MYCOPARASITES AND DECAY FUNGI: A STUDY OF THEIR ECOLOGICAL INTERACTIONS ON WOOD BLOCKS OF *BETULA PAPYRIFERA*

by

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Major Advisor

Second Reader

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ABSTRACT

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Keywords: *Betula papyrifera,* decay fungi, *Fomitopsis pinicola, Gliocladium roseum* mycoparasites, *Piptoporus betulinus*, *Trametes pubescens, Trichaptum biforme, Trichothecium roseum, Sesquicillium candelabrum, Verticillium tenerum.*

Decay fungi are an extremely important part of the forest ecosystem and provide essential ecosystem services including the breakdown of complex organic compounds and nutrient release. However, this important group of fungi are predated upon by mycoparasites; fungi which feed on other fungi. This study was conducted to examine the ecological interactions between various mycoparasites on selected species of wood decay fungi in relation to the level of decay observed in birchwood blocks, *Betula papyrifera*. Four species of wood decay fungi were chosen: *Trametes pubescens, Trichaptum biforme, Fomitopsis pinicola,* and *Piptoporus betulinus* to be tested with four different mycoparasites: *Gliocladium roseum, Verticillium tenerum, Trichothecium roseum,* and *Sesquicillium candalabrum. Trametes pubescens* exhibited the greatest average percent (%) decay overall with 56.35%, and could only be reduced to 41.68% in combination with *Gliocladium roseum. Verticillium tenerum* reduced average percent (%) decay to 5.46% when paired with *Fomitopsis pinicola*, but *Gliocladium roseum* and *Sesquicillium candalabrum* were observed to be the most consistent and successful in wood decay mitigation across all treatment combinations. The presence of mycoparasites negatively affects the overall ability of wood decay fungi to breakdown woody compounds, however, some are more successful than others, and some species of decay fungi show great resilience when faced with predation. Both entities perform essential ecosystem services.

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INTRODUCTION

WOOD DECAY

In a typical forest ecosystem, woody perennial plants comprise up to 70% of all aboveground biomass and account for a significant proportion of the belowground biomass due to complex root systems (Boddy and Watkinson 1995). Wood in itself is a complex organic structure comprised of three main compounds: cellulose, hemicellulose, and lignin (Worrall 2019). The process of wood decomposition involves enzymatic activities which chemically breakdown the three compounds into more usable forms within the forest ecosystem (Worrall 2019; Otjen and Blanchette 1986; Hammel *et al.* 2002). This process is relatively slow compared to other natural processes, and is highly dependent on several factors including: microclimate, the size of the wood, and the organism responsible for the breakdown of the compounds (Boddy and Watkinson 1995).

White-rot

Fungi that have the ability to breakdown all three components of wood through the process of depolymerization are known as white-rot fungi (De Groot 1972). White-rot decay is the most common type of wood decay, and yet only species of the Division Basidiomycota and some species of the Division Ascomycota are able to induce white-rot (Otjen and Blanchette 1986). The process is caused by enzymatic secretions by the fungi which completely depolymerize all woody components including lignin, which is brown in colour, leaving a white appearance (De Groot 1972; Worrall 2019; Hammel *et al.* 2002; Eaton 2000). The appearance of white-rot in wood is dependent on the interaction between the wood substrate and the type of fungi (Worrall 2019). White-rot

may present itself as stringy, spongy, laminated, mottled, white-pocketed, or as zone lines (Worrall 2019). This type of rot mainly attacks hardwood tree species and decays the innermost layers of woody cell walls and works its way outwards, decreasing the structural integrity of the wood with a noticeable and proportional decrease in weight (De Groot 1972).

Brown rot

Other species of decay fungi within the Division Basidiomycota cause what is known as brown rot, also known as cubical rot. This form of wood decay occurs when only the cellulose and hemicellulose components, the polysaccharides, are removed during the decay process (Worrall 2019; Curling *et al.* 2002; Green and Highley 1997; Eaton 2000). The lignin is left intact but slightly modified by oxidation and demethylation; it shrinks and becomes brittle, dark and cubical in appearance, and the structural integrity of the wood is significantly reduced (Green and Highley 1997; Curling *et al.* 2002; De Groot 1972; Eaton 2000). Brown-rot is predominantly found in softwood tree species, but can also be found on some hardwood species such as sugar maple (*Acer saccharum* Marshall) (Green and Highley 1997). It is known to cause significant structural damage to wood in-use and can cause the strength properties of wood to deteriorate before any weight-loss is detected (Green and Highley 1997).

Soft rot

Soft rot is a different kind of rot caused by members of the Division Ascomycota (Worrall 2019). This type of rot does not occur in living wood and predominantly affects wood in service and poses a major issue for industry (Worrall 2019; De Groot 1972). Wood exposed to high levels of moisture from wet soil, or directly exposed to

water over long periods of time is susceptible to this form of rot (De Groot 1972). The fungi attack the secondary walls of wood cells due to a decreased lignin content and create elongated cavities which gives the wood a spongy texture (Eaton 2000). This type of rot leaves a displeasing look of soft, spongy and brown wood as the outer cells are deprived of their polysaccharide components, while the inner cells are attacked by the fungi (Eaton 2000).

WHAT FUNGI ARE INVOLVED IN WOOD DECAY?

Fungi of forest ecosystems come from a vast range of genera and species, and have wide ecological implications. The Kingdom Fungi consists of several different Divisions of fungi characterized by their physiology, development, and structure (Alexopoulos and Mims 1979). Two Divisions of note include the Basidiomycota and the Ascomycota which include fungi that predate on plant and woody organic matter for its cellulose and/or lignin content (Alexopoulos and Mims 1979; Moore-Landecker 1972). These fungi are known as decay fungi, and are responsible for the breakdown of wood which is considered a complex organic material (Boddy and Watkinson 1995; Alexopoulos and Mims 1979). Decay fungi exist primarily as saprophytes, utilizing wood in dead trees and fallen logs, while a few are able to behave as facultative necrotrophic parasites and infect living trees. Of the latter, some occur as root parasites, while others decay in the heartwood of stems of living trees causing a typical heart rot type of decay (Moore-Landecker 1972).

The Division Basidiomycota gives rise to the class Hymenomycetes and class Gasteromycetes which are conspicuous groups of fungi that produce large fruiting bodies and reproductive structures known as basidiomata which produce spores during

their reproductive cycle (Alexopoulos and Mims 1979). Fungi of the class Hymenomycetes include bracket fungi, crust fungi, and mushrooms among others, while fungi of the class Gasteromycetes include puffballs, stinkhorns, and bird's nest fungi (Alexopoulos and Mims 1979). They are some of the only fungi capable of breaking down lignin via enzymatic activities, and are generally classified as those fungi which produce mycelium, and decompose wood and litter with mycelial enzymatic activities (Dix and Webster 1995; Moore-Landecker 1972).

The Division Ascomycota are a group of less conspicuous fungi that produce their spores in asci, which are sac-like structures within small inconspicuous fruiting bodies (Alexopoulos and Mims 1979). Fungi of the Division Ascomycota are typically found as saprophytes on dead organic and woody material, although many are parasites of herbaceous plants (Alexopoulos and Mims 1979).

ECOLOGICAL ROLE OF WOOD DECAY FUNGI

Dead woody debris and litter account for a significant proportion of the biomass found in a typical forest ecosystem (Boddy and Watkinson 1995). This biomass contains two important and very limited nutrients in the forest ecosystem: nitrogen and phosphorus. Without decomposer organisms such as decay fungi, these nutrients are trapped inside woody structures or are in unusable forms, and would remain unavailable to the ecosystem (Hiscox, *et al.* 2018; Otjen and Blanchette 1986; Boddy and Watkinson 1995; Dighton 2003). However, while decay fungi are able to break down the cell walls of wood and release the nitrogen and phosphorus contained within, hardly any of it is released into the ecosystem immediately (Boddy and Watkinson 1995). Instead, the fungi rapidly use these limiting nutrients to produce their reproductive structures or

increase the surface area of their mycelial mats (Boddy and Watkinson 1995). The nutrients do eventually reach the rest of the forest ecosystem by ways of organism interaction, mycelial grazing by animals, and leakage from mycelium when it starts to senesce (Boddy and Watkinson 1995; Dix and Webster 1995).

Decay fungi also release trapped carbon through the breakdown of woody compounds. Carbon is an essential nutrient in forest ecosystems, and the breakdown of woody structures allows the stored carbon to continue in the carbon cycle as carbon dioxide (CO_2) (Boddy and Watkinson 1995; Hiscox *et al.* 2018; Otjen and Blanchette 1987). Generally, most of the carbon stored is released within the first ten years of decomposition depending on moisture levels and temperature, though this is subject to variation (Van Der Wal *et al.* 2015).

DIFFICULTIES DECAY FUNGI FACE

Despite the very important role that fungi play in forest ecosystems they are often underappreciated (Stephenson 2010). Not only is there a great deal that science has yet to discover about fungi, but there is a prominent stigma that fungi are bad for the environment, disgusting, and overall weird. Decay fungi bear a large portion of this stigma due to their ability to decompose organic compounds, especially wood. Moreover, in professions such as urban forestry, decay fungi are viewed as the enemy as they are responsible for, or play a large part in, the failing of a given tree if they happen to be present (Terho *et al.* 2007).

Microclimatic conditions

Decay fungi require certain microclimatic conditions in order to succeed in their environment (Moore-Landecker 1972; Alexopoulos and Mims 1979; Pouska *et al.*

2016). Such conditions include but are not limited to: moisture content of their host, temperature of their surroundings, oxygen levels, host acidity, light, aeration, etc. (Alexopoulos and Mims 1979; Moore-Landecker 1972).

Decay fungi typically require moderate levels of moisture in their host substrate, however, Boddy and Rayner (1983) explain that extremely high levels of moisture and their associated low oxygen diffusion rates can actually inhibit mycelial growth, as can be seen with the lack of decay fungi present in submerged timber (Moore-Landecker 1972; Boddy and Rayner 1983; Pouska *et al.* 2016). Moisture content also includes the relative humidity of the surrounding air, and for maximum growth decay fungi require approximately 95-100% relative humidity (Moore-Landecker 1972). Growth tends to decline or plateau around 80-85% relative humidity, and few have the capability to grow below 65% (Moore-Landecker 1972). More specifically, decay fungi are unable to decompose wood without a wood moisture content of at least 20% (Moore-Landecker 1972). Standing trees/snags face more frequent changes in moisture content from external climatic conditions as compared with fallen woody debris exposed to soil moisture (Dix and Webster 1995; Pouska *et al.* 2016).

Temperature is another major factor in fungal growth success. As a general rule, fungal growth increases with temperature as it increases the enzymatic activities of the fungus which allows it to feed (Moore-Landecker 1972). The majority of fungi are considered mesophiles, meaning that they prefer moderate temperature for optimum growth (Dix and Webster 1995). The optimum range for fungal growth is a range that changes for different species and is debated, but generally can be anywhere between 5 and 30 degrees Celsius with the optimum range being between 20-25 degrees Celsius,

although specific species of fungi may fall outside this range (Moore-Landecker 1972; Dix and Webster 1995). Decay fungi colonizing wood must be able to handle a 20 to 30-degree temperature change during the course of the day, as wood can be warmed by the sun during the day but cools substantially overnight (Dix and Webster 1995). But, for some fungi, even small changes in temperature are enough to disrupt their physiological processes (Dix and Webster 1995; Moore-Landecker 1972). According to Pouska *et al.* (2016), standing trees and snags experience a warmer microclimate compared to deadwood on the forest floor, as the ground is cooler due to canopy cover and soil moisture (Pouska *et al.* 2016). Dix and Webster (1995) cite a study involving the temperatures of lodgepole pine slash piles and found that certain species of fungi were a dominant colonizer of the top of a slash pile which reached temperatures of 50-55 degrees Celsius, and others were more dominant in the cooler bottom of the pile (Dix and Webster 1995).

Nutrient requirements

Despite the seemingly destructive nature of decay fungi, they are an essential part of the forest ecosystem. Their inability to create food for themselves makes them completely dependent on their hosts for survival. As with any organic life form, decay fungi require various micro and macronutrients in order to be successful. Required macronutrients include: carbon, nitrogen, oxygen, hydrogen, phosphorus, potassium, magnesium, sulphur. The following micronutrients are required in much smaller quantities: boron, manganese, copper, iron, zinc and molybdenum (Moore-Landecker 1972; Alexopoulos and Mims 1979).

Of the macronutrients, several are relatively easy for the fungus to obtain;

oxygen from the atmosphere and hydrogen from the presence of water. However, nitrogen and carbon are harder to come by. Carbon presents a problem as it is required in such high volumes in order to create the fungus' physical structure, and accounts for nearly half of a fungus' dry weight (Moore-Landecker 1972). Carbon is obtained from breaking down carbohydrates, specifically the saccharide structures which are commonly found in wood (Moore-Landecker 1972). However, Dix and Webster (1995) point out that only certain species of fungi are able to tolerate high levels of carbon dioxide, and these colonizers can often be found within the heartwood of their host (Dix and Webster 1995)

Nitrogen is an element which is required by all organisms to synthesize amino acids and create proteins essential for growth (Moore-Landecker 1972). Unfortunately, it is also one of the most limiting nutrients within wood. Nitrogen can be obtained through recycling from senescent hyphae, parasitism of colonies of bacteria (Barron 1988), and the parasitism of invertebrates such as nematodes (Thorn and Barron 1984).

Presence of tannins and other chemicals

Tannins are antifungal compounds and are very abundant in woody matter in two different states: hydrolysable and condensed tannins (Dix and Webster 1995). Tannins are present in the greatest quantity in the bark layer of woody plants, and act as a deterrent to fungal colonization and inhibit enzyme production (Dix and Webster 1995). However, certain types of tannin-hydrolysing bacteria exist to break down the tannin compound through a chemical reaction with water, thus allowing the colonization of wood-decay fungi which were once inhibited from colonizing the woody material (Dix and Webster 1995). While the sapwood of most tree species possesses a moisture

content too high for wood decay fungi to colonize, the heartwood possesses certain fungitoxic/fungistatic properties as well as extractives to protect the tree from fungal colonization. These can include alkaloids, phenols, and resins, as well as a higher CO_2 concentration which only certain fungi are able to tolerate (Dix and Webster 1995; Boddy and Rayner 1983).

Grazing by mycophagous vertebrates and invertebrates

The large and conspicuous fruiting bodies of several types of fungi are often a sought-after food source for some types of biota. These fruiting bodies are rich in nitrogen and phosphorus; two nutrients not readily available in most forest ecosystems, and are often predated upon by various vertebrate animals and mycophagous invertebrates (Dighton 2003). Vertebrates such as squirrels, deer, and caribou are known to graze on mushroom fruiting bodies (Fogel and Trappe 1978). Furthermore, these vertebrates typically resort to consuming fungi when there are no other food sources available (Dighton 2003).

Mycophagous invertebrates, however, deliberately and consistently feed on certain types of Basidiomycete fruiting bodies and inhibit their growth (Dighton 2003). Several insects belonging to the insect order Diptera (flies) and order Collembola (springtails), produce larvae and adults which feed on the fleshy fruiting bodies or mycelium of these fungi, and thus severely impact their growth and reproductive success (Dighton 2003; Dix and Webster 1995). However, some fungi are able to produce secondary metabolites which make them unpalatable to some invertebrates and vertebrates; animals will not eat what they know or can sense will make them ill (Dighton 2003).

Fungal mycelium may also experience predation, this time from fungivorous invertebrates. These organisms graze on the fungus' mycelium, and the level to which grazing occurs is shown to have different effects on mycelial growth after the fact (Dix and Webster 1995). Though the scientific evidence is not all in agreement, a general trend is evident: light grazing from fungivorous invertebrates can stimulate mycelial growth under certain conditions, and over-grazing may reduce growth and lead to an increase in bacterial populations (Dix and Webster 1995). Scientists are unsure as to why light-grazing can increase mycelial growth, but some believe the introduction of faeces and other recycled nutrients into the environment may play a role (Dix and Webster 1995). A different study by Hanlon (1981) found that mycelial growth stimulation only occurred if the substrate possessed ample nutrients as the grazers only left the mycelium with recycled nutrients in very low quantities (Dix and Webster 1995; Hanlon 1981).

Nematodes are a unique type of invertebrate that inhabit woody debris, plant litter and soils, and are considered fungivorous, bacterivorous, predacious, or omnivorous depending on their morphology (Dix and Webster 1995; Swift *et al.* 1979). They are able to feed on the tissues and mycelium of decay fungi and release the stored nitrogen and carbon into the environment (Dix and Webster 1995). They can also damage or destroy fungal propagules which affects their physiological processes and can limit their success in the forest environment (Dighton 2003; McGonigle 1995).

MYCOPARASITISM

While decay fungi focus on the breakdown of complex organic compounds, they themselves are predated upon by parasitic fungi known as mycoparasites (Hiscox *et al*.

2018; Barnett and Lilly 1962). Mycoparasites, as with any other fungi, form biotrophic or necrotrophic relationships with their host, and such relationships are claimed to occur when the mycelium of one fungus gains nutrition from the mycelium of another (Hiscox *et al.* 2018; Jeffries 1995; Barnett and Lilly 1962; Kobayashi and Hillman 2005; Dix and Webster 1995).

Biotrophic relationships tend to be obligate for the mycoparasite as it cannot live independently of its host (Moore-Landecker 1972; Jeffries 1995; Barnett and Lilly 1962; Dix and Webster 1995). Nutrients are obtained directly from the mycelium of a living host via three specific host-parasite relationships: intracellular, haustorial, and fusion (Jeffries 1995). Intracellular biotrophic mycoparasitism involves the thallus of the parasite completely penetrating the hypha of the host while the cytoplasm remains alive (Jeffries 1995). Haustorial biotrophic mycoparasites use a short haustorial branch from their hypha to penetrate the hypha of the host while the cytoplasm remains alive (Jeffries 1995). Fusion biotrophic mycoparasites require direct or close contact with their host in order to develop micropores between the hyphae of each fungus, or a short penetrative hyphal branch will emerge from the parasite. The cytoplasm remains alive in this situation as well (Jeffries 1995). Biotrophic mycoparasites tend to have more restricted host ranges compared to necrotrophic mycoparasites as their specific host relationships are far more specialized (Jeffries 1995).

Necrotrophic relationships indicate a lethal relationship between the parasite and host, and the host may experience a decrease in fitness due to the lack of nutrient uptake and eventual mortality (Hiscox *et al.* 2018; Jeffries 1995; Moore-Landecker 1972; Dix and Webster 1995). Two types of necrotrophic relationships exist between parasite and

host: contact and invasive (Jeffries 1995). Contact necrotrophic/hyphal interference mycoparasites do not penetrate their host's cells, however, the parasite's hyphae cause degradation of the host cell's cytoplasm and hyphal lysis is possible (Jeffries 1995; Dix and Webster 1995). Invasive necrotrophic mycoparasites physically penetrate the host using hyphae, degrade the cytoplasm rapidly and is often followed by hyphal lysis (Jeffries 1995). According to Jeffries (1995), necrotrophic mycoparasites, regardless of their specific relationship with their host, have a much broader host range compared to biotrophic mycoparasites. They are also less specialized when it comes to specific host-parasite relationships, as necrotrophic mycoparasites tend to release an abundance of lytic enzymes and toxins into their environment, are overly destructive, and have no special penetration/infection structures (Jeffries 1995). They also have the ability to live independently of their hosts.

Mycoparasites are an essential part of the forest ecosystem as they are imperative in determining fungal succession and community structure (Hiscox *et al.* 2018; Jeffries 1995; Dix and Webster 1995). The relationships between decay fungi and mycoparasites, and evidence of such interactions, is an area of interest within forest ecology although it is still not fully understood (Barnett and Lilly 1962; Jeffries 1995; Hiscox *et al.* 2018).

OBJECTIVE

The objective of this study is to examine the interactions between various combinations of wood decay fungi and mycoparasites on inoculated birch wood blocks via rates of decay using dry weight data. The data collected from this study and results from statistical analysis will contribute to a better understanding of the interactions

between wood decay fungi and mycoparasites regarding their relationships *in vitro*, and to possibly extrapolate their interactions to the natural forest environment.

HYPOTHESIS

The null hypothesis (Ho) of this experiment consists of three parts:

1. There will be no differences in weight loss in the blocks inoculated with different wood decay fungi.
2. There will be no differences in the weight loss in the blocks inoculated with different mycoparasites.
3. Blocks inoculated with both mycoparasites and wood decay fungi will experience no differences in weight loss compared with blocks inoculated with wood decay fungi only.

The alternative hypothesis states:

The blocks inoculated with both a mycoparasite and wood decay fungus will experience differences in weight loss compared to the blocks inoculated with wood decay fungi only.

MATERIALS AND METHODS

WOOD BLOCK PREPARATION

On September 17, 2019; one hundred and twenty-five of $2cm^3$ wooden blocks of *Betula papyrifera* Marshall were numbered and individually placed onto separate aluminum weigh boats. These were placed in the drier located in BB 1046 and dried for two days at 100 degrees Celsius. Each block was weighed using a scale accurate to a milligram. Forceps were used to transfer the blocks to the scale and back to their weigh boats. After weighing, the blocks were rehydrated in a tub of water to later be used in the experiment.

BOTTLE PREPARATION

This experiment required the use of one hundred and twenty-five individual Quorpak (250mL) glass bottles, into which a mixture of 120mL of vermiculite (growing media) and 70mL of 2% malt extract broth were added. The rehydrated blocks were then placed into the bottles using forceps, buried in the vermiculite, and the bottles were lightly capped, covered with aluminum foil which was numbered and then placed into the autoclave for 30 minutes at 121 degrees Celsius.

INNOCULATION OF DECAY FUNGI AND MYCOPARASITES

This experiment required the use of four species of decay fungi: *Trametes pubescens* (Schum.: Fr.) Pil., *Trichaptum biforme* (Fr. In Klotzsch) Ryvarden., *Fomitopsis pinicola* (Fr.) Karst., and *Piptoporus betulinus* (Bull.: Fr.) Kar., and the use of four mycoparasites: *Gliocladium roseum* Bain., *Verticillium tenerum* (Nees ex Pers.) Link, *Trichothecium roseum* (Pers.) Link ex Gray, and *Sesquicillium candalabrum* (Bonord.) W. Gams. Cultures of both decay fungi and mycoparasites were grown on

Petri plates containing 2% malt extract agar, and stored in an incubator prior to experimental setup to allow for adequate growing time. From these cultures, 7mm plugs of inoculum were created using a cork borer and a spatula. All utensils were sterilized using 70% alcohol and a Bunsen burner.

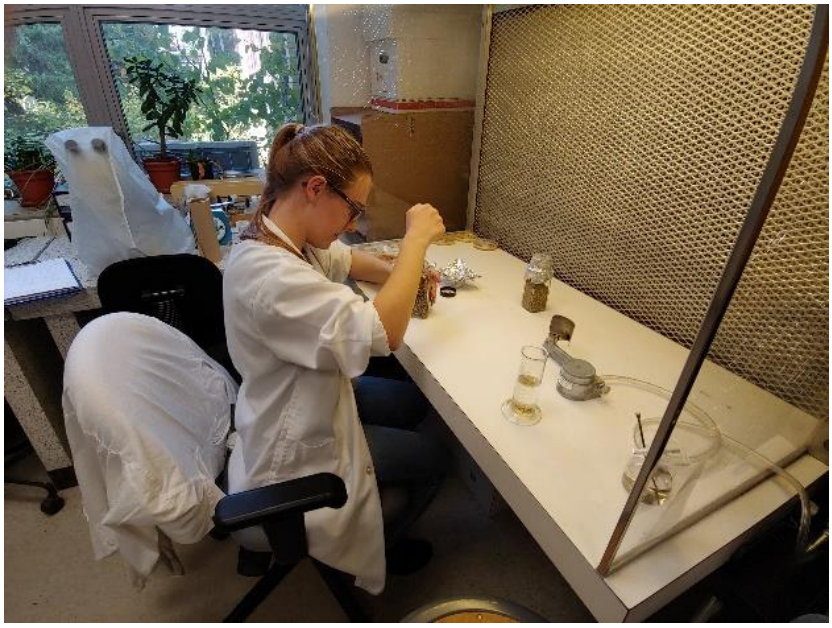
This experiment was designed to test each decay fungus alone, each mycoparasite alone, and decay fungi and mycoparasites together in different combinations. Each treatment was replicated five times to ensure an accurate representation of results. There were five control replicates with no fungi to serve as a baseline for comparison. Table 1 on page 17 provides an outline of the experimental design.

On October 1, 2019, bottles 1-65 were inoculated as per the experimental design in Table 1. One week later on October 8, 2019, the remaining bottles were inoculated. All inoculations took place under the transfer hood in the Forest Pathology Research Lab at Lakehead University. The workspace under the transfer hood was sterilized with 70% alcohol prior to work to ensure limited chances for contamination. Bottles were inoculated using inoculum plugs as mentioned previously. Two plugs of each respective fungus and/or mycoparasite species were aseptically placed onto the sides of the wooden blocks within the bottles using a sterilized metal spatula. The blocks were then reburied into the vermiculite using the spatula, and the edges of the bottle were placed over the Bunsen burner flame to further sterilize any possible contaminants. The caps were then tightly screwed on and the aluminum tops placed back over the bottles. All bottles were then placed into the incubator within the Forest Pathology Research Lab for four months to allow for adequate experimental run time. The bottles were arranged in a random

order within the incubator using a number drawing system, and were rearranged approximately three times. Figures 1 and 2 display the experimental setup process.

Source. Emily Rea

Figure 1. Inoculation work station



Source. Dr. Hutchison

Figure 2. Author inoculating bottles aseptically

Table 1. Experimental design

Bottle Numbers	Mycoparasite	Decay Fungi
1-5	control	Control
6-10	none	*Trametes pubescens*
11-15	none	*Trichaptum biforme*
16-20	none	*Fomitopsis pinicola*
21-25	none	*Piptoporus betulinus*
26-30	*Gliocladium roseum*	None
31-35	*Verticillium tenerum*	None
36-40	*Trichothecium roseum*	None
41-45	*Sesquicillium candalabrum*	None
46-50	*Gliocladium roseum*	*Trametes pubescens*
51-55	*Verticillium tenerum*	*Trametes pubescens*
56-60	*Trichothecium roseum*	*Trametes pubescens*
61-65	*Sesquicillium candalabrum*	*Trametes pubescens*
66-70	*Gliocladium roseum*	*Trichaptum biforme*
71-75	*Verticillium tenerum*	*Trichaptum biforme*
76-80	*Trichothecium roseum*	*Trichaptum biforme*
81-85	*Sesquicillium candalabrum*	*Trichaptum biforme*
86-90	*Gliocladium roseum*	*Fomitopsis pinicola*
91-95	*Verticillium tenerum*	*Fomitopsis pinicola*
96-100	*Trichothecium roseum*	*Fomitopsis pinicola*
101-105	*Sesquicillium candalabrum*	*Fomitopsis pinicola*
106-110	*Gliocladium roseum*	*Piptoporus betulinus*
111-115	*Verticillium tenerum*	*Piptoporus betulinus*
116-120	*Trichothecium roseum*	*Piptoporus betulinus*
121-125	*Sesquicillium candalabrum*	*Piptoporus betulinus*

Source. Dr. Hutchison

HARVESTING THE BLOCKS

Blocks were harvested in two rounds, one week apart, to account for the two rounds of initial inoculation and ensure equal time spent in the incubator for all bottles. On February 5, 2020, blocks 1-65 were harvested from their bottles, and on February 11, 2020, blocks 66-125 were harvested from their bottles. The harvesting process involved the use of sterile forceps, a spatula, and an autoclave-safe disposal bag for the used vermiculite.

The aluminum tops of the bottles were disposed of upon opening, and caps were removed. Photos were taken of one of the five bottles from every trial, and extra photos were taken of any abnormal mycelial growth within the trials to allow for accurate observational data. The blocks were then secured within the bottle using forceps and removed. Any adhering vermiculite, mycelium, or inoculum plugs were scraped off using a spatula into the autoclave bag, and blocks were placed onto corresponding numbered aluminum weigh boats.

Once all blocks from the harvest round had been removed, they were placed into the drying oven on their weigh boats for two days at 100 degrees Celsius. Blocks were then weighed using sterile forceps and a scale accurate to 0.000g. Weights were recorded in a data table along with the weights prior to inoculation.

STATISTICAL ANALYSIS

Results obtained from the experiment were converted into percent decay values in order to be compared, and this was done using the following formula:

$$Percent\ decay\ (\%) = \left(\frac{Dry\ weight\ (initial) - Dry\ weight\ (after)}{Dry\ weight\ (initial)}\right)\ x\ 100\%$$

Using these data, a univariate analysis (one-way ANOVA) was conducted using the IBM statistical software system SPSS Statistics. Results from the ANOVA test can be found in Appendix II. The analysis used the percent decay values as the response variable, with the presence of the various species of decay fungi, and the presence of mycoparasites as the fixed variables in order to detect significant differences and relationships using a P value of less than 0.05. After significant differences were

determined a Least Significant Difference (LSD) test was performed using the following formula:

$$LSD = {}^{t^{\propto}}/_{2, df^{s.e._{y1-y2}}}$$

Using the results of this test, a Post-Hoc test was then completed in order to determine if there is a significant difference between the means. All results from these tests can be found in Appendix II.

RESULTS

A summary of the average results is presented in Table 2, full dry weight results can be found in Appendix I. The twenty-four different treatments, each with five replicate bottles, are presented along with the five control replicate bottles which serve as a baseline for percent decay comparison. Bottles 6-25 contain no mycoparasites, and bottles 26-45 contain no decay fungi, and bottles 46-125 contain various combinations of decay fungi and mycoparasites as per the experimental design in Table 1. The average dry weight for each of the treatments, along with the calculated average percent (%) decay (difference in weight between average initial and average after weights listed as a percent) is listed in Table 2.

Observing treatments 2-5 where only wood decay fungi were present, *Trametes pubescens* was the most successful wood decay fungus with 56.35% decay. However, when paired with the mycoparasite *Gliocladium roseum*, the percent decay fell to 40.25%, a 16.10% decrease in average decay. The mycoparasite *Sesquicillium candalabrum* behaved in a similar manner to *G. roseum,* reducing decay to only 41.68%. The other mycoparasites were not as successful in their attempts to mitigate decay, with *Trichothecium roseum* being the worst of the group allowing for 58.99% decay over the incubation period.

The wood decay fungus *Trichaptum biforme* exhibited the lowest percent decay during its individual treatment, accomplishing only 37.93% decay. However, the mycoparasite *Sesquicillium candalabrum* was observed to have a substantial positive impact on the rate of decay, reducing it to 6.61%. *Verticillium tenerum* was observed to have the opposite effect and was entirely unsuccessful in reducing decay as it increased

from the baseline of 37.93% to 38.30%.

Fomitopsis pinicola presented a different situation from the other wood decay fungi as three of the four mycoparasites it was paired with demonstrated significant reductions in average decay. With a baseline average of 45.85% *F. pinicola* is the second most successful wood decay fungus within this experiment. Unlike the previous treatment, the mycoparasite *Verticillium tenerum* was observed to have the most significant impact on decay reduction when paired with *F. pinicola*, and reduced decay to just 5.46%; this was the greatest recorded reduction of decay across all treatments. In contrast, the mycoparasite *Trichothecium roseum* was observed to be the least successful allowing 56.39% decay over the incubation period; 10.54% more decay than the baseline treatment.

The final wood decay fungus, *Piptoporus betulinus*, was in the middle for baseline percent decay, accomplishing only 42.82% independently. The mycoparasite *Gliocladium roseum* was observed to be very successful in mitigating decay as it was able to reduce it to just 5.92%. The other three mycoparasites were not as successful as *G. roseum* but were still able to reduce decay to 21.12% or less.

Table 2. Summary of average results

Bottle numbers	Decay fungi	Mycoparasite	Average initial wood block weight (g)	Average wood block weight after experimental process (g)	Average percent decay (%)
1-5'	control	control	4.156	4.159	-0.067
6-10'	*T. pubescens*	none	4.845	2.115	56.351
11-15'	*T. biforme*	none	4.250	2.639	37.921
16-20	*F. pinicola*	none	4.325	2.342	45.850
21-25	*P. betulinus*	none	4.447	2.543	42.824
26-30	None	*G. roseum*	4.681	4.503	3.819
31-35	None	*V. tenerum*	4.322	4.183	3.225
36-40	None	*T. roseum*	4.634	4.490	3.095
41-45	None	*S. candalabrum*	4.737	4.527	4.433
46-50	*T. pubescens*	*G. roseum*	4.714	2.817	40.251
51-55	*T. pubescens*	*V. tenerum*	4.799	2.279	52.515
56-60	*T. pubescens*	*T. roseum*	4.948	2.029	58.998
61-65	*T. pubescens*	*S. candalabrum*	4.354	2.540	41.675
66-70	*T. biforme*	*G. roseum*	4.667	3.949	15.385
71-75	*T. biforme*	*V. tenerum*	4.472	2.759	38.302
76-80	*T. biforme*	*T. roseum*	4.768	3.051	35.997
81-85	*T. biforme*	*S. candalabrum*	4.638	4.332	6.610
86-90	*F. pinicola*	*G. roseum*	4.664	3.924	15.878
91-95	*F. pinicola*	*V. tenerum*	4.664	4.409	5.467
96-100	*F. pinicola*	*T. roseum*	4.447	1.939	56.389
101-105	*F. pinicola*	*S. candalabrum*	4.667	4.134	11.429
106-110	*P. betulinus*	*G. roseum*	4.510	4.243	5.920
111-115	*P. betulinus*	*V. tenerum*	4.859	3.980	18.098
116-120	*P. betulinus*	*T. roseum*	4.839	3.814	21.189
121-125	*P. betulinus*	*S. candalabrum*	4.589	3.903	14.953

Source. Appendix I

Table 3 outlines the results of the one-way ANOVA (Analysis of Variance) test which was conducted using the raw data found in Appendix I. The percent (%) decay was used as the response variable, and the species of decay fungi and mycoparasites acted as the fixed variables to determine if there is a significance between the relationships ($p<0.05$). The ANOVA test indicated that the relationship between the wood decay fungi and presence of the various mycoparasites showed significance, which translates to the mycoparasites having a significant effect on the decay caused by

the wood decay fungi. As can be noted from Tables 2 and 3, there are differences between the observed weights within the treatments involving wood decay fungi only, between the treatments involving mycoparasites only, and within treatments involving a combination of wood decay fungi and mycoparasites. Based on these data, we reject each component of the null hypothesis, and fail to reject the alternative hypothesis as it demonstrates significance.

Table 3. Results of univariate analysis

Source	df	Mean Square	Significance (p)
Decay fungi	4	7065.511	0.00
Mycoparasite	4	2496.080	0.00
Decay fungi*Mycoparasite	16	613.004	4.42×10^{-6}
Error	100	137.425	

Source. Appendix III, IBM SPSS

Due to the significant relationship presented by the decay fungi – mycoparasite interaction within the ANOVA test, a Least Significant Difference (LSD) test was conducted to determine which combinations presented the difference. Using the formula presented in the Materials and Methods section, an LSD value of 3.27 was calculated, and from this the data was able to be sorted into nine different groups: A - I. Treatments classified within groups F - I saw larger average % decay rates, indicating a relatively unsuccessful treatment. Treatments classified within groups A-E saw lower rates of average % decay which indicates a relatively successful treatment, with group A indicating the most successful treatments. Within the LSD tests, all species of decay fungi and mycoparasites are referred to using specific codes. The decay fungi are identified as follows: *T. pubescens* – TP, *T. biforme* – TB, *F. pinicola* – FP, and *P. betulinus* – PB; the decay fungus code creates the first half the treatment code. The mycoparasites are identified as follows: *G. roseum* – GR *V. tenerum* – VT, *T. roseum* –

TR, and *S. candalabrum* – SC; the mycoparasite code makes up the second half of treatment code. Table 4 outlines the treatments and their respective LSD groups.

Table 4. Treatments with their corresponding LSD groups based on average % decay

Treatment	Average percent decay (%)	LSD group
FP-VT	5.46%	A
TB-SC	6.61%	A
FP-SC	11.42%	B
PB-GR	11.42%	B
PB-SC	14.95%	C
TB-GR	15.38%	CD
FP-GR	15.87%	CD
PB-VT	18.09%	E
PB-TR	21.18%	E
TB-TR	35.99%	F
TB-VT	38.30%	F
TP-GR	40.25%	G
TP-SC	41.68%	G
TP-VT	52.51%	H
FP-TR	56.39%	I
TP-TR	58.99%	I

Source. Appendix II

In analyzing the treatment groups for significant relationships, there is a significant difference between the average decay rates between treatments involving *Fomitopsis pinicola*. When paired with *Trichothecium roseum*, the second greatest average percent decay for any treatment was observed at 56.39% within LSD group I. The other mycoparasite treatments fell within LSD groups A-CD with *Verticillium tenerum* producing the lowest average % decay of any treatment at 5.46%.

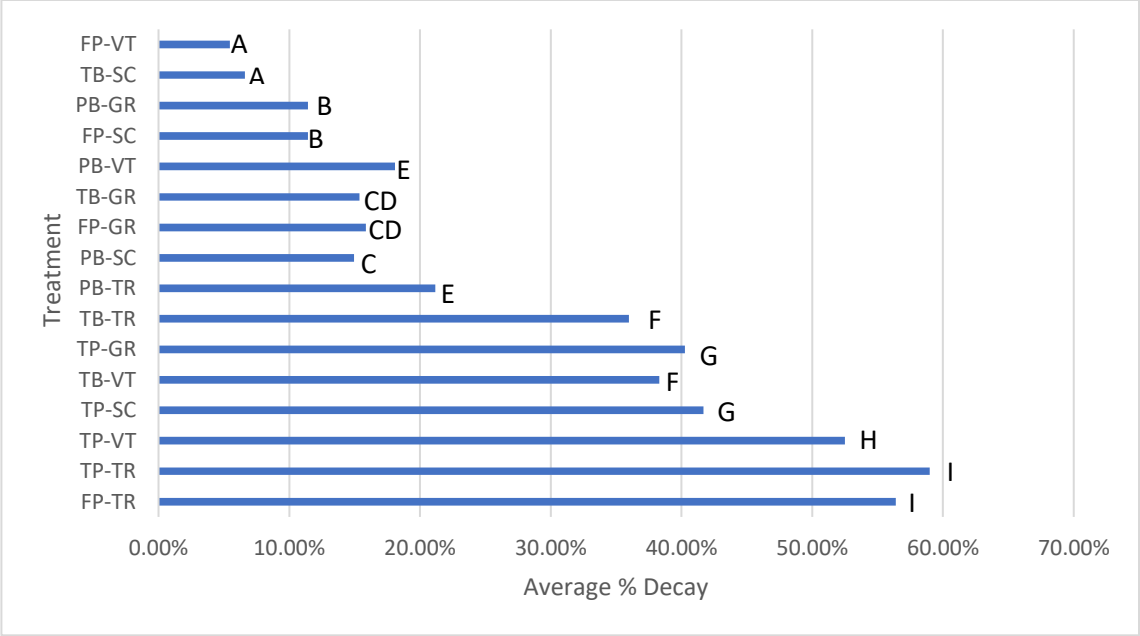
Trichaptum biforme exhibited a somewhat significant difference between the various treatments, with two treatments belonging to each LSD group F. The mycoparasites *Gliocladium roseum* and *Sesquicillium candalabrum* were able to reduce

the average percent decay to 15.38% and 6.61% and fall under LSD groups CD and A respectively.

Piptoporus betulinus was the only decay fungi to exhibit average percent (%) decay rates within LSD groups A-E for each mycoparasite treatment. When paired with *Trichothecium roseum* decay reached 21.18%, while *Gliocladium roseum* reduced average percent (%) decay to 11.42%.

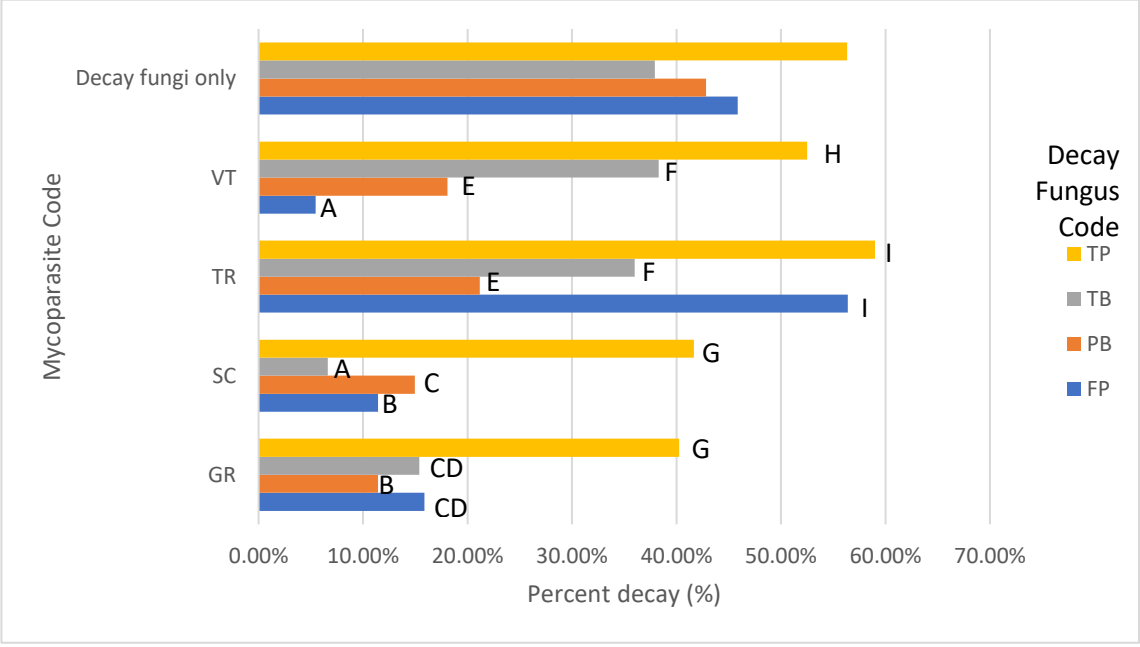
Trametes pubescens exhibited no significance between the various mycoparasite treatments as all average decay rates placed them within LSD groups G-I. When paired with *Trichothecium roseum* average percent (%) decay reached 58.99% which was the highest recorded for any treatment, and *Gliocladium roseum* was only able to reduce decay to 40.25%.

Figure 3 highlights the differences between the average percent (%) decay between each treatment, and depict a clear boundary between LSD groups A-E and F-I. Figure 4 further highlights the differences between the various treatments by comparing the success of each mycoparasite with each species of wood decay fungus, as well as the decay fungus only treatments. The LSD groups are listed beside each treatment to further illustrate the boundary between the two larger groups.



Source. Table 4, Appendix II

Figure 3. Percent (%) decay per treatment with LSD groups listed



Source. Table 4, Appendix II

Figure 4. Comparison of mycoparasite success in limiting % decay with LSD groups listed

DISCUSSION

Of the one hundred and twenty-five *Betula papyrifera* wood blocks, one hundred and twenty experienced changes in their weight during the course of the treatment process excluding the control replicates. However, the extent of that weight loss varied between treatments and within the treatment replicates. This change in weight was converted to a percent (%) decay which was used to compare the success of each of the treatments. Treatments which resulted in a very small calculated percent (%) of decay were considered more successful at mitigating decay from the present wood decay fungi than those treatments which resulted in higher percentages of decay.

Wood decay fungi have certain growing conditions required for optimal growth, some of which include appropriate growing temperature, moisture levels, and a suitable host to parasitize (Pouska *et al.* 2016). The climatic and habitual conditions remained the same for all treatments and replicates throughout the experimental process. All bottles were stored, at random, within the same incubator for the same duration of time. Therefore, they were all exposed to the same temperature, moisture content, pH, and light levels. As such, elements relating to the storage and climatic conditions experienced by the various treatments can be excluded as reasons for differences in the results.

Differences in wood decay rates may be due to the species of wood used for blocks, *Betula papyrifera*. White-birch is considered a hardwood tree species, and possesses different structural properties than those of softwood trees. All species of wood decay fungi within the experiment are specific to hardwoods only, except *Fomitopsis pinicola* which has the capability to predate upon hardwoods as well as

softwoods (Kuo 2020; Breitenbach and Kranzlin 1986). This lack of exclusivity is common in many fungal species, and may not impact the fungus' ability to decay the wood of its host (Lindblad 2000), however, its broad host range may indicate a lack resiliency under predation.

The species of wood decay fungi differ in their capabilities to decay wood, as white-rot and brown-rot fungi are able to degrade different components within the compound of wood. The two white-rot fungi: *Trametes pubescens* and *Trichaptum biforme* observed higher rates of decay overall, regardless of mycoparasitic treatment, as they are capable of degrading all portions of the woody compound: cellulose, hemicellulose and lignin (De Groot 1972; Worrall 2019; Hammel *et al.* 2002; Eaton 2000). The two brown-rot fungi: *Fomitopsis pinicola* and *Piptoporus betulinus* observed lower rates of decay overall, which is understandable as they are only capable of decaying cellulose and hemicellulose, but not lignin (Worrall 219; Curling *et al.* 2002; Green and Highley 1997; Eaton 2000).

As per Table 2, there was variation observed within the treatment trials themselves. This may due to potential contamination during the inoculation process despite attempts to minimize this, or due to some external circumstances out of our control. The increases in weights as compared to the control and decay fungi only treatments may be due to the growth of mycelium within the wood block, which was unable to be scraped off before drying and weighing. While the increases in weight were not substantial, they are still notable.

Mycoparasitism plays a significant role within the wood decay process as it limits the success the wood decay fungi have in regards to average percent (%) decayed.

The parasites feed on the growing mycelium created from the nutrients obtained through the wood decay process, thus decreasing the ability of the wood decay fungi to continue breaking down woody compounds (Hiscox *et al.* 2018; Barnet and Lilly 1962; Kobayashi and Hillman 2005; Dix and Webster 1995; Jeffries 1995). The responses observed from the various treatments are an indication as to the resilience each wood decay fungus possesses when predated upon by various species of mycoparasites. The results of this study indicate that the response varies for each species of wood decay fungus, and with each species of mycoparasite.

The mycoparasites also showed patterns of success in lowering the average percent (%) decay observed from treatments in comparison with the control and decay fungus only treatments. Overall, *Gliocladium roseum* and *Sesquicillium candalabrum* were the most successful and consistent in mitigating the effects of the wood decay fungi. However, the success the mycoparasites experienced in regards to decay mitigation is also the failure experienced by the wood decay fungi in fulfilling their purpose. In order to understand the interactions more closely, the individual wood decay fungi and mycoparasites will have their results analyzed separately.

TRAMETES PUBESCENS

Trametes pubescens induces white rot in hardwood trees, generally broadleaf hardwoods including white-birch, and is saprophytic in nature (Breitenbach and Kranzlin 1986; Kuo 2020). It typically grows in clusters, and can be found worldwide (Breitenbach and Kranzlin 1986; Kuo 2020; Dagne *et al.* 1994). Interestingly, a study by Dagne *et al.* (1994) identified the presence of an antifungal compound within the wood decay fungus, and this compound may have had a significant impact on the results of

this experiment.

Trametes pubescens saw the least decline in average percent (%) decay in each mycoparasitic trial. Furthermore, each treatment containing *T. pubescens* fell within the LSD groups G-I, indicating that the interactions were mostly unsuccessful at mitigating the decay of the wood decay fungus. This may indicate that the wood decay fungus is highly resilient to predation, or that the antifungal compound proposed by Dagne *et al.* (1994) is present and successful in mitigating the effects of the studied mycoparasites (Dagne *et al.* 1994). There is also evidence to suggest that limiting nitrogen availability, such as during a mycoparasitic interaction, increases the creation of laccases which are enzymes used in the wood degradation process (Galhaup *et al.* 2002). The mycoparasite treatments involving *Sesquicillium candalabrum* and *Gliocladium roseum*, however, were somewhat successful in reducing the rate of decay by approximately 29% as compared with the decay fungus only treatment.

TRICHAPTUM BIFORME

Trichaptum biforme, commonly known in North America as the purple-tooth polypore, induces white-pocket rot on hardwood limbs, logs, stumps and snags (Miller and Miller 2006: Kuo 2020). Fruiting bodies are produced between late spring and autumn, and generally occur is large clusters (Miller and Miller 2006). This species of wood decay fungus is one of the most common polypores in North America, and is spread across the continent (Miller and Miller 2006: Kuo 2020). A study by Yang *et al.* (2013) identified that *T. biforme* possesses some interesting pharmacological properties, including antifungal and antimicrobial properties (Yang *et al.* 2013). However, this was not enough to deter the mycoparasites or inhibit them from mitigating decay.

Trichaptum biforme experienced a significant reduction in average percent (%) decay within the treatments pairing the wood decay fungus with the mycoparasites *Gliocladium roseum* and *Sesquicillium candalabrum* respectively. As compared with the decay fungus only treatments, the two mycoparasites were able to reduce decay by 59% and 83% respectively which is significant to the other two mycoparasite treatments which could only reduce decay by 5% at best. Therefore, this affirms the LSD test grouping *G. roseum* and *S. candalabrum* within groups CD and A for significance.

FOMITOPSIS PINICOLA

Fomitopsis pinicola is one of the most conspicuous and widely distributed polypores in temperate and boreal forests across North America (Kuo 2020; Hogberg *et al.* 1999). It is considered a perennial saprophytic decay fungus, and can be found on standing or fallen trees, as well as stumps of both softwood and hardwood tree species (Breitenbach and Kranzlin 1986; Hogberg *et al.* 1999). This type of decay fungus induces brown rot, and is distributed widely across the globe (Breitenbach and Kranzlin 1986; Kuo 2020).

Fomitopsis pinicola experienced a significant decrease in average percent (%) decay in all treatments except when paired with the mycoparasite *Trichothecium roseum*. This interaction resulted in a higher rate of decay than what was observed in the wood decay fungus only treatment, and this is likely due to mycelial growth within the block that was unable to be scraped off before drying and weighing post-experiment. The other three mycoparasite treatments were very successful in reducing average decay, with *Verticillium tenerum* being the most successful and reducing decay by approximately 88% as compared to the decay fungus only treatments. These treatments

fell with LSD groups A-CD as they exhibit a significant difference between the average rates of decay for the other treatments, however, the treatment involving *T. roseum* was categorized as group I as it did not represent any significant improvement in rate of decay.

PIPTOPORUS BETULINUS

Piptoporus betulinus, commonly known as the birch polypore, is host specific to the genus *Betula* and saprophytic in nature (Breitenbach and Kranzlin 1986; Kuo 2020; Valaskova and Baldrian 2006). It produces brown rot in birch trees, and is has a large geographic range encompassing North America, Europe, and Asia (Breitenbach and Kranzlin 1986; Kuo 2020; Valaskova and Baldrian 2006).

Piptoporus betulinus was the only decay fungus to see improvements in average percent (%) decay within each mycoparasitic treatment, and, therefore, each treatment is categorized within LSD groups B-E. Of these treatments, *Gliocladium roseum* was observed to be the most successful at mitigating decay caused by the wood decay fungus, reducing decay by approximately 73% as compared with the decay fungus only treatment. The other mycoparasites were still successful in mitigating decay, but their effectiveness ranged from 50% to 65% decay reduction.

GLIOCLADIUM ROSEUM

Gliocladium roseum is a common fungus found within forest soils, and prefers a neutral to alkaline pH level (Domsch *et al.* 1993; Barnett and Lilly 1962). It is sensitive to NPK fertilizers and fungicides which are often used on agricultural crops to deter fungal infection (Domsch *et al.* 1993). Its range extends across the globe, and has an optimum growing temperature range between 28 and 30 degrees Celsius, although it can

grow decently well within the range of 20 to 35 degrees Celsius (Domsch *et al.* 1993). It is also a very well known and destructive mycoparasite (Domsch *et al.* 1993; Barnett and Lilly 1962).

Gliocladium roseum forms a contact necrotrophic relationship with its hosts, indicating a lethal relationship between the two fungi (Barnett and Lilly1962; Jeffries 1995). It was also one of the two most successful mycoparasites within the experiment. The parasitic fungus was able to reduce the decay in all of the treatments containing each of the four species of wood decay fungi, and only struggled with mitigating the enzymatic effects from *Trametes pubescens* which proved to be the most resilient species of wood decay fungi within the experiment. The mycoparasite's most successful treatment was with *Piptoporus betulinus*, as it was able to reduce the average percent (%) decay by 73%.

VERTICILLIUM TENERUM

Verticillium tenerum is a common fungus found within soils and decaying organic matter, including wood (Domsch *et al.* 1993). It has a worldwide distribution, no specified optimal pH range, and grows best within the temperature range of 15-25 degrees Celsius (Domsch *et al.* 1993). A study by Kuter (1984) determined that the genus *Verticillium,* including the species *V. tenerum,* are considered mycoparasites of soil-borne plant pathogens, and this includes wood decay fungi (Kuter 1984).

Verticillium tenerum saw only a 50% success rate as two of its treatments resulted in an LSD group classifications of F and H, while the other two were classified A and E. *Trametes pubescens* and *Trichaptum biforme* were deemed the less successful treatments (LSD groups F and H) as the mycoparasites were unable to reduce the

average percent (%) decay by more than 7%, and *T. biforme* exhibited an increase in average percent (%) decay. The treatments involving *Piptoporus betulinus* and *Fomitopsis pinicola* were very successful, and the interaction between *V. tenerum* and *F. pinicola* resulted in the largest decrease in average percent (%) decay of any treatment at 88%.

TRICHOTHECIUM ROSEUM

Trichothecium roseum is a relatively fast-growing fungus found on decaying plant material, however it can also be found within soils, although it is not considered a soil fungus like *Gliocladium roseum* (Domsch *et al.* 1993). It has a worldwide distribution, and is known for being a destructive mycoparasite (Domsch *et al.* 1993; Freeman and Morrison 1943). Optimal growth occurs between 15 and 25 degrees Celsius, and has an optimal pH of 6 although a wide range of pH levels can be tolerated (Domsch *et al.* 1993). The mycoparasite inhibits the growth of the host fungus' spores via toxins released through contact, indicating a contact necrotrophic relationship between mycoparasite and host wood decay fungi (Freeman and Morrison 1943; Jeffries 1995).

Trichothecium roseum was only successful in mitigating decay from the wood decay fungus within the *Piptoporus betulinus* treatment. While this treatment is considered to fall within the significant range and be categorized with LSD group E, the mycoparasite was only able to limit decay by 65% and resulted in the greatest average percent (%) decay for *P. betulinus*. Overall, this mycoparasite was the least successful of the four chosen, as the three other treatments fell under LSD groups F-I as less significant.

SESQUICILLIUM CANDALABRUM

Sesquicillium candalabrum is yet another soil fungus that is considered to be a mycoparasite (Domsch *et al.* 1993). It has an optimal growing temperature of 20 degrees Celsius, as well as a worldwide distribution (Domsch *et al.* 1993).

Sesquicillium candalabrum was the second of the two most successful mycoparasites used, as three of the four treatment pairings fell within the LSD groups A-C. The most successful treatment for this mycoparasite occurred when paired with the wood decay fungus *Trichaptum biforme*, as it was able to reduce decay by 83% as compared to the decay only treatment. However, *S. candalabrum* struggled to mitigate the effects of *Trametes pubescens,* as each mycoparasite had, which saw an average percent (%) decay rate of 41.68%.

CONCLUSIONS

The addition of various species of mycoparasites to different species of wood decay fungi resulted in varied outcomes, but a general trend was observed: the addition of mycoparasites to the wood decay fungus treatments resulted in lower observed average rates of decay on the wood blocks of *Betula papyrifera*. There were variations depending on which of the four wood decay fungi were paired with the four species of mycoparasites, as some mycoparasites were more successful overall, and some species of wood decay fungi were more resilient to predation.

The mycoparasites *Gliocladium roseum* and *Sesquicillium candalabrum* exhibited the greatest and most consistent success in limiting the average percent (%) decay experienced by the wood blocks of *Betula papyrifera*. Their success suggests that they were able to reduce the enzymatic processes the wood decay fungi rely on for growth. In contrast, *Verticillium tenerum* and *Trichothecium roseum* were not as successful in mitigating the decay processes of the wood decay fungi. Their average percent (%) decay rates were less consistent, and both struggled to mitigate the effects of the wood decay fungi *Trametes pubescens* and *Trichaptum biforme*.

Of the four species of wood decay fungi used, *Trametes pubescens* proved to be the most resilient as none of the four mycoparasites were able to limit the average percent (%) decay enough to suggest a significant difference. *Trametes pubescens* is known to possess antifungal compounds which may have led to this result; however, further research is required to determine the exact effects this compound may have on specific species of mycoparasitic fungi.

Each of the three null hypotheses were rejected as there were differences in the

observed weight loss within the wood decay only treatments, the mycoparasite only treatments, and especially within the combination treatments with both mycoparasites and wood decay fungi. We fail to reject the alternative hypothesis as the experiment confirmed that the blocks of *Betula papyrifera* inoculated with both a mycoparasite and a wood decay fungus do experience differences in weight loss compared with the blocks inoculated with wood decay fungi only.

The interactions between wood decay fungi and mycoparasites can be considered negative or positive depending on the circumstances, however, both entities performed their tasks as intended. They each perform an essential service within the forest ecosystem, and the extent of their interactions is not yet fully understood. The results of this thesis suggest that antifungal compounds may play a significant role in deterring mycoparasitic predation on certain species of wood decay fungi, but further studies and research are required to prove this theory. Furthermore, the results suggest that some mycoparasites are more successful in mitigating decay than others, but further studies are required to support this claim. More intensive and specific studies are required to understand the individual interactions between species of wood decay fungi and mycoparasites, and how they may relate to their interactions in the natural forest environment.

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APPENDIX I

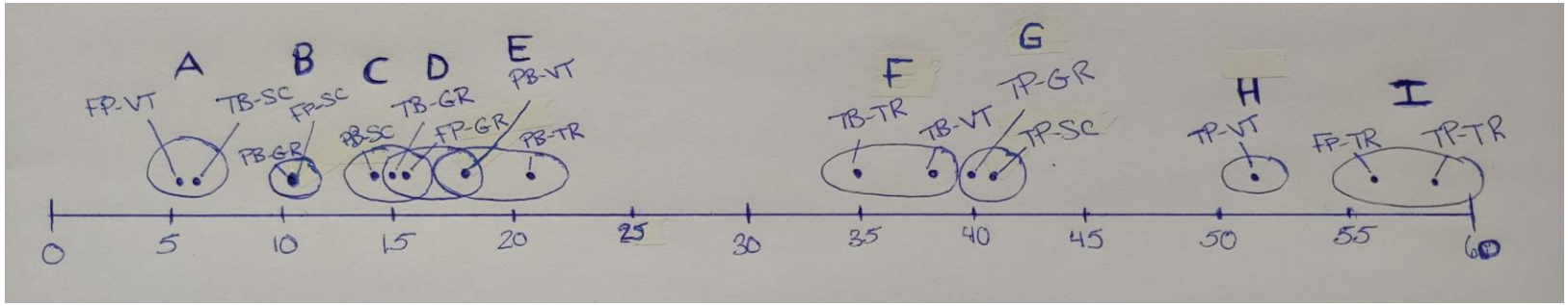
Block #	Decay Fungi	Mycoparasite	Before	After	%decay
1	none	none	4.398	4.411	-0.296
2	none	none	4.202	4.211	-0.214
3	none	none	3.600	3.598	0.056
4	none	none	4.349	4.346	0.069
5	none	none	4.232	4.229	0.071
6	*T. pubescens*	none	4.752	1.935	59.280
7	*T. pubescens*	none	4.736	2.025	57.242
8	*T. pubescens*	none	4.260	1.640	61.502
9	*T. pubescens*	none	5.791	2.998	48.230
10	*T. pubescens*	none	4.686	1.976	57.832
11	*T. biforme*	none	4.372	3.075	29.666
12	*T. biforme*	none	4.416	2.494	43.524
13	*T. biforme*	none	3.968	2.598	34.526
14	*T. biforme*	none	4.177	2.500	40.148
15	*T. biforme*	none	4.319	2.526	41.514
16	*F. pinicola*	none	4.339	2.480	42.844
17	*F. pinicola*	none	4.414	2.512	43.090
18	*F. pinicola*	none	4.034	2.030	49.678
19	*F. pinicola*	none	4.832	2.641	45.344
20	*F. pinicola*	none	4.008	2.048	48.902
21	*P. betulinus*	none	4.423	2.156	51.255
22	*P. betulinus*	none	4.622	3.286	28.905
23	*P. betulinus*	none	4.521	2.575	43.044
24	*P. betulinus*	none	3.964	2.120	46.519
25	*P. betulinus*	none	4.705	2.576	45.250
26	None	*G. roseum*	4.802	4.619	3.811
27	None	*G. roseum*	4.702	4.526	3.743
28	None	*G. roseum*	4.330	4.144	4.296
29	None	*G. roseum*	4.583	4.422	3.513
30	None	*G. roseum*	4.990	4.802	3.768
31	None	*V. tenerum*	4.213	4.071	3.371
32	None	*V. tenerum*	4.383	4.210	3.947
33	None	*V. tenerum*	4.211	4.079	3.135
34	None	*V. tenerum*	4.381	4.274	2.442
35	None	*V. tenerum*	4.422	4.279	3.234
36	None	*T. roseum*	4.932	4.787	2.940
37	None	*T. roseum*	4.544	4.397	3.235
38	None	*T. roseum*	4.949	4.792	3.172
39	None	*T. roseum*	4.293	4.164	3.005
40	None	*T. roseum*	4.451	4.312	3.123
41	None	*S. candalabrum*	4.539	4.336	4.472
42	None	*S. candalabrum*	4.784	4.564	4.599

Block #	Decay Fungi	Mycoparasite	Before	After	%decay
44	None	*S. candalabrum*	4.919	4.695	4.554
45	None	*S. candalabrum*	4.571	4.383	4.113
46	*T. pubescens*	*G. roseum*	5.290	3.524	33.384
47	*T. pubescens*	*G. roseum*	4.403	3.542	19.555
48	*T. pubescens*	*G. roseum*	4.603	2.295	50.141
49	*T. pubescens*	*G. roseum*	4.597	2.368	48.488
50	*T. pubescens*	*G. roseum*	4.679	2.355	49.669
51	*T. pubescens*	*V. tenerum*	5.217	2.368	54.610
52	*T. pubescens*	*V. tenerum*	4.635	2.619	43.495
53	*T. pubescens*	*V. tenerum*	4.934	2.048	58.492
54	*T. pubescens*	*V. tenerum*	4.457	1.902	57.326
55	*T. pubescens*	*V. tenerum*	4.754	2.458	48.296
56	*T. pubescens*	*T. roseum*	4.975	2.263	54.513
57	*T. pubescens*	*T. roseum*	4.857	1.841	62.096
58	*T. pubescens*	*T. roseum*	5.188	2.155	58.462
59	*T. pubescens*	*T. roseum*	4.793	2.174	54.642
60	*T. pubescens*	*T. roseum*	4.927	1.711	65.273
61	*T. pubescens*	*S. candalabrum*	4.068	3.771	7.301
62	*T. pubescens*	*S. candalabrum*	4.178	2.603	37.697
63	*T. pubescens*	*S. candalabrum*	4.631	2.829	38.912
64	*T. pubescens*	*S. candalabrum*	4.079	2.040	49.988
65	*T. pubescens*	*S. candalabrum*	4.815	1.455	69.782
66	*T. biforme*	*G. roseum*	5.363	4.085	23.830
67	*T. biforme*	*G. roseum*	4.270	3.392	20.562
68	*T. biforme*	*G. roseum*	4.999	4.578	8.422
69	*T. biforme*	*G. roseum*	4.275	4.080	4.561
70	*T. biforme*	*G. roseum*	4.427	3.609	18.478
71	*T. biforme*	*V. tenerum*	4.493	2.562	42.978
72	*T. biforme*	*V. tenerum*	4.249	2.573	39.445
73	*T. biforme*	*V. tenerum*	4.177	2.634	36.940
74	*T. biforme*	*V. tenerum*	4.783	3.012	37.027
75	*T. biforme*	*V. tenerum*	4.660	3.016	35.279
76	*T. biforme*	*T. roseum*	4.845	2.929	39.546
77	*T. biforme*	*T. roseum*	4.134	3.197	22.666
78	*T. biforme*	*T. roseum*	4.499	2.725	39.431
79	*T. biforme*	*T. roseum*	5.025	3.281	34.706
80	*T. biforme*	*T. roseum*	5.335	3.125	41.425
81	*T. biforme*	*S. candalabrum*	4.285	4.087	4.621
82	*T. biforme*	*S. candalabrum*	5.098	4.819	5.473
83	*T. biforme*	*S. candalabrum*	4.339	4.122	5.001
84	*T. biforme*	*S. candalabrum*	4.460	3.902	12.511
85	*T. biforme*	*S. candalabrum*	5.010	4.729	5.609
86	*F. pinicola*	*G. roseum*	4.471	3.800	15.008

Block #	Decay Fungi	Mycoparasite	Before	After	%decay
88	*F. pinicola*	*G. roseum*	4.024	3.739	7.083
89	*F. pinicola*	*G. roseum*	5.940	5.577	6.111
90	*F. pinicola*	*G. roseum*	4.380	2.865	34.589
91	*F. pinicola*	*V. tenerum*	4.606	3.498	24.056
92	*F. pinicola*	*V. tenerum*	4.853	3.291	32.186
93	*F. pinicola*	*V. tenerum*	4.913	3.956	19.479
94	*F. pinicola*	*V. tenerum*	4.259	3.319	22.071
95	*F. pinicola*	*V. tenerum*	4.690	7.982	-70.192
96	*F. pinicola*	*T. roseum*	4.827	1.773	63.269
97	*F. pinicola*	*T. roseum*	4.148	1.488	64.127
98	*F. pinicola*	*T. roseum*	4.181	3.352	19.828
99	*F. pinicola*	*T. roseum*	4.573	1.568	65.712
100	*F. pinicola*	*T. roseum*	4.506	1.516	66.356
101	*F. pinicola*	*S. candalabrum*	5.677	4.877	14.092
102	*F. pinicola*	*S. candalabrum*	4.200	3.930	6.429
103	*F. pinicola*	*S. candalabrum*	4.416	3.839	13.066
104	*F. pinicola*	*S. candalabrum*	4.411	3.869	12.287
105	*F. pinicola*	*S. candalabrum*	4.631	4.153	10.322
106	*P. betulinus*	*G. roseum*	4.799	4.563	4.918
107	*P. betulinus*	*G. roseum*	4.574	4.261	6.843
108	*P. betulinus*	*G. roseum*	4.217	3.945	6.450
109	*P. betulinus*	*G. roseum*	4.303	4.025	6.461
110	*P. betulinus*	*G. roseum*	4.659	4.423	5.065
111	*P. betulinus*	*V. tenerum*	4.883	3.971	18.677
112	*P. betulinus*	*V. tenerum*	4.638	3.796	18.154
113	*P. betulinus*	*V. tenerum*	5.285	4.424	16.291
114	*P. betulinus*	*V. tenerum*	5.017	4.082	18.637
115	*P. betulinus*	*V. tenerum*	4.472	3.625	18.940
116	*P. betulinus*	*T. roseum*	5.186	4.254	17.971
117	*P. betulinus*	*T. roseum*	4.469	3.310	25.934
118	*P. betulinus*	*T. roseum*	4.532	3.533	22.043
119	*P. betulinus*	*T. roseum*	4.825	3.858	20.041
120	*P. betulinus*	*T. roseum*	5.185	4.115	20.636
121	*P. betulinus*	*S. candalabrum*	3.989	3.157	20.857
122	*P. betulinus*	*S. candalabrum*	4.353	3.775	13.278
123	*P. betulinus*	*S. candalabrum*	4.754	4.113	13.483
124	*P. betulinus*	*S. candalabrum*	4.673	4.074	12.818
125	*P. betulinus*	*S. candalabrum*	5.176	4.395	15.089

APPENDIX II

LSD TEST GROUPING TREATMENTS WITH A VALUE OF 3.27 TO TEST FOR SIGNIFICANCES



LEGEND							
TREATENT	MEAN	TREATENT	MEAN	TREATENT	MEAN	TREATENT	MEAN
FP-VT	5.46%	PB-SC	14.95%	PB-TR	21.18%	TP-SC	41.68%
TB-SC	6.61%	TB-GR	15.38%	TB-TR	35.99%	TP-VT	52.51%
FP-SC	11.42%	FP-GR	15.87%	TB-VT	38.30%	FP-TR	56.39%
PB-GR	11.42%	PB-VT	18.09%	TP-GR	40.25%	TP-TR	58.99%

APPENDIX III

Tests of Between-Subjects Effects

Dependent Variable: %decay

Domsh *et al.* 1993	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	48054.425[a]	24	2002.268	14.570	0.000000000
Intercept	80973.552	1	80973.552	589.219	0.000000000
DecayFungi	28262.045	4	7065.511	51.414	0.000000000
Mycoparasite	9984.322	4	2496.080	18.163	0.000000000
DecayFungi * Mycoparasite	9808.057	16	613.004	4.461	0.000001418
Error	13742.511	100	137.425		
Total	142770.488	125			
Corrected Total	61796.936	124			

a. R Squared = .778 (Adjusted R Squared = .724)