraspecific fungal interactions of isolates of *T. abietinum & F. pinicola* on 1% Malt Extract Agar.

Figure 10 - Intraspecific antagonism of two brown rot isolates of *Fomitopsis pinicola*, in 1% MEA.
Interspecies interactions of two different isolates of either *F. pinicola* (brown rot) or *T. abietinum* (white rot)

Interspecies interactions showed more diverse patterns than intraspecific interactions (Figure 12). The general trend was that fungi initially repelled each other. More notably, however, was the suppression of the *Trichaptum abietinum* strains’ (WR1 & WR2) growth by the *Fomitopsis pinicola* isolate (BR2). Macroscopic observations suggest that BR2 (Figure 12, rows 3-4) had a much greater inhibitory effect than BR1 (Figure 12, rows 1-2) on the growth of the *Trichaptum abietinum* isolates. This is evidenced by the much smaller colony size when WR1 and WR2 are in presence of the BR2. However, in the BR1 & WR1 interaction, WR1 grew at a similar rate than BR1, and at day 18, we noted that WR1 was able to grow over or under the BR1 colony (Figure 12, row 1). However, BR1 reacted by producing a denser mycelial mass on the edge of the colony closest to WR1, which may be a defensive response to invasion.
Lodgepole pine wood decay from inoculation of one or two isolates of the same or different fungal species

We combined the *Fomitopsis pinicola* or *Trichaptum abietinum* isolates as shown in Table 1. We then measured the oven-dry mass of the lodgepole pine wood cubes after 12 weeks of incubation at room temperature to determine the percentage mass loss from the original wood cube weights for each fungal combination. Finally, we calculated an average for each combination (of 4-7 cubes for single isolate combinations and 8 cubes for different isolate combinations) as well as the standard deviation (Figure 13).

Figure 13 below shows that the weight losses on wood cubes inoculated with mycelia from the same parent colony differed more for the two brown rot isolates of *Fomitopsis pinicola* (BR1 and BR2) than for the two white rot isolates of *Trichaptum abietinum* (WR1 and WR2). BR1 and BR2 caused the highest and lowest levels of decay at 32% and 11%, respectively, while the two white rot isolates of *Trichaptum abietinum*, WR1 and WR2, had weight losses of 18.7% and 21.5%, respectively. When mycelia of two different isolates of the same species were paired (BR1 and BR2) or (WR1 and WR2), the results were different. For the brown rot pair, the percentage weight loss was ~10.4%, which was closer to that of the single isolate BR2 at ~10.7%. Also it was evidenced that the mycelial morphology showed low
colonization by both fungi (Figure 14, b1 and b2). The weight loss of the WR1 and WR2 pairing was ~19%; this decrease in wood mass was similar to those of each single isolate WR1 and WR2 at 19% and 22%, respectively. For the interspecies interaction between both isolates of *Fomitopsis pinicola* and *Trichaptum abietinum*, the wood weight losses were always lower than for the average of the higher decay fungus in the pair. For example, in the BR1 and WR2 pairing, BR1 decreased the wood weight by ~32% while WR2 showed a decrease of only 22%, but when BR1 was paired with WR2, the wood decrease was 24%. In addition, during colonization of wood, very distinct zones of mycelium boundaries were formed on wood (Figure 15), suggesting that distinct fungal territories have been formed. For one combination, BR1/WR1, the wood mass loss averaged 16%, which was lower than for the single isolates of BR1 at 32% and WR1 at 18.7%.

Figure 13 - Decay of lodgepole pine following 12 week incubation with different combinations of white and brown rot fungi isolates.

![Lodgepole pine decay following fungal incubation after 12 weeks](image)

Figure 14 - Mycelial morphology of decay fungus *Fomitopsis pinicola* of monoculture isolate (BR1) on wood cube (a) and biculture isolates (BR1/BR2) on wood cube (b1, b2) after 12 weeks incubation at room temperature.
Figure 15 - Mycelial morphology of decay fungi *Fomitopsis pinicola* (BR1) interacting with *Trichaptum abietinum* (WR2) on lodgepole pine wood cube after 12 weeks incubation at room temperature.
Discussion

Fungal growth and interaction on MEA
Fungal growth in MEA was consistent within replicates of one isolate. However, although all fungi we used were isolated from the same region (British Columbia, Canada), these isolates, even within the same species, grew differently in MEA. This was especially evident with our different isolates of our brown rot species, *Fomitopsis pinicola*, where one isolate grew much faster than the other in MEA. Given that isolates may have evolved to adapt to the chemistry and wood anatomy of specific hosts and sites, the physiological differences among these isolates are not unexpected as they may have resulted from the selective environmental pressures that the fungi have encountered. These differences could be due to adaptation to different pHs, temperature ranges, or variation in the host chemistry. It is well established that individuals in a population from the same or different locations vary and may not have the same genetic traits. For example, *F. pinicola* has been isolated from different hosts, including conifers in North America and hardwoods in England (Mattock, 2001; Son et al., 2011), and *T. abietinum* has been isolated from Norway spruce (*Picea abies*) in Norway, Sweden, Lithuania, Russia, and Finland as well as Lodgepole pines (*Pinus contorta*) in North America (Högberg, Holdenrieder, & Stenlid, 1999; Kauserud & Schumacher, 2003; Son et al., 2011).

In contrast to the more variable growth rate observed in our *F. pinicola* isolates, our white rot fungi, *Trichaptum abietinum*, had the faster growing isolates on MEA, and with both showing similar growth rates in our experiment. *T. abietinum*, often reported as a sap rot fungus, has been more frequently isolated from the sapwood than the heartwood of conifers, including pines (Allen & Pacific Forestry Centre, 1996; Son et al., 2011). Because of this reason, this species may have evolved to favor faster growth in an environment with higher nutrients and less harsh environmental conditions. The sapwood of trees whose pH is less acidic than the heartwood, and which contains less oleoresins than the latter (Gao, Breuil, & Chen, 1994), could all be contributing factors to an evolutionary adaptation to fast growth under more favorable conditions. The milder conditions of the sapwood may not have applied as severe evolutionary pressures on *T. abietinum* isolates. Conversely, *F. pinicola* which has been more frequently isolated in the heartwood of dead trees (Son et al., 2011), and which grew much slower in MEA, may have evolved to grow more slowly in more complex and toxic environments where it may first need to adapt to or modify to the environment before decaying it.

During some of our intraspecific interaction experiments we observed decreases in fungal growth. Fungi that were paired with isolates of the same species (BR1 with BR2 and WR1 with WR2) did not show an antagonistic reaction (i.e. zone of inhibition). However, the mycelia of the two *F. pinicola* isolates did not make contact and intermingle, in contrast to the two isolates of *T. abietinum*. Furthermore, during the early time phases of the interaction, mycelia of *F. pinicola* isolates appeared to control its environment around the colony by producing a ‘cloudy precipitate’ that appeared in the agar surrounding the colony. We hypothesize that this ‘cloudy precipitate’ may be crystals of oxalate. It has been shown that *F. pinicola* produces oxalic acid that can combine with metallic cations (e.g. calcium and iron) and form...
crystals of oxalate (Jarosz-Wilkolazka & Gadd, 2003; Munir, Yoon, Tokimatsu, & Hattori, 2001). It has been reported that this oxalic acid and some forms of oxalate can affect fungal growth; but some fungi can actively pump these chemicals out of the cell, through an oxalate efflux transporter (Watanabe et al., 2010). In addition, it has been shown in recent studies that white rot fungi can also produce oxalic acid. However, oxalic acid or oxalate are not produced in high concentrations and so they are not detected in artificial media. This is consistent with our observations, where no apparent form of precipitate was formed around the *T. abietinum* fungal isolate colonies, and with the hyphae of both colonies being able to intermingle. Furthermore, white rot fungi have been shown to be able to modify or decompose oxalic acid and oxalate, which in turn would not be significantly detrimental to its fungal growth (Munir et al., 2001; Watanabe et al., 2010).

In the interspecies interactions, the growth inhibition of *T. abietinum* by one of the *F. pinicola* isolates was evident. The colonies of *T. abietinum* preferentially grew away from the competing colonies of *F. pinicola* (BR2). This phenomenon was observed as early as 6 days after inoculation, suggesting that the brown-rot fungus *F. pinicola* may have produced volatile compounds which inhibit or slow down the growth of *T. abietinum* from some distance. We believe that such production of volatile compounds would be responsible for such effect, because a production of oxalic acid or oxalate might not be able to diffuse through agar as quickly, and likely would only produce a local effect, even in a closed environment. The production of such a volatile compound that inhibits competitive fungi (*T. abietinum* in this case) from a distance may be beneficial for *F. pinicola*, which could then occupy more resources where other fungi are not present and nutrients are available.

Meanwhile, in another interspecies interaction, when *T. abietinum* isolate WR1, our fastest growing isolate in MEA, interacted with the other brown rot isolate (BR1), the reaction was different. The white rot isolate’s growth did not appear to slow down as much as when paired with BR2. In contrast to when it was paired with BR2, WR1 colonies grew at a much higher rate when paired with BR1. Furthermore, after the WR1 mycelial front entered in contact with the BR1, the WR1 was able to bypass the contact zone and grow over or under the brown rot (BR1). The opposite was true with the other slower growing white rot isolate, WR2, which when paired with BR1, was also overgrown like when it interacted with BR2. Like other literature cited (Boddy, 2000), our evidence also suggests that the genetic diversity of each isolate as well an ability capture resources early, may be just as important factors as species diversity when it comes to successful fungal competition for space and resources.

**Decay patterns on lodgepole pine wood**

When our BR1 isolate was allowed to grow alone, it was very effective at decaying wood. This could be due to the greater efficiency of the fungus in producing some oxalic acid that results in making the wood more porous, as well as the ability of the fungus to produce the right enzymes to digest the carbohydrates from wood. It is likely that this fungus is well adapted to grow on this substrate, as it has also been often isolated from such wood species (Son et al., 2011). Furthermore, it is likely that the fungus releases a balanced amount of sugars from the cellulose and hemicellulose which are enough for fungal growth, but that do not inhibit the production of the degradative enzymes (Eriksson, Blanchette, & Ander, 1990). However, when BR1 was paired with any other fungus, decay decreased significantly, especially when paired with the other isolate of the same species (BR2) that was less active than BR1 in
decaying wood. This reduction in decay may result from mixing an active isolate with less efficient
decay fungi or from the wood heterogeneity encountered from the different wood blocks. This
contradicted our expectations, where we expected to see some synergistic effects from the increased
diversity of decay fungi in the wood cubes. When WR1 and WR2 (as well as BR1) were paired with BR2,
our slowest decay fungus, decay always showed a marked decreased than when the isolates grew by
themselves. This suggests that the observations on MEA, where BR2 inhibited the other fungi’s growth,
were reflected on our decay tests.

Conclusions
Although direct comparison of fungal pairings in MEA and in wood decay cannot always be made, some
overall patterns were apparent. Fungi allowed to grow by itself, whether on MEA or on wood blocks,
were almost always more successful whether measured by growth rate or decay. Fungi that appeared
to be able to produce volatile chemicals in MEA were also most effective at suppressing growth of
competing fungi in MEA as well as in wood. Fungi that were most successful in MEA growth (the white
rots) were not necessarily the most effective at decaying wood. This is normal, because wood is a very
complex material with many fungitoxic chemicals that can affect different fungi differently. Lastly,
overall decay patterns of interspecies fungal pairings were more homogenous (4.0% standard deviation)
than the collection of individual single isolate pairings (7.7% standard deviation). This suggests that
possible decay experiments which include multiple fungi may yield more consistent results as well as
resemble better decay which occurs in nature.
References


