THE IMPACT OF A FUNGUS-FEEDING NEMATODE (*APHELENCHOIDES* SP.) ON DECOMPOSITION OF TREMBLING ASPEN WOOD BY VARIOUS WOOD-DECAY FUNGI

by

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Major Advisor Second Reader

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ABSTRACT

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Keywords: *Aphelenchoides* sp., *Bjerkandera adusta, Cerrena unicolor, Climacodon septentrionale,* fungi, *Ganoderma applanatum,* grazing, *Hohenbuehelia grisea,* mycophagy, nematodes, nematophagous, *Sphaerobolus stellatus, Trametes pubescens*, wood-decay.

Grazing by fungus feeding invertebrates on fungal mycelium can potentially impact many important ecological processes such as the formation of mycorrhizas and the decomposition of wood and litter. A study was initiated to examine the impact of a fungus feeding nematode (*Aphelenchoides* sp.) on the decomposition of trembling aspen (*Populus tremuloides*) wood blocks under aseptic growing conditions by seven different decay fungi. These fungi were *Bjerkandera adusta*, *Cerrena unicolor*, *Climacodon septentrionale*, *Ganoderma applanatum*, *Hohenbuehelia grisea*, *Sphaerobolus stellatus*, and *Trametes pubescens*. Results based on dry weight measurements of wood blocks before and after inoculation and in the presence or absence of nematodes revealed that four of the seven fungi exhibited lower rates of decay in the presence of nematodes. These fungi were *T. pubescens*, *G. applanatum*, *C. septentrionale* and *S. stellatus*. The other three fungi had slight increases in decomposition of wood blocks in the presence of nematodes. It is suggested that *B. adusta* and *C. unicolor* may have responded to grazing by producing enhanced mycelial growth and thus enhanced enzymatic activity. *Hohenbuehelia grisea* is a known nematophagous fungi, capturing and consuming nematodes as a supplementary source of nitrogen, thus accounting for enhanced decomposition in the presence of nematodes.

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INTRODUCTION

WOOD DECAY

Wood is a very important substance in our world and is of considerable anatomical and chemical complexity; supporting rich communities of fungal species in a variety of microbial niches (Dix and Webster 1995). A key component in the healthy life cycle of a forest is the presence of wood-inhabiting fungi which are extremely important in the process of nutrient and carbon cycling in temperate forests. Wood rotting fungi are, by definition, those which can bring about significant weight loss and structural change in woody tissues (Dix and Webster 1995).

Wood is composed of mostly cellulose, which is a polymer of sugars, and lignin which is a complex heterogeneous polymer that is made up of several phenol-containing compounds. Trees differ in their wood structure, as does the enzymatic potential of the fungi for decomposition. The degree of decomposition on the substrate and the method used to do so will depend on the ability to degrade different cell types and cell-wall constituents (Schwarze *et al*. 2000).

Coarse woody debris makes a large contribution to immobilized nutrients such as Phosphorus (P) and Nitrogen (N) that can be readily utilized by wood-decay fungi (Stenlid *et al*. 2008). Fungi which decay wood are some of the few organisms that can utilize these nutrients by secreting an array of enzymes with the unique ability to disassemble the complex molecules that comprise wood (cellulose, lignin and hemicellulose) and recycle it back into the ecosystem (Boddy and Watkinson 1995).

TYPES OF WOOD DECAY

However, not all fungi secrete the same array of enzymes to disassemble wood, and thus not all fungi attack wood in the same way. They may be distinguished by differences in colour, solubility, strength, dimensional stability, pulping properties and the chemical composition of the decayed wood (Cowling 1961). As a result, one might expect to see one of the following three types of rot: white rot, brown rot and soft rot depending on the enzymatic arsenal of the fungus and the host that it is attacking (Dix and Webster 1995). In some cases, wood-decay fungi may use several different methods of attack on the same tree (Dix and Webster 1995).

White Rot

White rot is the most common type of decay and normally leaves a bleached appearance due to the oxidation of lignin in wood and may occur uniformly, leaving the wood spongy or stringy. White rot may also appear as a selective decay or a pocket rot (Goodell *et al*. 2008). White rot fungi possess both cellulolytic and lignin degrading enzymes, therefore having the potential to degrade the entirety of the wood structure under the correct environmental conditions (Goodell *et al*. 2008).

White rot fungi are the most efficient lignin degraders in nature, but erode the cellulose and hemicellulose components in wood as well (Dix and Webster 1995). White rot can also be broken down into white-pocket, white-mottled, and white-stringy based on macroscopic characteristics. These are all dependent on the fungal species, wood species and ecological conditions (Schmidt 2006). Another unique characteristic to white rotters, is that they may produce zone lines demarking territory between decay

and non-decayed wood which is called "spalting" and can be seen in early stages of decay. From microscopic and ultrastructural investigations, two main types of white rot have been distinguished (Liese 1970).

In the simultaneous white rot or "corrosion rot", carbohydrates and lignin are almost uniformly degraded at the same time and at a similar rate at all stages of decay (Schmidt 2006). This is done by the hyphae of the wood-decay fungi penetrating the cell wall and secreting an array of degrading enzymes (Liese 1970). Thus a lysis zone develops under the hypha, and in turn the hypha produces grooves in the wall which is gradually reduced in thickness, similar to the way a river erodes the ground (Schmid and Liese 1964; Liese 1970).

In selective white rot, or otherwise known as successive (sequential) white rot, the degradation of lignin and hemicellulose is greatest in the early stages of attack (Liese 1970). Further, fungi showing successive white rot contain small, elongated cavities within wood tissue, where the lignin and hemicelluloses are "selectively" (preferentially) degraded, and the greatest part of the cellulose remains (Blanchette 1984). This is more commonly known as "white-pocket rot".

White-rot fungi predominantly attack hardwoods and cause the greatest decrease in the strength properties of wood versus the further discussed forms of wood-decay.

Brown Rot

Brown rot fungi are much less common than white rot fungi and are more frequently found on softwoods than on hardwoods. Brown rot fungi degrade wood by metabolizing the carbohydrates cellulose and hemicelluloses found in the cell wall of

wood by non-enzymatic and enzymatic action and leave the lignin in wood unaltered, causing the brown mottled appearance (Liese 1970). As the brown rot fungi attack the cellulose along the cell wall, it breaks it up into short pieces, causing a significant amount of strength loss, making wood very brittle and easily breakable under tension (Liese 1970). Strength properties of wood decayed by white and brown rot fungi were reviewed by Hartley (1958) and was concluded that at comparable stages of decay, as measured by weight loss, brown-rot fungi reduce the strength of wood more than do white-rot fungi. This can be the reason for brown-rotted wood being more friable than white-rotted wood.

Brown-rot fungi colonize the wood by using their microhyphae to penetrate through pits in the cell wall, where they spread into the longitudinal tissue (Liese 1970). The microhyphae then grow inside the cell lumina and with the use of enzymes, penetrate through the relatively resistant tertiary wall containing high lignin and diffuse into the secondary wall, where they degrade the carbohydrates completely (Liese 1970). Contrary to white-rot fungi, brown-rot fungi do not typically cause lysis zones around their hyphae, but instead the hyphae are surrounded by slime layers (Schmidt 2006).

Soft Rot

Soft rot, originally termed by Findlay and Savory (1954), differs from white and brown rot, as it occurs when wood loses mechanical strength and becomes wet and spongy (Dix and Webster 1995). Soft rot fungi grows mainly inside the woody cell wall and degrades cellulose and hemicellulose, however cellulolytic agents do not diffuse as far into the cell wall compared to brown rot (Liese 1964). Furthermore, the lignin

content in wood is either attacked minimally or not at all in the initial stage, in turn leaving it to resemble brown rot. The distribution of lignin due to its restriction to cellulose access, is of particular importance as it determines which parts of the wall are attacked preferentially by brown and soft-rot fungi (Dix and Webster 1995). Soft rot fungi typically attack wood of higher moisture content, and can create unique cavities in the wood cell wall (Goodell *et al*. 2008).

WHAT FUNGI CAUSE DECAY?

Robert Hartig, better known as the father of forest pathology proved that wood decay is not caused by all fungi, but is in fact caused by fungi belonging to one of the two taxonomic groups being the Basidiomycota (basidiomycetes) and the Ascomycota (ascomycetes) (Hartig 1874).

The basidiomycetes are the largest and most important group of fungi as they are one of the few groups of organisms capable of degrading lignin in wood, and are responsible for brown rot, and most white rot. Examples of basidiomycetes include mushrooms and bracket fungi that one would see walking through the forest. A study done by Gilbertson and Ryvarden (1986) identified nearly 500 different basidiomycetes responsible for decaying wood of living and dead trees in North America.

The ascomycetes belong to another large group of fungi, however only a few ascomycetes are responsible for wood decay which include soft-rot and some white rot. These fungi are more commonly known as the cause of leaf diseases and cankers on trees (Breitenbach and Kranzlin 1986). The fruiting structures on ascomycetes are also much less conspicuous than those of the basidiomycetes, with an example being cup-fungi.

ECOLOGICAL ROLE OF WOOD-DECAY FUNGI

Fungi carry out many ecosystem services, all being of a particular importance. A key component in the temperate forest is the presence of wood-inhabiting fungi, as they play an extremely important role in nutrient and carbon cycling.

As dead wood litter contributes to a large percentage of most forest ecosystems, it forms a reservoir of immobilized mineral nutrients such as nitrogen (N) and phosphorus (P). These nutrients remain unavailable to primary producers until they are released by decomposer organisms, such as wood-decay fungi (Boddy and Watkinson 1995).

Wood-decay fungi are also some of the few organisms that can utilize the carbon (C) assimilated into wood components such as cellulose, lignin and recycle it back into the ecosystem (Boddy and Watkinson 1995). As decomposition proceeds, relative concentrations of N and P in the wood increase (*i.e.*, carbon/nitrogen ratio decrease) and carbon (C) is lost in the form of CO_2 (Boddy and Watkinson 1995). Wood-decay fungi then use the excess nutrients for the production of reproductive structures and foraging mycelium.

This ability for accumulated nitrogen (N) and phosphorus (P) to move within the mycelial networks of wood-decay fungi enables fungi to play key roles as wood decomposers and root symbionts (Watkinson *et al.* 2006). Mycelial networks act as both a reservoir and a distribution system as nutrients are conserved and often relocated for many metres and can even aid in the establishment of mycelia in new resources (Boddy and Watkinson 1995). This whole process, in turn, is a major driving force in the soil

formation of forest ecosystems in the temperate forests of northern Ontario (Watkinson *et al.* 2006).

Mycelium and fruiting bodies of wood-decay fungi are also important food sources for many other organisms and wildlife that contribute to overall ecosystem services.

Obstacles Faced

Like all living organisms, wood-decay fungi require certain factors for growth and survival including water, oxygen, optimal temperature range, a digestible substrate (wood), a favourable pH range as well as various chemical growth factors (Zabel and Morrell 2012).

In order for fungi to grow on wood, they must be able to tolerate certain chemical and physical stresses including high levels of tannins, phenols and other antifungal aromatics present in wood (Dix and Webster 1995). This can be overcome by some wood-decay fungi, as for example, heart-rot basidiomycetes which produce an array of enzymes including laccases and tyrosinases in order to detoxify wood (Dix and Webster 1995).

Although important, decay of wood is a very slow process for many reasons. One reason being the presence of lignin in the cell walls of the bulk of tissues (Dix and Webster 1995). Lignin coats the cell wall polysaccharides and chemically combines with them to form lignocellulose, a substance that is very resistant to microbial degradation. Therefore, unless wood-decay fungi can chemically modify or degrade lignin, it will protect the cell wall from being penetrated by the hyphae (Dix and Webster 1995).

Fungi are obligate aerobic organisms and require moderate amounts of oxygen for respiration (Zabel and Morrell 1992). Another limiting factor in the ability for wood-decay fungi to contribute to ecosystem processes are low levels of oxygen (O_2) and high levels of carbon dioxide (CO_2) in the substrate. In the natural environment reduced levels of oxygen and high levels of carbon dioxide can occur in wood and influence the ability for wood-decay fungi to inhabit these areas as oxygen is needed for growth.

Moisture levels will also play a major role in the ability for wood-decay fungi to succeed in their environment (Griffin 1977). Only a few wood-decay fungi species can degrade wood below the fiber saturation point (ca. 25%) (Ammer 1963). Optimal moisture levels for the growth of wood-decay fungi are not known but many tests have suggested that the optimal wood-moisture levels for most wood-decay lie between 40%-80% (Scheffer 1973; Schwarze *et al*. 2000).

Temperature plays a significant role in the success of wood-inhabiting fungi as well. In general, temperature limits for the growth of most fungi lie between 0 and 45 degrees Celsius. Previous studies have been done in order to understand the success rates of wood inhabiting fungi based on temperature. Humphrey and Siggers (1933) studied the growth rate of 56 species of wood-decay fungi at temperatures ranging from 0 to 40°C and found 12 species with an optima below 24°C; 42 with an optima between 24 and 32°C and 10 with an optima above 32°C.

Nitrogen concentrations in wood can also inhibit the growth and ability for wood-decay fungi to succeed. There have been many studies done that prove high nitrogen contents in wood can lead to higher rates of degradation by wood-decay fungi, or cause increased disposition for decay (Hungate 1940, Kollmann 1951, Platt *et al*.

1965, Cowling and Merrill 1966, Garrett 1970). However, it is assumed that wood is a substance that happens to be low in nitrogen, and nitrogen is essential for living organisms (Cowling and Merrill 1966). Due to these low levels of nitrogen, there are some wood-decay fungi that will parasitize invertebrates, including nematodes, in soil and utilize their nutrients (Thorn and Barron 1984, Tzean and Liou 1993).

As wood-decay fungi are rich in nutrients, they act as an important food supply to many grazing animals in the environment. The average nitrogen and phosphorus levels in fungal mycelium can range from 3.7% to 5.32% and 0.55% to 0.7%, respectively (Flanagan and Van Cleve 1977; Bååth and Söderström 1979). Vegetative mycelium represents over 99% of the fungal colony biomass and offers a valuable source of nutrients for soil fauna (Frankland 1982). Not only are the survival and growth of these grazing animals dependent upon the extensive hyphal systems and fruiting bodies produced by lignicolous and mycorrhiza-forming fungi, but the animals have effects on the fungi through the dispersal of spores or reduction in the fecundity of the fungi (Dighton 2003). In turn, fungal grazers alter fungal species richness by selectively eliminating or promoting certain species over others and ultimately effect fungal growth and respiration (Newell 1984*a*, Newell 1984*b*; McGonigle 2007; Hanlon and Anderson 1979; Bengtsson *et al*. 1993; Hedlund and Augustsson 1995; Bretherton *et al*. 2006; Tordoff *et al*. 2008).

Invertebrates are one example of grazing animals that can change the competitive strength and the overall reproductive success of wood decay fungi, which play a major role in important processes (Dighton 2003). Invertebrates that graze on fungi include amoebae (Chakraborty *et al*. 1985), nematodes (Townshend 1964; Riffle

1971), enchytraeid annelids (Hedlund and Augustsson 1995; Jaffee *et al*. 1997), mollusks (Silliman and Newell 2003), mites (Mitchell and Parkinson 1976), collembolans (Visser and Whittaker 1977; Shaw 1985), adult insects (Weber 1966; Newton 1984), and insect larvae (Fletcher *et al*. 1989; Shaw 1992).

Nematodes are extremely important invertebrates contributing a large portion to nutrient cycling in ecosystems. Nematodes are among the most abundant multicellular organisms in the world and are ubiquitous in soil and may feed on a variety of fungal species, resulting in them being of great ecological importance (Blaxter 2011; Bongers and Bongers 1998). Nematodes are varied in their habitats, feeding habits and general biology. Nematodes are very common in dung, rotting wood and also occur in the sea as well as fresh water (Dix and Webster 1995). It is estimated that there are approximately 29 million nematodes per square metre of mixed deciduous forest soil (Bernard 1992).

Based on their anterior morphology, nematodes can be defined as fungivores, bacterivores, predators or omnivores with a diet that consists of algae, fungal spores, protozoa and other nematodes (Swift *et al*. 1979; Twinn 1974). Fungivorous nematodes pierce hyphae of wood-decay fungi with their stylets and feed on the fluid protoplasm of the fungus using a pumping action (Figure 1), leaving empty hyphal walls behind (Freckman and Baldwin 1990; Siddiqui and Taylor 1969). Invertebrate grazing can influence mycelial growth and physiology of wood-decay fungi. Grazing by invertebrates can damage or destroy fungal propagules, affecting species richness by promoting or eliminating the introduction of a fungal species and affecting the relative abundance and community diversity (McGonigle 1995). The impact of gazing by fungivorous nematodes can be severe, killing all aerial hyphae and reducing growth

drastically (Shafer *et al*. 1981). Extensive feeding by mycophagous nematodes disrupts mycelial networks, affecting the ability of wood-decay fungi to carry out ecological functions such as decomposition and mycorrhizal relationships (Ingham 1988; Gera Hol and Cook 2005; Boddy and Jones 2008).

Figure 1. Photo taken by Dr. Hutchison of *Aphelenchoides* sp feeding on hyphae

However, in order for wood-decay fungi to successfully reproduce and contribute to the ecosystem, some have evolved to defend themselves from invertebrate grazing.

OBJECTIVE

This thesis will analyze the effects of the fungal-feeding nematode *Aphelenchoides* sp. on the ability of seven wood decay fungi to decay pre-weighed wood blocks of trembling aspen. The objective is to understand the relationship between wood-decay fungi and hyphal grazing nematodes. As we know, nematodes consume the vegetative hyphae through selective cropping or feeding, and due to this, the flasks inoculated with both wood-decay fungi and nematodes should, in theory, not be able to decay the wood blocks as efficiently as compared to the flasks containing decay fungi only. However, we know that some wood-decay fungi have evolved defence mechanisms against grazing. Some of the fungi selected are known to employ such defence mechanisms.

HYPOTHESIS

The Null hypothesis (*Ho*) for this thesis project consists of two parts:

1. The *Aphelenchoides* sp. will have no effect on the decomposition of the wood blocks by the various wood-decay fungi.
2. Those decay fungi with known defence mechanisms against the hyphal grazing will have no effect on *Aphelenchoides* sp.

MATERIALS AND METHODS

WOOD BLOCK PREPARATION

On September 20, 2016; 150 wood blocks of *Populus tremuloides* Michx. which were approximately 1.5-2.0 cm^3 in size, were individually numbered and placed on aluminum weigh boats and dried for 72 hours at 100 degrees Celsius. They were later removed with forceps and weighed (Figure 2). The weight was recorded (see Appendix II) for each block.

Figure 2. Dried wood blocks being weighed

FLASK PREPARATION

120 mL of a vermiculite-peat mixture (10:1) was added to the 150 glass Erlenmeyer flasks (250 mL). Each flask was numbered and the corresponding block was added to each flask, making sure to cover it in the vermiculite-peat mixture. Seventy mL of a 2% malt extract broth (20g malt extract, 1g yeast extract, 1000 mL water) was added to each flask. Each flask was then plugged with a cotton ball wrapped in cheese

cloth in order to prevent contamination. A piece of aluminum foil was then used to cover the mouth of the flask and was also numbered to correspond to the number of wood block (Figure 3). The flasks were autoclaved for 1 hour at 121 degrees Celsius and approximately 1.7 kg/cm^2 pressure.

Figure 3. Flasks prepared with vermiculite-peat mixture and malt extract broth before being autoclaved.

EXPERIMENTAL DESIGN

Table 1 and Figure 4 display the layout of the experimental design for investigating the effects of the fungus-feeding nematode *Aphelenchoides* sp. on the decay of wood blocks of *Populus tremuloides* by seven different wood-decay fungi. The seven wood-decay fungi used are *Trametes pubescens* (728), *Bjerkandera adusta* (297), *Ganoderma applanatum* (089), *Cerrena unicolor* (433), *Climacodon septentrionale* (426), *Hohenbuehelia grisea* (606) and *Sphaerobolus stellatus* (589) (Appendix I).

The last three fungi used (*Climacodon septentrionale, Hohenbuehelia grisea, Sphaerobolus stellatus)* are known to have fungal defence mechanisms present. Wood-decay fungi were obtained from Lakehead University's Mycological Herbarium Fungal

Culture Collection (Thunder Bay, Ontario). Fungi were selected that are known to decay deciduous wood.

Table 1. Experimental Design

Flask Numbers	Nematodes Present (Y) Yes (N) No	Fungi
1-10	N	Control (none)
11-20	N	*Trametes pubescens*
21-30	Y	
31-40	N	*Bjerkandera adusta*
41-50	Y	
51-60	N	*Ganoderma applanatum*
61-70	Y	
71-80	N	*Cerrena unicolor*
81-90	Y	
91-100	N	*Climacodon septentrionale*
101-110	Y	
111-120	N	*Hohenbuehelia grisea*
121-130	Y	
131-140	N	*Sphaerobolus stellatus*
141-150	Y	

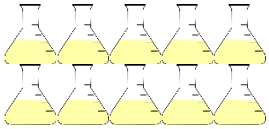
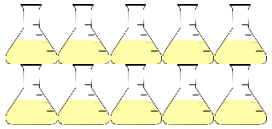
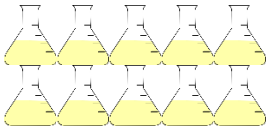
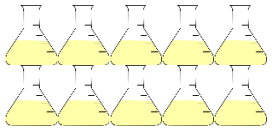
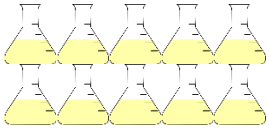
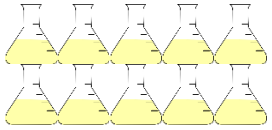
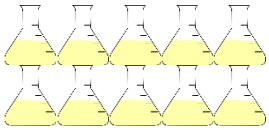
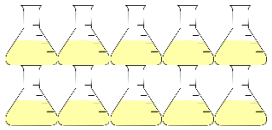
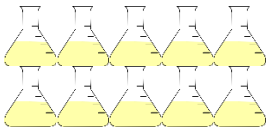
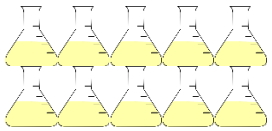
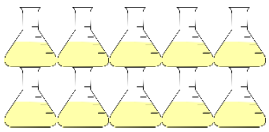
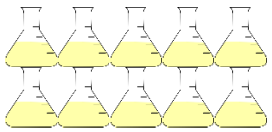
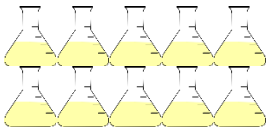
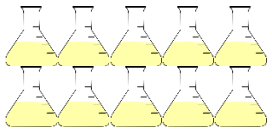
Treatment	No Nematodes	Nematodes
Trametes pubescens		
Bjerkandera adusta		
Ganoderma applanatum		
Cerrena unicolor		
Climacodon septentrionale		
Hohenbuehelia grisea		
Sphaerobolus stellatus		

Figure 4. Experimental design showing 10 flasks for each fungus containing no nematodes, and the other 10 containing nematodes.

Note:  represents a 250 mL Erlenmeyer flask.

INOCULATION OF FLASKS WITH WOOD-DECAY FUNGI

On October 18th and 21st, 2016, the 150 flasks were inoculated with the respective fungus to each numbered grouping of flasks. In order to do this, the first step was to sterilize all surfaces under the transfer hood (located in BB1046) with 70%

ethanol. Tools used were also kept in 70% ethanol between inoculations and when not in use. Flasks were taken one at a time and placed in the transfer hood, where the foil and cotton plugs were removed, the mouth of the flask flamed by a Bunsen burner. Two agar plugs (7mm in diameter) per flask were cut from the margin of vigorously growing cultures with a flame sterilized cork borer, and placed on two sides of the wood block with a sterilized long-handled spatula (Figure 5). The mouth of the flask was then flamed again, and the cotton plug and foil were placed back on the flask. Once 140 flasks were inoculated, the flasks were incubated in the dark at 20°C until mycelium was visible in each flask, meaning the fungi and wood blocks were ready to be inoculated with nematodes.

Figure 5. Author inoculating wood-decay fungi on wood blocks (left) and example of *Cerrena unicolor* agar plugs from petri dish (right).

INOCULATION WITH *APHELENCHOIDES* SP.

On November 19, 2016, a small volume of sterilized water was added to agar dishes containing cultures of *C. macrocephalus* containing the nematode *Aphelenchoides* sp. The cultures were swirled and the water containing nematodes pooled into a sterile beaker. A one mL sample was taken and added to a

haemocytometer to determine the density and was found to be approximately 200 nematodes/mL. Using an Eppendorf micropipette, a 1mL aliquot of suspended nematodes was aseptically added to each set of flasks (Figure 6) designated to receive nematodes (Table 1). All inoculations were done aseptically in the transfer hood in BB1046. Once inoculated, all flasks were incubated in the dark at 20°C until blocks were removed at the end of the incubation period (March 2017) (Figure 7).

Figure 6. Author using Eppendorf micropipette to inoculate 200 nematodes/mL of *Aphelenchoides* sp. (top) and sterilize flask using Bunsen burner (bottom).

Figure 7. 150 Erlenmeyer flasks in incubator for incubation at 20°C

ADDITION OF EXTRA WATER

An additional 30 mL of sterile water was aseptically added to each flask on January 13th in order to ensure that adequate moisture levels of the vermiculite-peat mixture were conducive for fungal growth.

HARVESTING WOOD BLOCKS

On March 3rd, 2017, all flasks were removed from incubation (Figure 8). One at a time, each flask was emptied of all substances, and each wood block was scraped clean, and placed on their respective numbered aluminum foil weigh boats. Once this was done, the wood blocks were placed back in the drying oven for 72 hours at 100°C. The weights were then recorded for all 150 wood blocks (see Appendix II).

Figure 8. Mycelium present in Erlenmeyer flasks before harvesting and drying wood blocks for statistical analysis

STATISTICAL ANALYSIS

The percent decay for each of the 150 wood blocks were recorded by using the dry weights of the wood blocks before and after inoculations:

$$\text{Percent decay (\%)} = \left(\frac{\text{Dry weight (before)} - \text{Dry weight (after)}}{\text{Dry weight (before)}}\right) * 100$$

Using a statistical software system, SPSS Statistics 17.0 (SPSS Inc. 2008), a univariate analysis (one-way ANOVA) was done (Appendix IV) using the percent decay (%) as the response variable, with fixed variables being the fungus species used, and the presence of nematodes in order to detect significant relationships with a p value of less than 0.05. Once significances were found, the least significant difference (LSD) was found. This was done by using the following formula:

$$LSD = t\alpha/_{2,df} s.e_{y1-y2}$$

A Post-Hoc test was then done in order to determine if the 14 means were significantly different from each other and can be found in Appendix V.

RESULTS

PERCENT DECOMPOSITION

A summary of the results are presented in Table 2. The eight treatments (each with 20 flasks) are shown, seven with their respective wood-decay fungi and one treatment being the control (10 flasks). The first set of 10 flasks for each wood-decay fungus do not contain nematodes, and the second set of 10 flasks do contain nematodes. The average dry weights of each of the 150 wood blocks are summarized before and after inoculations, with the average percent difference (%).

Trametes pubescens was the most successful at decaying wood blocks of *Populus tremuloides*, however it did experience a decline in decay in the presence of the fungus-feeding nematode *Aphelenchoides* sp. The average percent difference between dry weights of wood blocks before and after inoculation with *Trametes pubescens* without the presence of nematodes was 23.72%, and in presence of nematodes was 19.24%.

Ganoderma applanatum exhibited similar results to *Trametes pubescens*, as there was a decrease in the rate of decay in the presence of the fungus-feeding nematode *Aphelenchoides* sp. The average percent difference between dry weights of wood blocks before and after inoculation with *Ganoderma applanatum* without the presence of nematodes was 8.79%, and with nematodes 2.13%.

Bjerkandera adusta exhibited the second greatest ability to decay wood blocks of *Populus tremuloides* compared to *T. pubescens.* Unlike *T. pubescens, B. adusta* exhibited an increase in the rate of decomposition in the presence of the fungus-feeding

nematode *Aphelenchoides* sp. with an average percent difference of 19.11% versus 10.93% in the absence of nematodes.

Cerrena unicolor exhibited similar results to *Bjerkandera adusta* as the average percent difference between dry weights of wood blocks before and after inoculation increased in the presence of nematodes (5.10%) versus without nematodes (3.75%), but this was only slightly statistically significant. This can also be said of the next fungus used, *Hohenbuehelia grisea,* whose average decay (%) also increased in the presence of nematodes (0.75%) versus without nematodes (0.27%) but likewise was not statistically significant. However, *H. grisea* also exhibited the least amount of decay compared to all other six fungi used.

The remaining wood-decay fungi used, *Climacodon septentrionale* and *Sphaerobolus stellatus*, exhibited a small decrease in the average percent difference in dry weights of wood blocks before and after inoculation, with an average percent difference of 1.66% and 1.56% respectively, in the presence of fungus-feeding nematodes versus 2.55% and 2.33% respectively, without nematodes. Neither of the differences were statistically different.

Table 2. Summary table showing average dry weights before and after inoculation and their average percent differences (percent decay %).

Treatment	Fungi	Presence of Nematodes	Average Weight (Before)	Average Weight (After)	Average Percent Difference (%)
1	Control	N	1.203	1.204	-0.20
2	*Trametes pubescens*	N	1.476	1.143	23.72
		Y	1.596	1.289	19.24
3	*Bjerkandera adusta*	N	1.814	1.619	10.93
		Y	1.672	1.361	19.11
4	*Ganoderma applanatum*	N	1.746	1.595	8.79
		Y	1.770	1.731	2.13
5	*Cerrena unicolor*	N	1.534	1.477	3.75
		Y	1.216	1.205	5.10
6	*Climacodon septentrionale*	N	1.678	1.634	2.55
		Y	1.757	1.727	1.66
7	*Hohenbuehelia grisea*	N	1.348	1.345	0.27
		Y	1.133	1.124	0.75
8	*Sphaerobolus stellatus*	N	1.579	1.546	2.33
		Y	1.532	1.507	1.56

Table 3 shows the results from the one way ANOVA that was run using the average percent decay (%) as the response variable compared against the presence of nematodes and the wood-decay fungi used in order to find significances ($p < 0.05$). The relationship between the wood-decay fungi used and the presence of nematodes did show a significance. Therefore, there was a significant impact on the percent of decay in the presence of nematodes. There was also a significance shown in the wood-decay fungi used, therefore the species of fungi also impacted the amount of decay present.

Table 3. Results from univariate analysis (one way ANOVA).

	df	Mean Square	Significance (p)
Fungi used	6	1246.67	0.00
Nematodes	1	5.57	0.38
Interaction	6	111.39	0.00
Error	126	7.114	

A Least Significant Difference (LSD) test was done using the appropriate formula and a value of 2.36 was calculated. A Post-Hoc test was then done in order to find significances between each treatment. By using the LSD value and the average percent difference in decay (%) per each species of wood-decay fungi, treatments were grouped into A, B, C, D, E, F or a combination in order to find significances between treatments (Table 4). If treatments belong to different groupings, they exhibit a significant difference. For the ease of analysis, treatments were given codes: TP - *Trametes pubescens*; with (N) for no nematodes present and (Y) for nematodes present, this continues for the following 6 wood-decay fungi: BA – *Bjerkandera adusta,* GA – *Ganoderma applanatum,* CU – *Cerrena unicolor,* CS – *Climacodon septentrionale,* HG – *Hohenbuehelia grisea,* SS – *Sphaerobolus stellatus.*

When analyzing the treatment groups for significances, there is a significant difference exhibited between treatments of *Trametes pubescens* in the presence or absence of *Aphelenchoides* sp. In the absence of nematodes the percent decomposition was greater (23.72%) with an LSD group F, versus in the presence of nematodes decomposition decreased (19.24%) with an LSD group E.

Similarly, *Ganoderma applanatum* exhibited a significant difference in the percent rate of decomposition in the presence of nematodes (2.13%) with an LSD group AB, versus in the absence of nematodes (8.79%) in grouping D.

Bjerkandera adusta also exhibited a significant difference in decay between treatments in the presence or absence of the fungus-feeding nematode *Aphelenchoides* sp. However unlike the first two wood-decay fungi discussed, *B. adusta* exhibited an increase in decomposition in the presence of fungus-feeding nematodes. Percent

decomposition in the absence of nematodes was 10.93% and in grouping D, while the percent decomposition in the presence of nematodes was 19.11% and in grouping E.

Similarly, *Cerrena unicolor* exhibited a slight significant increase in decomposition with the presence of nematodes (5.10% and in grouping C) versus without nematodes (3.75% and in grouping BC), this overlap in group C shows there is a slight significance in these results.

The last three wood-decay fungi; *Climacodon septentrionale, Sphaerobolus stellatus* and *Hohenbuehelia grisea* did not show a significant difference in the rate of decomposition in the presence or absence of the fungus-feeding nematode *Aphelenchoides* sp.

Table 4. Treatments with their respective average percent decay (%) sorted from smallest to largest, and LSD group.

Treatment	Average Percent Decay (%)	LSD group
HG (N)	0.27	A
HG (Y)	0.75	A
SS (Y)	1.56	AB
CS (Y)	1.66	AB
GA (Y)	2.13	AB
SS (N)	2.33	AB
CS (N)	2.55	AB
CU (N)	3.75	BC
CU (Y)	5.10	C
GA (N)	8.79	D
BA (N)	10.93	D
BA (Y)	19.11	E
TP (Y)	19.24	E
TP (N)	23.72	F

Figure 9 depicts the average percent difference in decay for each wood-decay fungus treatment. LSD groupings are also shown to depict significant differences, as treatments belonging to a different letter groupings represent a significance in results.

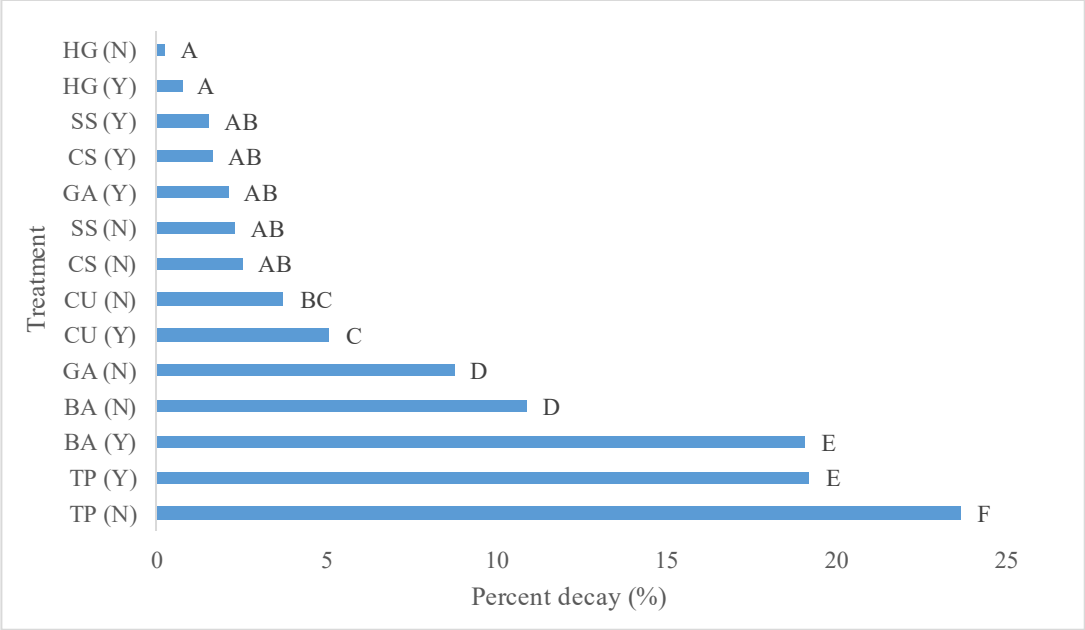


Figure 9. Figure showing the percent decay (%) corresponding to each treatment, with the LSD grouping labelled.

Figures 10 and 11 depict the final results showing the percent decay for each treatment (%). The results for the percent decay without the presence of nematodes is represented in blue, while the percent decay with the presence of nematodes is represented in orange. The LSD codes for each treatment are also displayed.

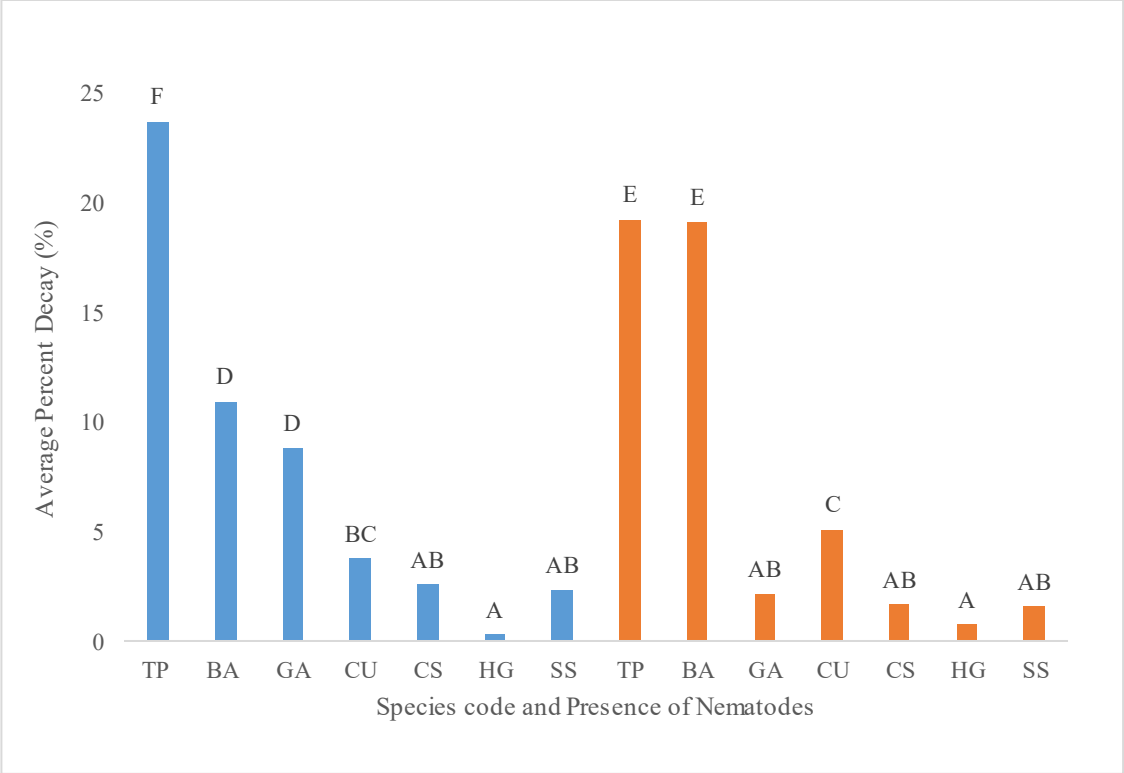


Figure 10. Average percent decay (%) for each treatment of wood-decay fungi, with the presence of nematodes shown in orange.

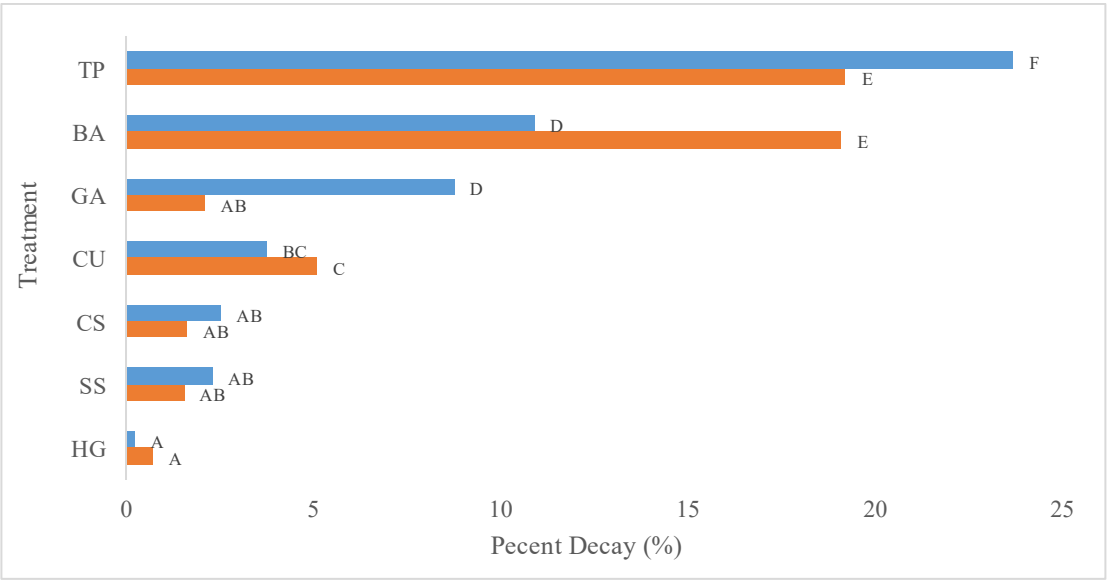


Figure 11. Average percent decay (%) for each treatment of wood-decay fungi, with the presence of nematodes shown in orange, versus no nematodes shown in blue.

DISCUSSION

All 140 flasks inoculated with wood-decay fungi were able to produce mycelium and ultimately cause decay and weight loss to the *Populus tremuloides* wood blocks. However, the degree of decomposition varied among the seven species of fungi, as well as with the presence or absence of the nematode *Aphelenchoides* sp.

Four of the wood-decay fungi used did exhibit a reduction in their rate of decomposition due to the presence of nematodes. These species were *Trametes pubescens, Ganoderma applanatum, Climacodon septentrionale* and *Sphaerobolus stellatus.* The other three wood-decay fungi used did not exhibit this pattern and in fact increased the amount of decomposition in the presence of the fungus-feeding nematode *Aphelenchoides* sp. These species were *Bjerkandera adusta, Cerrena unicolor* and *Hohenbuehelia grisea.*

Wood-decay fungi require certain factors for growth and survival including water, oxygen, optimal temperature range, a digestible substrate, a favourable pH range as well as various chemical growth factors (Zabel and Morrell 2012). With this knowledge, it can be assumed that the seven wood-decay fungi used were provided with optimum growing conditions.

As trees differ in anatomical structure as well as structural differences in individual cell-wall layers, all tree species differ in their ability to provide optimal conditions for fungal enzymes to break them down. The seven wood-decay fungi used in this experiment were all white-rot fungi and are known to prefer wood from deciduous trees, therefore making *Populus tremuloides* a suitable host. Although this is true, the rate of decay for each species of wood-decay fungi may vary depending on the

enzymatic arsenal of each species and the anatomical and structural traits of the *Populus tremuloides* wood blocks.

One factor that can lead to differences in the rate of decay for each of the seven species of wood-decay fungi used in this experiment is the rate of growth per fungus species. Riffle (1971) noted that fast growing fungi which produce mycelium at a higher rate than nematode consumption were more successful than slow growing fungi which cannot produce mycelium at a higher rate than the consumption rate by nematodes. With this knowledge, we can assume slower growing fungi will be more impacted from the fungus-feeding nematode *Aphelenchoides* sp. thus affecting decay. While *S. stellatus*, *C. septentrionale*, *G. applanatum* were slow growing and were indeed most affected by grazing, *T. pubescens* exhibited rapid growth.

Although we know that wood-decay fungi can be affected in their success rate by grazing by invertebrate species such as *Aphelenchoides* sp., we also know that some wood-decay fungi possess antifeedant mechanisms. Nematophagous fungi can possess adhesive structures which can be diverse in size and shape, ranging from unmodified adhesive hyphae to adhesive knobs and three-dimensional nets (Gray 1987). Some species possess constricting rings in order to disable nematodes. In the presence of nematodes, the rings induce the rapid inflation of cells and trap the nematode which has entered the ring (Higgins and Pramer 1967). Some fungi possess crystalline structures along their hyphae which mechanically damage the cuticle of nematodes (Luo *et al*. 2004; Luo *et al*. 2007). Chemical antifeedants are another defence mechanisms used by some species of fungi in order to deter predators and protect substrate territory (Janzen 1977). These fungal chemical antifeedants can be presented to the potential grazer in

three ways. Exotoxins are secreted from the hyphae into the surrounding environment, creating a gradient preventing gazing from organisms including nematodes (Tanney 2011). Fungi may also produce toxins from specialized structures including toxocysts and secretory cells (Tanney 2011). Another strategy used by some fungi in order to deter predators involves the ingestion of fungal cytoplasmic toxins (Tzean and Liou 1993). This may be the most efficient defence strategy in terms of metabolic cost compared to exotoxins which must be constantly secreted in order to maintain an effective concentration in the substrate (Tanney 2011)

Forms of antifeedant mechanisms that have evolved among wood-decay fungi include the function of gloeocystidia (Tanney and Hutchison 2011), the presence of setae on perithecia of *Chaetomium* spp. (Wicklow 1979), the production of toxin droplets from secretory cells of *Conocybe lactea* and *Panaeolina foenisecii* (Hutchison *et al*. 1996), the production of cytoplasmic toxins in fungal hyphae (Shaw 1985; Tzean and Liou 1993), the release of toxic volatiles and antibiotics from fungal hyphae (e.g.: Hayashi *et al*. 1981, Riffle 1971, Stadler *et al*. 1993; Stadler *et al*. 1994; Rohlfs *et al*. 2007), and the presence of calcium oxalate crystals on hyphae (Böllmann *et al*. 2010).

Of the seven fungi utilized, three possess well documented antifeedant and/or nematophagous abilities. *Hohenbuehelia grisea* produces sticky hour glass knobs on the hyphae to capture nematodes and eventually consume them as a nitrogen source (Barron and Dierkes 1977). This possibly explains the greater rate of decay in the aspen wood blocks in the presence of *Aphelenchoides* sp. The other two, *Sphaerobolus stellatus* employs gloeocystidia as an antifeedant which releases an oily material and encapsulates the heads of nematodes thus immobilizing them (Tanney and Hutchison

2011), while *Climacodon septentrionale* employs secretory cells which secrete toxic droplets to kill nematodes (Tanney and Hutchison 2012). It is interesting that these latter two species were ineffective in reducing grazing on their respective hyphae.

In order to better understand the interaction between the fungus feeding nematode *Aphelenchoides* sp. and the seven species of wood-decay fungi used in this experiment, we will further look at the results per species.

TRAMETES PUBESCENS

Trametes pubescens exhibited the highest rate of decay compared to the other six wood-decay fungi used. *Trametes pubescens* also exhibited higher decay rates in the treatment without the presence of *Aphelenchoides* sp. (23.72 % difference) versus in the presence of *Aphelenchoides* sp. (19.24%). When doing the LSD test, significant differences were found between these two treatments, suggesting that the fungus-feeding nematode *Aphelenchoides* sp. were able to successfully graze on the hyphae and inhibit the success and rate of decay by *T. pubescens*. *Trametes pubescens* may have favoured the wood block species *Populus tremuloides* over the six other wood-decay fungi used, as it was originally isolated from *P. treumuloides* (Appendix I).

BJERKANDERA ADUSTA

Bjerkandera adusta differed from *Trametes pubescens* as there was a significant increase in the rate of decay in the presence of *Aphelenchoides* sp. (19.11%) compared to the treatment without nematodes (10.93 %). As *Bjerkandera adusta* is not known to have any defence mechanisms against grazing, we can only assume the fast rate of growth and possible growth response to grazing may explain the observed results.

Bjerkandera adusta is a well-known primary colonizer of dead wood and exhibits a ruderal strategy (Dix and Webster 1995).

Fungal growth is known to be dependent on grazing intensity, as growth may increase when fungi are subjected to low intensity grazing and may decrease under high intensity grazing (Hanlon and Anderson 1979; Hanlon 1981; Ingham *et al.* 1985; Moore *et al.* 1988; Hedlund and Augustsson 1995). This fungal response to grazing intensity suggests that activity is increased up to an optimal value, after which further increases in grazing intensity begin to reduce fungal activity (Hanlon 1981; Ek *et al.* 1994). When invertebrate grazing occurs at optimal densities, fungal growth may be enhanced through the selective pruning of senescent hyphae, releasing immobilized nutrients (Hanlon 1981; Moore *et al.* 1988). Selective grazing may also remove toxin-accumulating hyphae, allowing nutrients to be further utilized, increasing fungal growth (Hanlon 1981).

GANODERMA APPLANATUM

The third wood-decay fungus used, *Ganoderma applanatum*, showed the greatest significant difference in the rate of decomposition between treatments. The treatment with *Ganoderma applanatum* and host wood block *Populus tremuloides* alone exhibited an average decay rate of 8.75% (LSD group D), versus the treatment with *Ganoderma applanatum,* wood block and fungal grazing nematode which exhibited an average decay rate of 2.13% (LSD group AB). From these results, we know that grazing from *Aphelenchoides* sp. had a significant effect on the ability of *Ganoderma applanatum* to successfully decay wood blocks of *Populus tremuloides*. Although *Ganoderma applanatum* did not have the highest rates of decay versus the other six

wood-decay fungi used, it exhibited the greatest influence by invertebrate grazing. It is possible that *Ganoderma applanatum* provided the most preferred food source for *Aphelenchoides* sp. compared to the other six wood-decay fungi used. *Ganoderma applanatum* was also one of the slowest growing fungi used, and even the lightest of grazing may have impacted rates of decay. *Ganoderma applanatum* exhibits a stress tolerant strategy which includes a slower growth rate (Dix and Webster 1995).

CERRENA UNICOLOR

The fourth wood-decay fungus used was *Cerrena unicolor* and like *Bjerkandera adusta,* exhibited an increase in decomposition in the presence of the fungus-feeding nematode *Aphelenchoides* sp. However, there was only a slight significance between the first treatment where *Cerrena unicolor* was inoculated onto *Populus tremuloides* wood blocks alone with an average percent decay of 3.75% (LSD grouping BC) versus the treatment with *Cerrena unicolor* and the fungus feeding nematode *Aphelenchoides* sp. inoculated onto wood blocks of *Populus tremuloides* with an average percent decay of 5.10% (LSD grouping C). Similar to *Bjerkandera adusta*, *Cerrena unicolor* is not known to possess defence mechanisms against hyphal grazing, therefore we can only assume *B. adusta* responded to low intensity grazing by the fungus-feeding nematode *Aphelenchoides* sp. by exhibiting enhanced growth and enzyme activity through the selective pruning of senescent hyphae, thus releasing immobilized nutrients (Hanlon 1981; Moore *et al*. 1988). Selective grazing may also remove toxin-accumulating hyphae, allowing nutrients to be further utilized, increasing fungal growth (Hanlon 1981).

CLIMACODON SEPTENTRIONALE

The fifth wood-decay fungi used, *Climacodon septentrionale*, did show a difference in the rate of decay between the treatment with nematode species *Aphelenchoides* sp. versus without nematodes. The treatment with *C. septentrionale* inoculated alone with wood blocks showed an average percent decay of 2.55%, while the treatment with *C. septentrionale*, wood block and nematode species *Aphelenchoides* sp. exhibited an average percent decay of 1.66%. However, this difference did not prove to be significant when doing the LSD test, placing both treatments in grouping AB.

Tanney (2011) undertook an investigation on the presence of antifeedant mechanisms in *C. septentrionale* while in the presence of mycophagist nematodes. The study found that when nematodes were introduced to cultures of *C. septentrionale,* they became immobilized in the aerial mycelia by means of toxin droplets produced by secretory cells. When nematodes came into contact with secretory cell droplets, their movement became restricted, enveloping portions of the nematode body. When enveloped, the nematodes were found to struggle in motion in an attempt to locate adjacent hyphae, causing droplets to coalesce and further capture nematodes. This continued until nematodes fully ceased in motion and ultimately died. Hyphae were never found to penetrate the nematode cuticle (Tanney 2011).

In the investigation done by Tanney (2011), it was found that nematodes degraded at inconsistent rates. The reason for this was thought to be due to the fact that experiments took place on a nutrient rich medium, which may negate the requirement to consume exogenous nutrient sources by the fungus (Tanney 2011). It is possible that trapped nematodes are used as a dietary supplement and that other organic substrates

such as lignin or cellulose are the main energy sources. The ability to capture and consume is most useful when nitrogen is a limiting factor at sites of high microbial activity in order to supplement nitrogen (Thorn and Barron 1984). Tanney hypothesized that if his experiment was done in a nutritionally starved medium, different results would have been obtained.

HOHENBUEHELIA GRISEA

When studying the effects of *Aphelenchoides* sp. on *Hohenbuehelia grisea,* there was an increase shown in the rate of decay while in the presence of fungus-feeding nematodes, with an average percent decay of 0.75% in the weight of wood blocks; compared to the treatment without nematodes (0.27%). However, this was not statistically significant.

A study done by Barron and Dierkes (1977) found *Hohenbuehelia* as the perfect state of *Nematoctonus*. The genus *Nematoctonus* was erected by Drechsler (1941) in discovering two fungi found to be parasitic on nematodes. Fungal parasites of nematodes are broadly classified as either predaceous or endoparasitic, however the genus *Nematoctonus* is unique in that some species are endoparasitic and some are predaceous (Barron 1977). *Nematoctonus* fungi are capable of capturing nematodes with hourglass-shaped adhesive knobs (Drechsler 1949), which is the case with *H. grisea.*

SPHAEROBOLUS STELLATUS

Sphaerobolus stellatus, similar to *Climacodon septentrionale*, exhibited a slight decline in decay when in the presence of fungus-feeding nematodes compared to their absence, but this was not statistically significant. Like *C. septentrionale* and *H. grisea*, *S. stellatus* is known to have the ability to immobilize mycophagous nematodes.

The surface mycelium of *Sphaerobolus* colonies produce gloeocystidia which encapsulate and immbolize fungus-feeding nematodes such as *Aphelenchoides* sp. When the nematode inserts its stylet into the gloeocystidium, oleaginous contents solidify and form a persistent cap on the head of the nematode. This persistent cap restricts the movement of the nematode, not allowing it the ability to feed on fungal hyphae (Tanney 2011)

Tanney (2011) found that although gloeocystidia occurred in high densities, it was restricted to the agar surface, allowing nematodes to successfully graze on hyphae submerged in agar. It was also found that the majority of immobilized nematodes appeared to be adults. This can be due to the fact that juvenile nematodes are able to shed their encapsulating material through moulting, which has been found to facilitate the removal of parasites in other organisms (Whitney 1982).

It is possible that *S. stellatus*, similar to *C. septentrionale*, was not able to produce mycelium at a quick enough rate to surpass the rate of grazing by the fungus-feeding nematode *Aphelenchoides* sp. It is also possible that mostly juvenile nematodes that were grazing *S. stellatus* hyphae were are able to escape encapsulation by shedding material through moulting.

CONCLUSION

Fungus feeding nematodes such as *Aphelenchoides* sp. do have an impact on ecological functions such as wood decay. However, some wood-decay fungi possess defence mechanisms and are able to immobilize nematodes and utilize their nutrients. Therefore, among seven wood-decay fungi tested, there was either an increase or decrease in the rate of decay of the *Populus tremuloides* wood blocks while in the presence of the fungus-feeding nematode *Aphelenchoides* sp.

Trametes pubescens and *Ganoderma applanatum* exhibited the largest decline in the rate of observed decay while in the presence of the fungus-feeding nematode *Aphelenchoides* sp. suggesting that the nematodes were able to successfully graze on their hyphae and impact enzyme activity. In contrast, *Bjerkandera adusta* and *Cerrena unicolor* displayed an increase in decay in the presence of fungus-feeding nematodes. Although there is little explanation for this, it was suggested that optimal grazing can enhance vigorous hyphal growth and thus promote better enzymatic activity.

The last three wood-decay fungi in this experiment have all proven in previous studies to possess hyphal defence mechanisms against hyphal grazing. *Climacodon septentrionale* and *Sphaerobolus stellatus* did not show significant differences in their rates of decay while in the presence or absence of fungus-feeding nematode *Aphelenchoides* sp. However, *Hohenbuehelia grisea* did exhibit an increase in the rate of decomposition when in the presence of the fungus-feeding nematode *Aphelenchoides* sp. This increase in decomposition in the presence of nematodes can be explained by the ability of *H. grisea* to consume nematodes for nitrogen, thus it may have been able to increase the rate and success of decomposition.

In order to better understand the relationship between the fungus-feeding nematode *Aphelenchoides* sp. and wood-decay fungi, it is suggested that more studies be done with larger sample sizes in order to achieve more accurate results. The length of experiment can be increased, varying optimal temperatures can be used, a greater amount of fungus-feeding nematodes may be introduced into each flask, and the species of host wood block can all be investigated further in order to see varying results.

Although many antifeedant mechanisms are known to exist among wood-decay fungi, the effects of these may be investigated on other mycophagist organisms with various feeding mouthparts such as collembola and mites in order to provide more insight into these interactions (Tanney 2011). The results from this thesis conclude further research must be done in order to fully understand the relationship between fungi and their co-inhabiting fauna.

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APPENDICES

APPENDIX I

COLLECTION INFORMATION FOR WOOD-DECAY FUNGI

- *Bjerkandera adusta* (297)
  10 November 2004
  Lakehead University campus, Thunder Bay, Ontario
  Isolated by O. Bott
  Isolated from spore print from basidioma on deciduous tree

- *Cerrena unicolor* (433)
  22 November 2005
  Lakehead University campus, Thunder Bay, Ontario
  Isolated by P. Gammond
  Isolated from spore print from basidoma

- *Climacodon septentrionale* (426)
  September 2005
  Thunder Bay, Ontario
  Isolated by L.J. Hutchison
  Isolated from tissue pieces from basidioma on stem of living *Acer saccharum*

- *Ganoderma applanatum* (089)
  18 November 2002
  Thunder Bay, Ontario
  Isolated by S. Hill
  Isolated from tissue pieces from basidioma

- *Hohenbuehelia grisea* (606)
  2007
  London, Ontario
  Isolated by R.G. Thorn
  Host substrate unknown

- *Sphaerobolus stellatus* (589)
  23 October 2007
  Lakehead University campus, Thunder Bay, Ontario
  Isolated by N. Coomes-Johnson
  Isolated from peridioles from basidiomata on decayed wood

- *Trametes pubescens*
  3 October 2014
  Lakehead University campus, Thunder Bay, Ontario
  Isolated by L.J Hutchison
  Isolated from tissue pieces from basidioma on fallen *Populus tremuloides* lo

APPENDIX II

DRY WEIGHTS OF *POPULUS TREMULOIDES* BEFORE AND AFTER INOCULATIONS WITH AVERAGE PERCENT DIFFERENCE (DECAY) (%)

Number of wood block	Dry weight of wood block (Before)	Dry weight of wood block (After)	% Difference (Decay)	Number of wood block	Dry weight of wood block (Before)	Dry weight of wood block (After)	% Difference (Decay)
1	1.020	1.025	-0.49	87	0.929	0.915	1.51
2	1.088	1.098	-0.92	88	1.081	0.984	8.97
3	1.271	1.251	1.57	89	1.350	1.352	-0.15
4	1.763	1.778	-0.85	90	0.872	0.869	0.34
5	1.012	1.019	-0.69	91	1.692	1.651	2.42
6	0.946	0.948	-0.21	92	1.564	1.532	2.05
7	1.242	1.246	-0.32	93	1.837	1.777	3.27
8	1.602	1.606	-0.25	94	1.750	1.730	1.14
9	1.095	1.095	0.00	95	1.670	1.649	1.26
10	0.972	0.970	0.21	96	1.693	1.626	3.96
11	1.241	0.925	25.46	97	1.804	1.689	6.37
12	1.360	1.037	23.75	98	1.806	1.786	1.11
13	1.764	1.432	18.82	99	1.547	1.499	3.10
14	1.450	1.057	27.10	100	1.413	1.401	0.85
15	1.553	1.158	25.43	101	1.402	1.390	0.86
16	1.572	1.205	23.35	102	1.751	1.743	0.46
17	1.275	0.911	28.55	103	1.489	1.474	1.01
18	1.574	1.228	21.98	104	2.053	2.025	1.36
19	1.563	1.250	20.03	105	1.876	1.843	1.76
20	1.581	1.222	22.71	106	1.596	1.582	0.88
21	1.489	1.152	22.63	107	1.832	1.695	7.48
22	1.618	1.379	14.77	108	1.986	1.961	1.26
23	1.411	1.121	20.55	109	1.713	1.693	1.17
24	1.735	1.460	15.85	110	1.868	1.861	0.37
25	1.700	1.340	21.18	111	1.183	1.179	0.34
26	1.183	0.983	16.91	112	1.299	1.283	1.23
27	1.745	1.377	21.09	113	1.353	1.338	1.11
28	1.690	1.337	20.89	114	1.121	1.119	0.18
29	1.829	1.496	18.21	115	1.471	1.478	-0.48
30	1.561	1.244	20.31	116	1.141	1.132	0.79
31	2.151	1.976	8.14	117	1.333	1.337	-0.30
32	1.752	1.564	10.73	118	1.332	1.333	-0.08

Number of wood block	Dry weight of wood block (Before)	Dry weight of wood block (After)	% Difference (Decay)	Number of wood block	Dry weight of wood block (Before)	Dry weight of wood block (After)	% Difference (Decay)
33	2.081	1.874	9.95	119	1.646	1.649	-0.18
34	1.782	1.596	10.44	120	1.599	1.597	0.13
35	1.721	1.527	11.27	121	1.120	1.111	0.80
36	1.459	1.299	10.97	122	1.218	1.212	0.49
37	1.868	1.668	10.71	123	1.124	1.126	-0.18
38	2.031	1.814	10.68	124	1.194	1.191	0.25
39	1.517	1.284	15.36	125	1.009	1.008	0.10
40	1.780	1.584	11.01	126	1.160	1.157	0.26
41	1.256	0.913	27.31	127	0.914	0.911	0.33
42	1.953	1.688	13.57	128	1.310	1.268	3.21
43	1.934	1.664	13.96	129	0.996	0.992	0.40
44	1.740	1.381	20.63	130	1.284	1.261	1.79
45	1.568	1.220	22.19	131	1.670	1.626	2.63
46	1.763	1.455	17.47	132	1.868	1.855	0.70
47	1.810	1.590	12.15	133	1.566	1.537	1.85
48	1.501	1.187	20.92	134	1.609	1.575	2.11
49	1.538	1.193	22.43	135	1.301	1.274	2.08
50	1.652	1.314	20.46	136	1.743	1.719	1.38
51	1.617	1.475	8.78	137	1.296	1.270	2.01
52	1.829	1.632	10.77	138	1.093	1.008	7.78
53	1.896	1.695	10.60	139	1.818	1.784	1.87
54	1.518	1.367	9.95	140	1.830	1.814	0.87
55	1.659	1.446	12.84	141	1.807	1.812	-0.28
56	1.696	1.485	12.44	142	1.302	1.288	1.08
57	1.598	1.480	7.38	143	1.543	1.534	0.58
58	1.931	1.880	2.64	144	1.480	1.470	0.68
59	1.870	1.725	7.75	145	1.745	1.701	2.52
60	1.850	1.763	4.70	146	1.546	1.525	1.36
61	1.783	1.749	1.91	147	1.223	1.217	0.49
62	1.718	1.676	2.44	148	1.182	1.129	4.48
63	1.689	1.659	1.78	149	1.735	1.688	2.71
64	1.741	1.718	1.32	150	1.742	1.707	2.01
65	1.530	1.493	2.42				
66	1.649	1.630	1.15				
67	2.154	2.090	2.97				
68	1.752	1.715	2.11				
69	1.963	1.894	3.52				

Number of wood block	Dry weight of wood block (Before)	Dry weight of wood block (After)	% Difference (Decay)				
70	1.716	1.687	1.69				
71	1.472	1.429	2.92				
72	1.197	1.156	3.43				
73	2.000	1.950	2.50				
74	1.399	1.351	3.43				
75	1.713	1.660	3.09				
76	1.446	1.369	5.33				
77	1.570	1.514	3.57				
78	1.677	1.633	2.62				
79	1.491	1.398	6.24				
80	1.370	1.310	4.38				
81	1.681	1.614	3.99				
82	1.524	1.305	14.37				
83	1.327	1.289	2.86				
84	1.121	1.099	1.96				
85	1.213	1.181	2.64				
86	1.065	0.911	14.46				

APPENDIX III

DATA RUN THROUGH SPSS IN STATISTICAL ANALYSIS

Flask #	Name	Nematodes	Percent decay (%)
11	*Trametes pubescens*	N	25.46
12	*Trametes pubescens*	N	23.75
13	*Trametes pubescens*	N	18.82
14	*Trametes pubescens*	N	27.1
15	*Trametes pubescens*	N	25.43
16	*Trametes pubescens*	N	23.35
17	*Trametes pubescens*	N	28.55
18	*Trametes pubescens*	N	21.98
19	*Trametes pubescens*	N	20.03
20	*Trametes pubescens*	N	22.71
21	*Trametes pubescens*	Y	22.63
22	*Trametes pubescens*	Y	14.77
23	*Trametes pubescens*	Y	20.55
24	*Trametes pubescens*	Y	15.85
25	*Trametes pubescens*	Y	21.18
26	*Trametes pubescens*	Y	16.91
27	*Trametes pubescens*	Y	21.09
28	*Trametes pubescens*	Y	20.89
29	*Trametes pubescens*	Y	18.21
30	*Trametes pubescens*	Y	20.31
31	*Bjerkandera adusta*	N	8.14
32	*Bjerkandera adusta*	N	10.73
33	*Bjerkandera adusta*	N	9.95
34	*Bjerkandera adusta*	N	10.44
35	*Bjerkandera adusta*	N	11.27
36	*Bjerkandera adusta*	N	10.97
37	*Bjerkandera adusta*	N	10.71
38	*Bjerkandera adusta*	N	10.68
39	*Bjerkandera adusta*	N	15.36
40	*Bjerkandera adusta*	N	11.01
41	*Bjerkandera adusta*	Y	27.31
42	*Bjerkandera adusta*	Y	13.57

43	*Bjerkandera adusta*	Y	13.96
44	*Bjerkandera adusta*	Y	20.63
45	*Bjerkandera adusta*	Y	22.19
46	*Bjerkandera adusta*	Y	17.47
47	*Bjerkandera adusta*	Y	12.15
48	*Bjerkandera adusta*	Y	20.92
49	*Bjerkandera adusta*	Y	22.43
50	*Bjerkandera adusta*	Y	20.46
51	*Ganoderma applanatum*	N	8.78
52	*Ganoderma applanatum*	N	10.77
53	*Ganoderma applanatum*	N	10.6
54	*Ganoderma applanatum*	N	9.95
55	*Ganoderma applanatum*	N	12.84
56	*Ganoderma applanatum*	N	12.44
57	*Ganoderma applanatum*	N	7.38
58	*Ganoderma applanatum*	N	2.64
59	*Ganoderma applanatum*	N	7.75
60	*Ganoderma applanatum*	N	4.7
61	*Ganoderma applanatum*	Y	1.91
62	*Ganoderma applanatum*	Y	2.44
63	*Ganoderma applanatum*	Y	1.78
64	*Ganoderma applanatum*	Y	1.32
65	*Ganoderma applanatum*	Y	2.42
66	*Ganoderma applanatum*	Y	1.15
67	*Ganoderma applanatum*	Y	2.97
68	*Ganoderma applanatum*	Y	2.11
69	*Ganoderma applanatum*	Y	3.52
70	*Ganoderma applanatum*	Y	1.69
71	*Cerrena unicolor*	N	2.92
72	*Cerrena unicolor*	N	3.43
73	*Cerrena unicolor*	N	2.5
74	*Cerrena unicolor*	N	3.43
75	*Cerrena unicolor*	N	3.09
76	*Cerrena unicolor*	N	5.33
77	*Cerrena unicolor*	N	3.57
78	*Cerrena unicolor*	N	2.62
79	*Cerrena unicolor*	N	6.24
80	*Cerrena unicolor*	N	4.38

81	*Cerrena unicolor*	Y	3.99
82	*Cerrena unicolor*	Y	14.37
83	*Cerrena unicolor*	Y	2.86
84	*Cerrena unicolor*	Y	1.96
85	*Cerrena unicolor*	Y	2.64
86	*Cerrena unicolor*	Y	14.46
87	*Cerrena unicolor*	Y	1.51
88	*Cerrena unicolor*	Y	8.97
89	*Cerrena unicolor*	Y	-0.15
90	*Cerrena unicolor*	Y	0.34
91	*Climacodon septentrionale*	N	2.42
92	*Climacodon septentrionale*	N	2.05
93	*Climacodon septentrionale*	N	3.27
94	*Climacodon septentrionale*	N	1.14
95	*Climacodon septentrionale*	N	1.26
96	*Climacodon septentrionale*	N	3.96
97	*Climacodon septentrionale*	N	6.37
98	*Climacodon septentrionale*	N	1.11
99	*Climacodon septentrionale*	N	3.1
100	*Climacodon septentrionale*	N	0.85
101	*Climacodon septentrionale*	Y	0.86
102	*Climacodon septentrionale*	Y	0.46
103	*Climacodon septentrionale*	Y	1.01
104	*Climacodon septentrionale*	Y	1.36
105	*Climacodon septentrionale*	Y	1.76
106	*Climacodon septentrionale*	Y	0.88
107	*Climacodon septentrionale*	Y	7.48
108	*Climacodon septentrionale*	Y	1.26
109	*Climacodon septentrionale*	Y	1.17
110	*Climacodon septentrionale*	Y	0.37
111	*Hohenbuehelia grisea*	N	0.34
112	*Hohenbuehelia grisea*	N	1.23
113	*Hohenbuehelia grisea*	N	1.11
114	*Hohenbuehelia grisea*	N	0.18
115	*Hohenbuehelia grisea*	N	-0.48
116	*Hohenbuehelia grisea*	N	0.79
117	*Hohenbuehelia grisea*	N	-0.3
118	*Hohenbuehelia grisea*	N	-0.08

119	*Hohenbuehelia grisea*	N	-0.18
120	*Hohenbuehelia grisea*	N	0.13
121	*Hohenbuehelia grisea*	Y	0.8
122	*Hohenbuehelia grisea*	Y	0.49
123	*Hohenbuehelia grisea*	Y	-0.18
124	*Hohenbuehelia grisea*	Y	0.25
125	*Hohenbuehelia grisea*	Y	0.1
126	*Hohenbuehelia grisea*	Y	0.26
127	*Hohenbuehelia grisea*	Y	0.33
128	*Hohenbuehelia grisea*	Y	3.21
129	*Hohenbuehelia grisea*	Y	0.4
130	*Hohenbuehelia grisea*	Y	1.79
131	*Sphaerobolus stellatus*	N	2.63
132	*Sphaerobolus stellatus*	N	0.7
133	*Sphaerobolus stellatus*	N	1.85
134	*Sphaerobolus stellatus*	N	2.11
135	*Sphaerobolus stellatus*	N	2.08
136	*Sphaerobolus stellatus*	N	1.38
137	*Sphaerobolus stellatus*	N	2.01
138	*Sphaerobolus stellatus*	N	7.78
139	*Sphaerobolus stellatus*	N	1.87
140	*Sphaerobolus stellatus*	N	0.87
141	*Sphaerobolus stellatus*	Y	-0.28
142	*Sphaerobolus stellatus*	Y	1.08
143	*Sphaerobolus stellatus*	Y	0.58
144	*Sphaerobolus stellatus*	Y	0.68
145	*Sphaerobolus stellatus*	Y	2.52
146	*Sphaerobolus stellatus*	Y	1.36
147	*Sphaerobolus stellatus*	Y	0.49
148	*Sphaerobolus stellatus*	Y	4.48
149	*Sphaerobolus stellatus*	Y	2.71
150	*Sphaerobolus stellatus*	Y	2.01

APPENDIX IV

UNIVARIATE ANALYSIS WITH PERCENT DECAY AS THE RESPONSE VARIABLE AND PRESENCE OF NEMATODES AND SPECIES OF WOOD-DECAY FUNGI AS FIXED VARIABLES

Tests of Between-Subjects Effects

Dependent Variable: % decay

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8153.913[a]	13	627.224	88.172	.000
Intercept	7413.516	1	7413.516	1042.152	.000
Name	7480.036	6	1246.673	175.250	.000
Nematodes	5.565	1	5.565	.782	.378
Name * Nematodes	668.312	6	111.385	15.658	.000
Error	896.321	126	7.114		
Total	16463.750	140			
Corrected Total	9050.234	139			

a. R Squared = .901 (Adjusted R Squared = .891)

APPENDIX V

LSD TEST DONE GROUPING TREATMENTS WITHIN A VALUE OF 2.36 IN ORDER TO TEST FOR SIGNIFICANCES

LEGEND			
TREATMENT	MEAN	TREATMENT	MEAN
HG (N)	0.27	CU (N)	3.75
HG (Y)	0.75	CU (Y)	5.10
SS (Y)	1.56	GA (N)	8.79
CS (Y)	1.66	BA (N)	10.93
GA (Y)	2.13	BA (Y)	19.11
SS (N)	2.33	TP (Y)	19.24