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ABSTRACT

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Keywords: Balsam Fir, Cambium, Endophyte, Fir Broom Rust, *Foveostroma abietinum, Melampsorella caryophyllacearum*, *Phoma*

A total of 241 isolation attempts were made, with 233 yielding cultures from 61 branches collected from two balsam fir trees. The most commonly found fungal endophyte in healthy branches of balsam fir was *Foveostroma abietinum*, followed by *Geniculosporium* sp. and *Zythiostroma pinastri*, while in witches' broom twigs the most common fungal endophyte was *Phoma* sp. 1*,* followed by *Foveostroma abietinum,* and *Thelebolus caninus.* A modification of Good's Hypothesis was used to calculate the percentage of total biodiversity likely to be found in the surveyed twigs, and the values of 46.6% and 55.6% were obtained for healthy twigs and witches' broom twigs respectively. Approximately half of the fungal endophyte diversity is represented in this study according to the modification of Good's Hypothesis, however, further research should be conducted to further understand the fungal endophyte diversity in both healthy and witches' broom twigs.

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LITERATURE REVIEW

MORPHOLOGY OF BALSAM FIR

Balsam fir [*Abies balsamea* (L.) Mill.] is a medium-sized coniferous tree species that typically grows to heights up to 25 m and 70 cm in diameter (Farrar 2006). The longevity of this species is usually 125 years; however, some trees live up to 200 years. The typical form of *Abies balsamea* is conical with a spire-like crown that extends to the ground in open conditions, but an elevated crown in well stocked areas. The trunk below the crown is slightly tapered with a shallow root system that penetrates no deeper than 30 cm (Johnson and Warren n.d. and Wile n.d.). Bark found on this species is greyish and smooth with prominent resin blisters when young; the bark breaks into irregular brownish scales in old age. The wood is light, soft, weak and somewhat brittle with little contrast between the earlywood and latewood (Farrar 2006). The needles are flat, resinous, 15 to 25 millimeters long with a blunt or notched tip, and two medial resin canals. On the upper surface of the leaf, the colour is typically a dark green and, on the underside, there are usually 10-12 bands of stomata. Needles are arranged in 2 ranks, shorter on the upper side of the twig (Harlow *et al.* 2001).

Balsam fir is a monecious species with female flowers located near the top of the tree and male flowers produced further down the crown. The arrangement of the flowers ensures that there is cross fertilization since the pollen is spread through wind. The

juvenile seed cones are formed in the leaf axils of the previous year's twig and appear erect, composed of many bracts that cross over a large scale containing two inverted basal ovules. Once mature, the female cones are four to 10 cm long, appearing cylindrical and erect with bracts shorter than the scales (Harlow *et al.* 2001; Farrar 2006). The pollen cones are soft, catkin-like, and pendulous, located in the leaf axils of the previous year's twig. Male flowers disintegrate following pollination in late spring (Wile n.d.). The seed of balsam fir occurs in pairs on the upper side of the cone scales. The seeds are typically three to six millimetres in length with an ovoid appearance, conspicuous resin vesicles and a broad terminal wing that is purple to brown. Balsam fir seeds are distributed by wind and small mammals. The seeds of balsam fir contain dormant embryos and will only germinate following stratification in moist soils for 90 to 240 days (Hart 1959).

TAXONOMY OF BALSAM FIR

Balsam fir are gymnosperms belonging to the Pinaceae family in the order Coniferales. This species belongs to the genus *Abies,* which is the second largest in the family Pinaceae. The genus is the most taxonomically complex and widely distributed throughout East Asia, North and Central America, Mediterranean and Southern Europe and is comprised of 40 recognized species, however, the taxonomic classification of the genus is greatly disputed and widely varies (Semerikov and Semerikova 2015). In a 1950's monograph, the genus *Abies* is subdivided into two subgenera consisting of *Pseudotorreya* Franco and *Sapindus* Franco with balsam fir belonging to the section

Balsameae Engelm, which falls in the *Sapindus* subgenus and subfamily *Abietoidea* There are two recognized varieties of balsam fir, *Abies balsamea var. balsamea* and *Abies balsamea var. phanerolepis* (Bakuzis and Hansen 1965).

SILVICULTURE OF BALSAM FIR

Balsam fir is a species that prefers a cold climate and an abundance of moisture for best development and is widely distributed across North America (Hart 1959). In Canada, it is found from Newfoundland to northwestern Alberta and from New England to northern Minnesota in the United States (Koubaa and Zhang 2008). However, this species achieves greatest growth in southeastern Canada and the Maritime Provinces. Balsam fir can be found planted outside of its natural range as ornamentals in many European countries such as Germany, Norway and Finland (Bakuzis and Hansen 1965). This species has the ability to grow on all soil types from heavy clays to a rocky surface and is able to tolerate a wide range of soil pH although they grow best on deep, well-drained soils containing an abundance of organic material. Balsam fir grows on a variety of upland and lowland sites such as mountain slopes and glaciated uplands, peatland, swamps and alluvial flats (Koubaa and Zhang 2008).

This species is typically found in mixed coniferous stands and deciduous-coniferous mixed stands. Other tree species associated with balsam fir in upland sites may include white spruce [*Picea glauca* (Moench) Voss], white birch (*Betula papyrifera* Marsh), *Populus* spp*,* American beech (*Fagus grandifolia* Ehrh.) *etc*. In lowland sites, species associated with balsam fir include black spruce (*Picea mariana* Mill.), black ash

(*Fraxinus nigra* Marsh.), tamarack (*Larix laricina* K. Koch), and more (Koubaa and Zhang 2008). However, balsam fir is considered a shade tolerant species in its natural range, so it has the ability to reproduce under its own canopy and form pure stands (Hett and Loucks 1976). Layering is not an important means of regeneration for balsam fir and typically only does this in extreme conditions in the northern part of its range. Balsam fir are prolific seed producers and can easily regenerate through sexual reproduction and seed dispersal by wind and small mammals which typically causes a dispersal distance of 20 to 60 m. Although in some instances, when the soil is moist, the lower branches of balsam fir may layer when in contact with the ground. (Hart 1959).

ECONOMICS USES OF BALSAM FIR

Balsam fir is a conifer species with a variety of economic uses. The wood of this species is typically used for lumber and pulpwood. Previously, it was believed that due to the low density and strength, knots and poor treating qualities, that balsam fir wood was poor and limited in its use. However, through studies, it is now believed that dimension lumber is acceptable in the marketplace under the category spruce-pine-fir (SPF) (Koubaa and Zhang 2008; Bakuzis and Hansen 1965). Due to its physical characteristics, balsam fir lumber produces high quality 2” x 4” and 2” x 6” studs for frame construction. Lumber from balsam fir may also be used for products such as building construction, sheathing, roofing and subflooring, and prefabrication (Govett and Sinclair 1983). The wood may also be used for mine timbers, boxes, crates, house siding, and poles. Balsam fir is also typically used for pulp because of its good fibre

length (three to four mm), and quality. The pulp is used to manufacture a range of products including paper, tissues, paperboard and newsprint (Bakuzis and Hansen 1965). Wood from balsam fir killed by spruce budworm can also be used for energy and chemicals. Research completed by Barnes and Sinclair (1984), concluded that the gross heat of combustion for the calorific value of living balsam fir showed negligible differences to that of the dead trees (Barnes and Sinclair 1984).

As a non-timber forest product (NTFP), balsam fir is traditionally used for Christmas trees due to its symmetrical shape, abundance of needles, and fragrance (Bakuzis and Hansen 1965). Another common NTFP produced from balsam fir are essential oils derived from the twigs or needles of the tree (Mohammed 1999). Oil from this species has a turpentine-like odour and is highly volatile making it a common fragrance in the production of air fresheners, cleaners, detergents, and disinfectants. Balsam fir resin, found in the bark blisters, has been collected to make Canada balsam (USDA 2010). This substance is a turpentine that can be used to make permanent microscope slides because of its properties that allow it to be amorphous when dried and it will not crystallize with age as well as its poor thermal and solvent resistance (Devi 2017). Canada balsam has also traditionally been used in optics as an invisible glue for glass such as lens elements, however, this has since been replaced by UV-cured epoxies (Devi 2017).

DISEASES AFFECTING BALSAM FIR

Balsam fir is susceptible to infection from many rusts and other fungi that cause a reduction in vigour and defoliation. Bakuzis and Hansen (1965), compiled a list of fungi associated with balsam fir based on previous research. The list consisted of 262 species of parasitic and saprophytic fungi found on balsam fir, however, the list may not be complete. The majority of fungi on the list are described as causing disease of living trees or growing on dead needles, twigs, bark, and wood. It consisted of 164 species of Basidiomycetes, 66 species of Ascomycetes, and 32 species of Fungi Imperfecti (Bakuzis and Hansen 1965).

According to Bakuzis and Hansen (1965), seedlings of the genus *Abies* can become infected by damping-off-fungi in the genera *Fusarium, Rhizoctonia, Pythium* and *Phytophthora.* However, since it is not widely planted in nurseries, there are few reports of these occurrences. During winters producing large amounts of snowfall, snow mold caused by *Phacidium infestans* (P.Karst.) has been found. This pathogen causes the most damage to young conifers that have not yet reached heights above snow levels. This can lead to injury resulting in the browning and death of needles and rarely death of terminal buds (Roll-Hansen 1989).

Blights and needle casts are also a significant group of diseases that attack balsam fir that causes portions of, whole, and groups of needles and twigs to be killed, and can even infect the stem forming witches' brooms (Bakuzis and Hansen 1965).The most important disease causing fungi responsible belong to the genus *Rehmiellopsis* of the family Sphaeriaceae, genera *Lophodermium* and *Hypodermella* of the family

Hypodermataceae, and the genera *Phacidium* and *Bifusella* of the family Phacidiaceae. According to Bakuzis and Hansen (1965), *Bifusella faullii* Darker is considered the most serious of these, attacking whole needles and killing current years growth on balsam fir.

Although cankers are typically more common on hardwoods than softwoods, there are many instances of cankers on balsam fir. Canker fungi usually enter through wounds caused by atmospheric conditions or insects and cause necrosis of cortical tissue resulting in an open wound on the infected tree (Bakuzis and Hanen 1965; Horst 2013). These formations can girdle stems or trunks of trees killing water-conducting tissues that lead to prominent dieback of the tree or death (Horst 2013). According to Quirke and Hord (1955), cankers found on balsam fir in Ontario are most commonly located on immature trees along the borders of a stand and on mature trees growing on dry, shallow soils.

Major diseases found in balsam fir include: balsam fir tip blight [*Delphinella basameae* (Rostr.)], fir broom rust [*Melampsorella caryophyllacearum* (DC.) J. Schroet], caliciopsis canker (*Caliciopsis pinea* Peck), red flag of balsam fir [*Fusicoccum abietinum* (R.Hartig) Prill. & Delacr.], witches-broom of blueberry [*Pucciniastrum goeppertianum* (Kuehn) Kleb.], Armillaria root rot (*Armillaria* spp.) and tomentosus root rot [*Inonotus tomentosus* (Fr.) Teng] (Koubaa and Zhang 2008).

FIR BROOM RUST

Rusts are obligate parasites belonging to the order Uredinales of the division Basidiomycota meaning they produce basidia and basidiospores. There are five different spore producing structures found on rust fungi; spermagonium (0), aecium (I), uredinium (II), telium (III), and basidium (IV). The fir broom rust fungus (*Melampsorella caryophyllacearum*) is heteroecious, meaning its life cycle occurs on two hosts, and macrocyclic, containing all spore states (Cummins and Hiratsuka 2003). This species is native to Canada infecting *Abies*, its aecial host, and *Ceratium* spp. and *Stellaria* spp, its telial host. Fir broom rust is a systemic species which means that the rust may be found in areas where the other host may not be present (NRCAN 2015).

Infection of balsam fir begins in the spring when basidiospores found on chickweed are carried by wind and infect the buds of a tree when they begin to open (Ziller 1974). The mycelium slowly spreads throughout the tissue and infected areas become recognizable during the following autumn as elongate swellings. Spermagonia and aecia develop on the infected needles which fall from the tree in August following the shedding of the aeciospores (Ziller 1974). The aeciospores then infect the leaves of chickweed and begin to produce uredinia a few weeks following initial infection (USDA n.d.). Urediniospores then spread on the leaves of chickweed and will overwinter on perennial chickweed as systemic mycelium (Ziller 1974). The following spring, mycelium of the pathogen will grow into the new shoots and extend into the leaves followed by the production of uredinia and telia. Teliospores will then germinate and release basidiospores, thus completing the life cycle of the rust fungus (Ziller 1974).

This rust causes a tissue malformation known as a ‘witches’ broom’ which form as a result of the infection causing excessive bud formation that elongates, producing compact twigs that have a bushy appearance (Littlefield 1981). In fungus species that produce witches’ brooms such as *Melampsorella caryophyllacearum,* the witches’ brooms are perennial and can persist for 15 to 20 years due to the mycelium surviving for years in woody tissue. The needles formed on witches’ brooms are shorter and broader when compared to healthy needles and fall off annually during August. Chlorophyll content is also greatly reduced in perennial witches’ brooms and cause a great deal of nutrient drain on the host (Littlfield 1981; Ziller 1974). In some cases, witches’ brooms caused by the fir broom rust fungus may be mistaken for mistletoe infections or brooms caused by physiological abnormalities (NRCAN 2015).

There are multiple methods that can be used to manage fir broom rust. One of the methods suggested to break the disease cycle and control the spread of this pathogen is to eradicate all chickweed in the area (Eshenaur and Lamb 2013). If the chickweed is located in an area where it cannot be controlled, it is recommended to consider planting tree species other than susceptible firs (Eshenaur and Lamb 2013). However, Ziller (1974) states that the removal of trees with main stem infections during the early stages of the stand would be a sufficient control measure (Ziller 1974). Pruning is also a measure that can be used to reduce the occurrence of infection and rid infected fir trees of already developed witches’ brooms (Ziller 1974; Eshenaur and Lamb 2013).

FUNGAL ENDOPHYTES

Fungal endophytes are species of fungi that can be found in tree species without causing noticeable disease (Brader *et al.* 2017). Fungal endophytes are extremely common and have been found in almost all vascular plant species examined living asymptomatically and systemically (Faeth and Fagan 2002). Based on previous research, members of the Ascomycota, Basidiomycota, Deuteromycota, and a few Oomycota have been isolated as endophytes (Cerkauskas and Sinclair 1996)

Typically, endophytes can be divided into two groups: those that are ubiquitous and are isolated from a wide array of hosts in various geographical conditions, and those that show a certain degree of host specificity, following a pattern characteristic of obligate antagonistic symbionts (Crous *et al*. 2000). According to Petrini (1996), these can further be divided into two ecological groups: clavicipitaceous systemic grass endophytes and the endophytes found in trees and shrubs which includes non-clavicipitaceous grass endophytes. Endophytic fungi can be transmitted from one generation to the next through systemic infection or vertical transmission which occurs through host seeds or vegetative propagules (Cates *et al.* 2014; Crous *et al*. 2000). However, vertical transmission is imperfect and seedling germination and growth rates presents an issue for fungal growth (Cates *et al.* 2014). Endophytes may also be spread through horizontal transmission. This occurs when infection takes place through air-borne spores and is closely correlated with seasonal rainfall (Crous *et al.* 2000).

Endophytic fungi can have various affects on the host species' biochemistry and physiology and can influence multitrophic networks and ecosystems (Unterseher 2011). Some of these endophytes are commensals and cause no effects, some are mutualistic and promote growth, whereas some are pathogenic on some plant species (Brader *et. al* 2017). Fungal endophytes found in woody tissues often appear as primary wood decay fungi on dying or dead branches. When a tree becomes weak or stressed from diseases or other outside factors, some endophytes can become pathogenic and cause further damage to a tree. Whereas dark septate endophytes found in the rhizosphere functionally coincide with soil fungi, mycorrhizal fungi, saprotrophic fungi, and obligate and pathogenic fungi. In leaves, the endophytes are comprised of heterogenous assemblages of mutualists, latent pathogens, parasites, saprobes and facultative entomopathogens (Unterseher 2011). Fungal endophytes are considered an important component of fungal biodiversity, however, the range of ecological functions of endophytes in woody plants is poorly understood and there is still little known about endophytes and plant species relationships (Brader *et al.* 2017; Elissetche *et al.* 2011; Unterseher 2011).

METHOD AND MATERIALS

On September 23, 2019, twig samples were collected by Alexis Dunn and Dr. Hutchison at Jack Haggerty Forest from healthy branches of two balsam fir (*Abies balsamea*) and witches' brooms on the same trees. Twig samples were removed from the tree using secateurs that were previously sterilized with 70 percent ethanol. Once removed, samples were placed in plastic bags and labelled according to the tree harvested from (one or two) and whether they were healthy (H) or collected from witches' brooms (B). The twig samples were stored in a freezer located in the forest pathology teaching lab in the Braun Building until isolations were performed.

A two percent malt extract agar medium (10.0 g malt extract, 0.5 g yeast, 7.5 g agar, and 500 mL of water) was used for isolation of fungi. The medium was made in quantities of 500 mL and placed in 1 L flasks to mix. Spatulas and weigh boats were used to weigh out each ingredient on a scale, using clean tools between each ingredient. After all ingredients were weighed, aluminum foil was placed over the mouths of the flasks. The medium was then sterilized in an autoclave for 20 minutes at 121 °C. The flasks were removed and placed into a water bath until cool enough to handle. The agar was then poured into sterile Petri dishes (90 mm diameter). The dishes were left to harden in a transfer hood for 24 hours to reduce the condensation on the underside of the lids. Petri dishes were then wrapped with Parafilm® to prevent the medium from drying.

Isolations were made from September to November 2019 from the collected twigs. The samples were placed in 70 percent ethanol alcohol for one minute to ensure surface contaminants were killed. All tools, secateurs, scalpels, needle nose pliers, and

tweezers, were also sterilized with 70 percent ethanol and flamed using a gas burner prior to use. The pliers were used to hold and stabilize the twigs and secateurs were used to remove the two alcohol-soaked ends of the twigs to prevent the alcohol from potentially killing the fungi. The scalpel was then used to cut the bark and expose the cambium. Four chips of the cambium were removed from one end of the twig, then placed in a Petri dish containing the agar medium using tweezers. To reduce the risk of the dishes being contaminated by bacteria, a small quantity of antibiotics (streptomycin sulphate and penicillin G) were put on the medium of each dish using a sterilized needle and then sealed with parafilm. This process was then completed for the other end of the sample.

In total, sixteen healthy twigs and nine broom twigs collected from tree one, and twenty healthy twigs and sixteen broom twigs from tree two were used. The plates were categorized by which tree the twig was collected from, whether it was from healthy branches or a broom, the twig number, and which end of the twig it was from (*e.g.,* T1B-T1-E1 = Tree1 Broom, Twig 1, End 1). In total, there were initially 122 agar plates (two plates for each twig from two trees). The plates were placed in an incubator at approximately 20°C for the fungi to grow out. If more than one type of fungus grew out, samples were transferred to a new Petri dish and assigned a roman numeral along with the original identification number on the dish (*.eg.,* TIB-T1-E1-i). Identifications began December 9, 2019 utilizing a compound light microscope and a dissecting microscope to identify samples. Initially, most of the samples were not sporulating, so some cultures were scratched with a sterile needle, and all dishes were placed in an illuminated chamber to promote sporulation. Taxonomic literature was also utilized to identify

cultures down to the species level if possible. The identified fungi, along with its associated tree, twig number and Petri dish number can be found in Appendix I. Photographs of unique fungi were also taken using an Olympus E-330 digital camera mounted on a Nikon Eclipse 440 phase contrast microscope.

RESULTS

Sixty-one branches in total were obtained from the two balsam fir trees at Jack Haggerty forest. A total of 242 Petri dishes were inoculated from the branches and included: original isolations, transfers, bacteria, overgrown cultures, and those with no growth. The complete list of all isolates can be found in Appendix I.

Tables 1 and 2 summarize the fungi that were isolated and identified to genus and some to species. Isolates that did not sporulate were categorized based on morphology and given a sterile number. Bacteria and yeasts found on the plates were not identified.

Table 1. Taxa of fungi isolated from healthy twig of balsam fir.

Taxa	Total	Frequency
Bothrodiscus berenice	1	0.68%
Botrytis-like sp.	1	0.68%
Coniothyrium sp. 1	1	0.68%
Coniothyrium sp. 2	1	0.68%
Foveostroma abietinum	10	6.85%
Geniculosporium sp. 1	7	4.79%
Geniculosporium sp. 2	2	1.37%
Geotrichum-like sp.	1	0.68%
Gloeosporidiella-like sp.	1	0.68%
Lecythophora sp. 2	2	1.37%
Nigrospora sphaerica	1	0.68%
Phoma sp. 1	2	1.37%
Phoma-like sp.	4	2.74%
Prosthemium sp.	1	0.68%
Thelebolus caninus	1	0.68%
Zignoella sp.	1	0.68%
Zythiostroma pinastri	5	3.42%
Sterile #1	2	1.37%
Sterile #2	4	2.74%
Sterile #3	10	6.85%

Table 1. (continued)

Sterile #4	17	11.64%
Sterile #5	1	0.68%
Sterile #6	11	7.53%
Sterile #7	1	0.68%
Sterile #8	1	0.68%
Sterile #9	3	2.05%
Sterile #10	4	2.74%
Sterile #12	8	5.48%
Sterile #13	1	0.68%
Sterile #14	3	2.05%
Sterile #15	1	0.68%
Sterile #16	2	1.37%
Sterile #17	1	0.68%
Sterile #18	1	0.68%
Sterile #19	1	0.68%
Sterile #20	1	0.68%
Sterile #21	1	0.68%
Sterile #22	4	2.74%
Sterile #24	14	9.59%
Sterile #25	8	5.48%
Sterile #28	1	0.68%
Sterile #29	1	0.68%
Unknown Sporulating sp.	1	0.68%
Yeast-like	1	0.68%
Total	146	100.00%

Based on the total number of isolates made during this study, the healthy branches of balsam fir had the greatest amount of diversity with 44 different taxa represented by 146 isolates out of the 241 total isolates. Of all isolates taken from healthy balsam fir twigs, *Foveostroma abietinum* (Peck) DiCosmo was the most commonly occurring species isolated, comprising of 10 (6.71 %) of the 146 isolates from healthy twigs. *Foveostroma abietinum* (Fig. 1) is the anamorphic state of *Dermea*

balsamea (Peck) Seaver which is a weakly parasitic fungus that causes dieback of branches and leaders of trees (Funk 1981). The sterile isolate that had the greatest occurrence in healthy balsam fir twigs was sterile #4 which was comprised of 17 isolates (11.41%).

Table 2. Taxa of fungi isolated from balsam fir twigs infected with the causal agent of fir broom rust.

Taxa	Total	Frequency
Alternaria alternata	1	1.11%
Chaetomium cochliodes	1	1.11%
Cladosporium cladosporioides	2	2.22%
Cladosporium sphaerospermum	1	1.11%
Coniothyrium fuckelii	1	1.11%
Coniothyrium sp. 1	1	1.11%
Foveostroma abietinum	4	4.44%
Gloeosporidiella-like sp.	2	2.22%
Lecythophora sp. 1	2	2.22%
Lecythophora sp. 3	2	2.22%
Mortierella sp. 1	2	2.22%
Mortierella sp. 2	1	1.11%
Papulospora sp.	1	1.11%
Penicillium frequentans	1	1.11%
Penicillium sp.	1	1.11%
Phoma sp. 1	5	5.56%
Phoma sp. 2	1	1.11%
Thelebolus caninus	4	4.44%
Trichoderma koningii	3	3.33%
Ulocladium chartarum	2	2.22%
Zignoella sp.	2	2.22%
Zythiostroma pinastri	1	1.11%
Sterile #3	3	3.33%
Sterile #6	8	8.89%
Sterile #9	6	6.67%
Sterile #10	6	6.67%
Sterile #11	1	1.11%

Table 2. (continued)

Sterile #12	1	1.11%
Sterile #16	1	1.11%
Sterile #20	1	1.11%
Sterile #22	3	3.33%
Sterile #23	2	2.22%
Sterile #24	4	4.44%
Sterile #25	5	5.56%
Sterile #26	2	2.22%
Sterile #27	1	1.11%
Not Grown	5	5.56%
Total	90	100.00%

The amount of diversity found in witches' broom twigs was lower than that of the healthy twigs with 36 different taxa comprising of 90 isolates total. *Phoma* sp. 1 was the most commonly occurring species found with 5 isolates (5.56 %). *Phoma* species belong to the division Ascomycota and are cosmopolitan phytopathogens that commonly occur in soil, organic matter, plants and aquatic systems (Bennett *et al.* 2018, Domsch *et al.* 1993). Out of the sterile isolates, sterile #8 occurred most frequently with 8 isolates (8.89%).

Table 3 shows the taxa, including sterile isolates that were found in both healthy balsam fir twigs and twigs infected with the causal agent of fir broom rust.

Table 3. Taxa, including sterile isolates found in both healthy balsam fir twigs and fir broom rust twigs.

Taxa	Total
Coniothyrium sp. 1	2
Foveostroma abietinum	14
Gloeosporidiella-like sp.	3
Phoma sp. 1	7
Thelebolus canninus	5
Zignoella sp.	3
Zythiostroma pinastri	6
Sterile #3	13
Sterile #6	19
Sterile #9	9
Sterile #10	10
Sterile #12	9
Sterile #16	3
Sterile #20	2
Sterile #22	7
Sterile #24	18
Sterile #25	13
Total	143

Out of all taxa identified, only 17 taxa were isolated from both healthy twigs and witches' broom twigs. The taxon that was most commonly isolated was *Foveostroma abietinum* (Figure 1) with 14 of the 143 isolates. The taxa that were less frequently isolated were *Gloeosporidiella-like* sp. (Figure 2) and *Zignoella* sp. both of which had three isolates. Sterile #6 was the most commonly occurring of all sterile isolates found.

Species isolated from both healthy twigs and fir broom rust twigs include taxa that are known to be pathogenic including: *Lecythophora* spp. (Damm *et al.* 2010), *Bothrodiscus berenice* (Berk. & Curt.) Groves (Figure 3)– causes branch mortality – (Funk 1981), *Geniculosporium* spp. – causing cankers and dieback – (Chesters and Greenhalgh 1964), *Nigrospora sphaerica* Sacc. (Figure 4) (Ahmed and Hameed 2013), and *Coniothyrium fuckelii* Sacc., a wound pathogen causing cankers (Alfieri 1969).

Saprophytic taxa were also commonly found and include: *Zignoella* spp. (Fernandez *et al.* 2006), *Zythiostroma pinastri* (Karst.) Hoehn. (Gremmen 1977), *Prosthemium* sp. (Figure 5) – more commonly found as an endophyte – (Hirayama *et al.* 2010; Barr *et al.* 2005), *Alternaria alternata* (Akagi *et al.* 2013), *Mortierella* spp. (Adhikari *et al.* 2015), *Papulospora* sp. (Figure 6) (Hotson 1942), *Trichoderma koningii* Oudem. (Borner *et al.* 1998), and *Ulocladium chartarum* (INSPQ 2016a). *Cladosporium cladosporioides* (Fresen.) de Vries and *C. sphaerospermum* Penz. are saprophytes that are commonly found as endophytes (Bensch *et al.* 2010), while *Penicillium* spp. are ubiquitous and commonly found as contaminants on various substrates (Domsch *et al.* 1993; Ellis n.d.),

Other than pathogens and saprophytes, *Thelebolus caninus* (Auersw.) Jeng & J.C. Krug is a coprophilous fungus (Nyberg and Persson 2002), and *Chaetomium* spp. are widespread in soil, on decaying plant materials, and are also known as soft-rot fungi in softwood and hardwood timber (INSPQ 2016b).

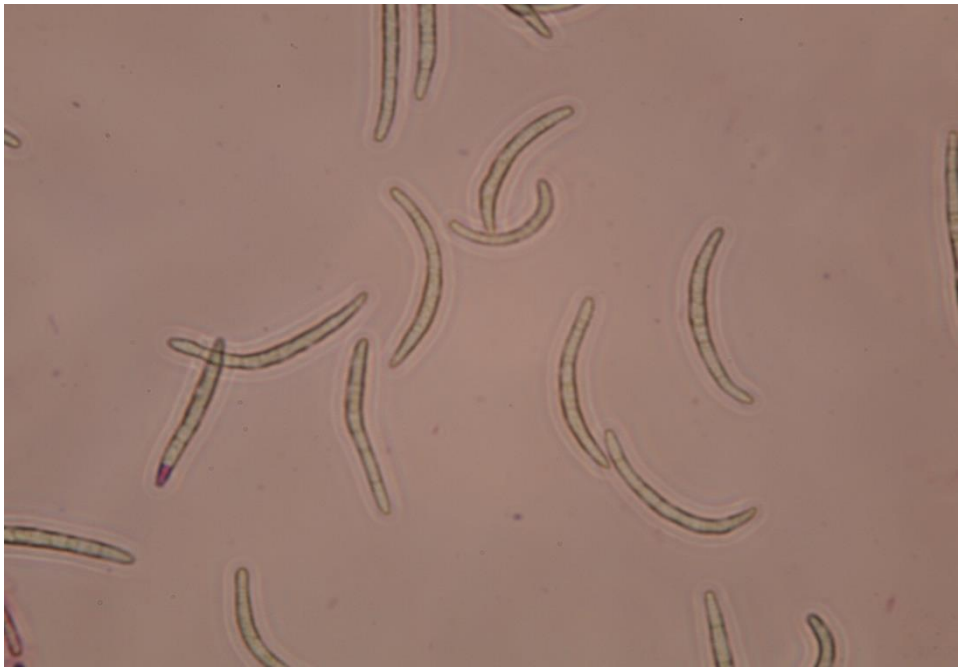

Figure 1.Conidia of *Foveostroma abietinum*.

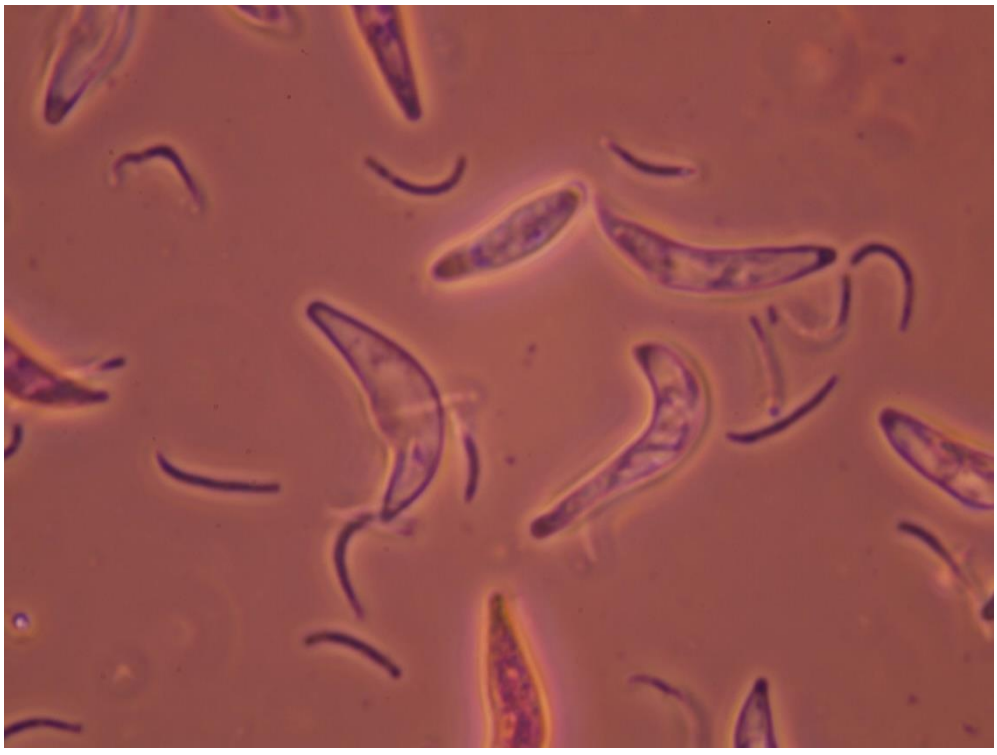

Figure 2. Boomerang-shaped conidia of *Gloeosporidiella*-like sp.

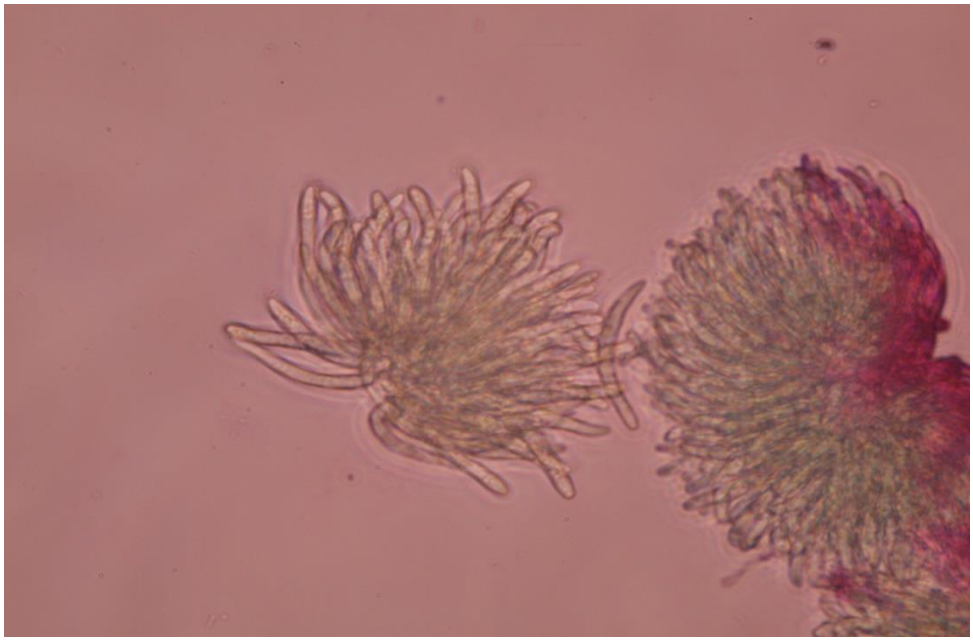

Figure 3. Glomerule of *Bothrodiscus berenice*.

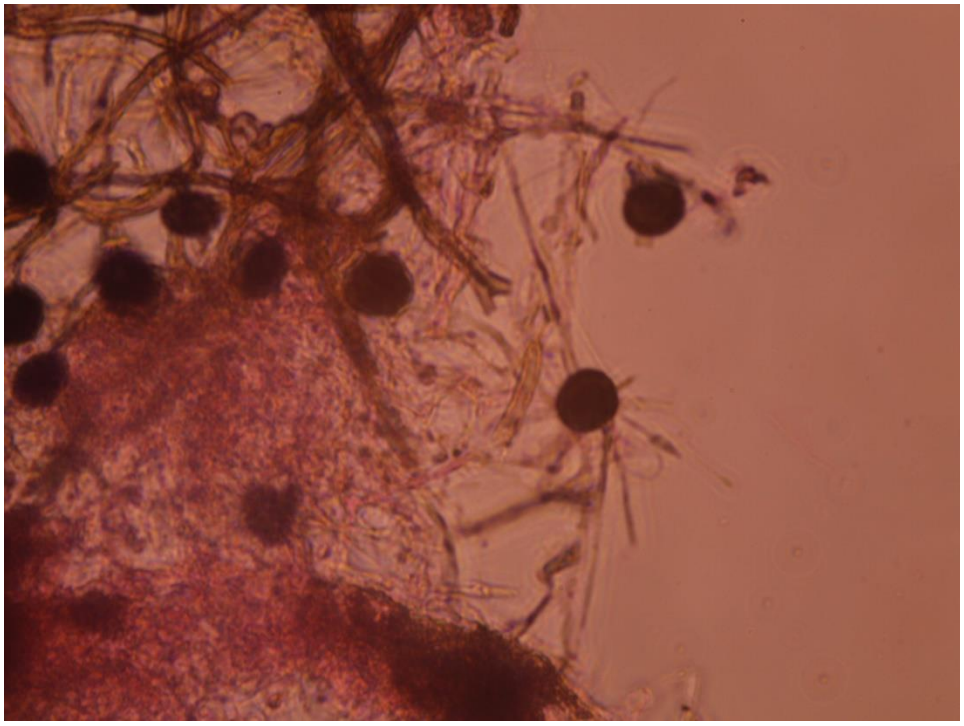

Figure 4. Conidia of *Nigrospora sphaerica.*

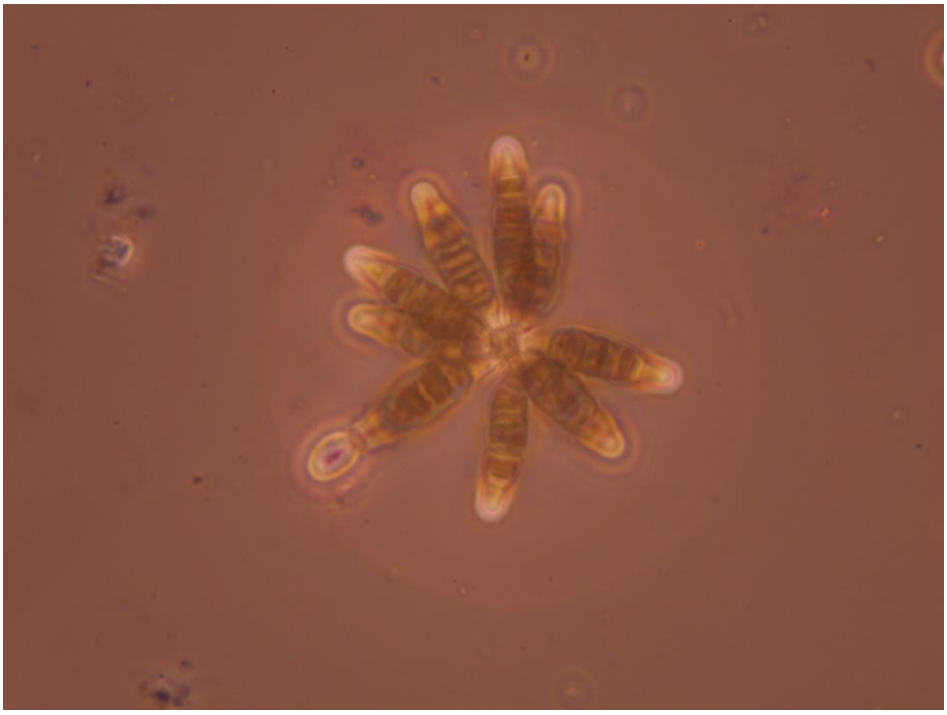

Figure 5. Conidium of *Prosthemium* sp.

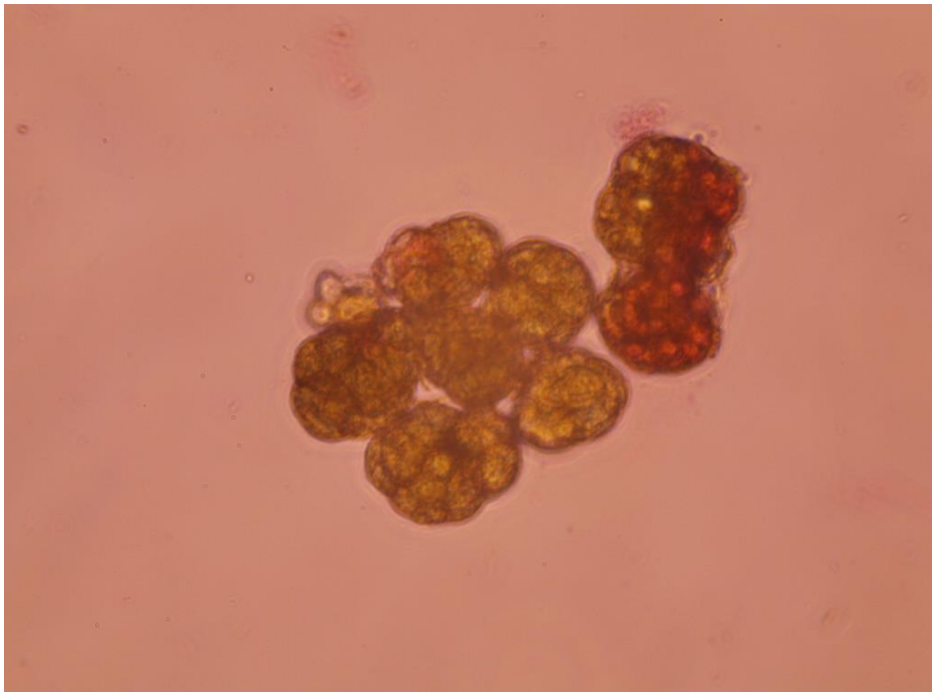

Figure 6. Conidia of *Papulospora* sp.

DISCUSSION

A COMPARISON OF HEALTHY TO BROOM BALSAM FIR TWIGS

The fungal endophyte diversity isolated from healthy branches of balsam fir was not widely different from that of the broom twigs. In healthy branches, 44 different species were found with 24 being found only once whereas in broom twigs, 36 different species were found, of which 16 were only found once. Of the species isolated from broom twigs, 17 were also found in healthy twigs.

Foveostroma abietinum was the most commonly isolated species from healthy twigs. This fungus is a parasitic pathogen found on wounded trees causing canker and dieback of balsam fir. It has simple conidia that are unicellular and acerose when young and become one to four celled and falcate at maturity (Groves 1946). As previously mentioned, it is the imperfect state of *Dermea balsamea* which is one of the most common causal agents of dieback in balsam fir found in Ontario and is usually represented by *F. abietinum* on stems and branches (Raymond and Reid 1961). Although this species is pathogenic, it was not causing disease symptoms in the tree. This fungus was most likely in a latent stage, waiting for more favourable conditions for disease to develop, such as stress, poor environmental conditions, or wounding (Pirttila and Wali 2009). According to Hord and Quirke (1955), extremely dry growing conditions accounted for increased incidences of dieback caused by this fungus.

Isolates of *Phoma* sp. 1 were the most commonly encountered from witches' broom twigs. The genus *Phoma* possesses relatively round pycnidia containing unicellular colourless to yellow conidia borne from phialides lining the walls of the pycnidium (Bennett *et al.* 2018; Irinyi *et al.* 2006). The genus was previously grouped in with the anamorphic Coelomycetes due to similar morphological characteristics, however it has since been moved to the class Dothideomycetes and belonging to the family Didymellaceae (Bennett *et al.* 2018). They are often isolated from a wide array of substrates, particularly as plant pathogens and soil-borne saprophytic and opportunistic fungi (Irinyi *et al.* 2006).

The genera of fungi found only in healthy twigs of balsam fir include plant pathogens such as *Bothrodiscus berenice* and saprophytes such as *Prosthemium*. The genera of fungi that were only found in witches' brooms twigs were similar in ecological function and include saprophytes such as *Alternaria alternata*, decay fungi such as *Chaetomium cochliodes,* and pathogens such as *Coniothyrium fuckelii*. Of the species found only in witches' broom twigs, *Alternaria* spp. and *Cladosporium* spp. have been known to be associated with rust fungi. Representatives of these genera can act as parasites of rust fungi using their mycelium to colonize the uredinia and urediniospores, destroy the cytoplasm, and eventually kill the colonized spores (Littlefield 1981). Of the seven genera found in both healthy twigs and broom twigs, four were plant pathogens, two were saprophytes, and one was a coprophilous fungus (Table 3).

Plants are colonized by a great number of microorganisms, primarily commensals – with no known function – or mutualists, and in some instances, endophytes may be pathogens – waiting for favourable conditions – or saprobes that

have colonized wood early to have a competitive advantage to other saprobes (Brader *et al.* 2017; Pirttila and Wali 2009). Endophytes are typically categorized into non-pathogenic and pathogenic, although the criteria for pathogenic endophytes are unclear since most isolated endophytes are only tested on a single or few plant species. Since this is the case, endophytes may not show deleterious effects on the tested plant, however they may show pathogenicity on other hosts (Brader *et al.* 2017). Not only this, but pathogenicity may also be altered by environmental factors, genotypes, and biotic interactions that have been shown to affect pathogen tolerance or resistance (Pirttila and Wali 2009; Brader *et al.* 2009). Endophytes are heterotrophic organisms that utilize the same substrate, and thus compete for resources. As a result, many endophytes possess the ability to produce antagonistic compounds to other colonizers that alter the defence mechanisms of the host tree or inhibit the growth or pathogenic function of fungi (Pirttila and Wali 2009; Brader *et al.* 2017). The explanations provided by Brader *et al.* (2017) and Pirttila and Wali (2009), may account for the large variety of phytopathogens found in the twigs that were not causing apparent disease.

DIFFERENCE IN ENDOPHYTE DIVERSITY BETWEEN HEALTHY AND BROOM TWIGS

The number of fungal species found in healthy twigs was greater than in broom twigs in this study. Due to the low number of twigs collected from only two balsam fir trees, this study cannot fully represent the fungal diversity of healthy and broom twigs.

The sampling efficiency of this study, using Good's Hypothesis (Good 1953) modified by Moore and Holdeman (1974), was:

$$1 - \left(\frac{Number\ of\ species\ found\ once}{Total\ number\ of\ species}\right) \times 100$$

This equation represents the percentage of the total biodiversity likely to be found in the survey. Using this equation,

Healthy balsam fir twigs:

$$1 - \left(\frac{24}{44}\right) \times 100 = 45.5\%$$

Broom twigs:

$$1 - \left(\frac{16}{36}\right) \times 100 = 55.6\%$$

The above calculations show that 45.5% and 55.6% of the total biodiversity of healthy twigs and broom twigs were likely found in this study, respectively.

In both calculations, the total biodiversity found is relatively high with just under half and slightly over half of the biodiversity being isolated for the healthy twigs and the broom twigs, respectively. The twigs of healthy balsam fir and witches' broom twigs used in this study were taken from a natural setting in which the species composition of the stand was a mixture of balsam fir and other boreal conifers. According to Kehr and Kowalski (1996), the diversity of plant communities has the potential to greatly

influence the extent of endophyte colonization which has been seen through host specific endophytes colonizing non host plants when in mixed stands. Species diversity of endophyte populations in fir has also been shown to be affected by the type of forest management in a stand. Sieber-Canavesi and Sieber (1987), they found that clear cuts and plantations eliminate or reduce the transmission of endophytic fungi. Spontaneously grown trees (*i.e.,* trees grown under normal conditions) were also found to possess more endophytes than their planted counterparts (Sieber-Canavesi and Sieber 1987). Since the twigs were collected from a natural stand it can account for a higher diversity of endophytes found, and thus a higher total biodiversity isolated during this study.

Fungal endophytes found in tree hosts are typically transmitted horizontally which means they are spread through air-borne spores or rainfall (Crous *et al.* 2000; Pirtilla and Wali 2009). Within plant tissues, the spread of endophytic fungi is affected by a number of factors, one of which is host nutrients as endophytes are heterotrophic. Since endophytes are heterotrophic and take resources from the host plant, it can account for the lower diversity found (and higher total biodiversity calculated) in broom twigs than in the healthy twigs because witches' brooms caused by *Melampsorella caryophyllacearum* lead to a great deal of nutrient drain to the host, and therefore may not have sufficient nutrients in the twigs for endophyte colonization. Not only this, but endophyte infections can occur a number of ways, and theoretically, infections can occur from systemic infections from other tissues (Crous *et al.* 2000). This means that endophytes infecting the foliage of balsam fir may be transmitted to the twigs that the needles are located on. Based on previous studies, it has been found that old tissues of trees, such as older foliage, are typically more heavily colonized by endophytes than

younger tissues (Bernstein and Carroll 1977). This could account for the higher diversity of fungal endophytes found in healthy twigs of balsam fir compared to broom twigs. Typically, balsam fir foliage remains on the tree for several years before falling off, providing more time for colonization by endophytes, however, the foliage found on witches' brooms caused by *Melampsorella caryophyllacearum* fall off annually, and therefore, provide less time for colonization.

COMPARISON OF ENDOPHYTES FOUND IN OTHER RESEARCH

Petrini *et al.* (1989) isolated fungi from needles of balsam fir and galls of *Paradiplosis tumifex* Gagne on balsam fir. Thirty-five taxa were isolated from the needles with pathogens and saprophytes composing of the majority of the fungi found. Fungi such as *Foveostroma abietinum, Geniculosporium* sp., *Phoma* sp., and *Zythiostroma pinastri* were also isolated and were the only fungi that overlapped with this study.

Johnson and Whitney (1989) isolated endophytes from the needles of a single balsam fir tree. 771 isolates were obtained from the needles of the balsam fir tree with three taxa comprising of 90% of the isolates, all of which were saprophytic and pathogenic. Other fungi isolated from the needles that coincided with this study only included *Cladosporium* sp. and *Penicillium* spp.

Both of the previous studies only used the needles of balsam fir whereas this study used the cambium of twigs. Most studies examining endophytes in conifers have only been done on the needles, including balsam fir, and so far, there is little knowledge

on endophytic fungi found in bark or wood (Pirttila and Wali 2009). As a result, of these differences, it is reasonable to assume that the fungi found in this study are different than those that have been presented.

There were no studies found on fungal endophytes associated with witches' brooms caused by *Melampsorella caryophyllacearum.* Further studies are required to determine the full endophyte diversity found in both broom twigs and healthy twigs of balsam fir.

STERILE CULTURES

A total of 29 different sterile taxa, otherwise known as mycelia sterilia, were found during this study and were unable to be identified as a result of the cultures not sporulating (Guo *et al.* 1998). Theses sterile taxa represented 60.6% of the total number of isolates in this study. Carroll *et al.* (1982), isolated from evergreen shrubs in western Oregon and found that 15% of the fungi would not sporulate. According to Fisher *et al.* (1994), there are also varying isolation frequencies of mycelia sterilia depending on the different sites and tissues examined. They found that the frequencies of mycelia sterilia ranged from 11.2% to 41.3% and concluded that it is important to develop methods to promote sporulation of these sterile cultures.

Guo *et al.* (1998) performed a study that examined endophytic fungi isolated from Chinese fan palm [*Livistona chinensis* (Jacq.) R.Br. ex Mart.]. Of the 778 endophytes isolated, 52.2% of the isolates did not sporulate. In an attempt to promote sporulation, the leaves were cut into strips, sterilized in an autoclave at 121 °C for 20

minutes, and then isolates were transferred onto agar plates and the sterilized leaf strips were also added. In some instances, this promoted the formation of fruiting bodies on the leaf and allowed for identification.

Another method that may be employed to identify mycelia sterilia is to examine dead samples of the tissues being isolated from and examine them for sporulating structures (Bills 1996). To add to this, it is sometimes possible to rehydrate and incubate these dead plant materials in moist chambers to promote active sporulation. In living tissues, it is also possible to girdle a stem to release latent endophytes from the bark and sapwood to encourage sporulation *in situ* (Bills 1996).

CONCLUSION

There has been a great deal of research to examine foliar endophytes of balsam fir, however, there is little to be found in regard to twig inhabiting endophytes in healthy and a witches' broom twigs on this tree species. There was little contrast in endophyte diversity between healthy and broom twigs with approximately half of the fungal endophyte diversity found in both. More studies are required to determine the total biodiversity of each, and such studies may potentially lead to endophytic species that can be used as biological control agents against certain pathogens or induce resistance to them. If more time was available, further measures to promote sporulation of sterile cultures should be utilized for a more accurate measurement of biodiversity. This study only used twigs from two balsam fir trees located at Jack Haggerty forest and future research should involve isolating fungal endophytes from a larger number of trees over a larger geographic range.

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APPENDIX

APPENDIX I: LIST OF FUNGI ISOLATED IN STUDY

Tree #	Healthy/ Broom	Dish #	Transfer ID	Resulting Fungus
1	HEALTHY	T1H-T3-E1-X		Sterile #22
		T1H-T6-E1-X		Botrytis-like sp.
		T1H-T8-E2-X		Sterile #25
		T1H-T11-E2-X		Sterile #4
		T1H-T11-E2-X		Sterile #24
		T1H-T12-E2-X		Sterile #18
		T1H-T14-E1-X		Phoma sp. 1
		T1H-T15-E1-X		Zythiostroma pinastri
		T1H-T15-E2-X		Sterile #24
		T1H-T15-E2-X		Sterile #4
		T1H-T16-E1-X		Sterile #19
1	HEALTHY	T1H-T1-E1		Sterile #14
		T1H-T1-E1		Sterile #3
		T1H-T1-E1		Sterile #24
		T1H-T1-E2		Sterile #24
		T1H-T1-E2		Sterile #4
		T1H-T2-E1		Sterile #6
		T1H-T2-E2		Bacteria
		T1H-T3-E1		Sterile #4
		T1H-T3-E2	i	Sterile #4
		T1H-T3-E2	ii	Sterile #4
		T1H-T4-E1		Sterile #4
		T1H-T4-E1		Sterile #24
		T1H-T4-E2		Sterile #10
		T1H-T4-E2		Sterile #4
		T1H-T4-E2	i	Sterile #4
		T1H-T5-E1		Foveostroma abietinum
		T1H-T5-E1		Sterile #6
		T1H-T5-E1		Sterile #24
		T1H-T5-E2		Geniculosporium sp. 1
		T1H-T6-E1		Sterile #24
		T1H-T6-E1		Sterile #4
		T1H-T6-E2		Sterile #4
		T1H-T6-E2		Sterile #25
		T1H-T6-E2	i	Sterile #4

		T1H-T7-E1		Sterile #2
		T1H-T7-E2		Sterile #25
		T1H-T8-E1		Sterile #6
		T1H-T8-E1		Sterile #24
		T1H-T8-E2		Geniculosporium sp. 1
		T1H-T9-E1		Sterile #20
		T1H-T9-E1		Sterile #25
		TIH-T9-E2		Sterile #24
		TIH-T9-E2		Sterile #4
		T1H-T10-E1		Sterile #24
		T1H-T10-E1		Sterile #6
		T1H-T10-E2		Sterile #25
		T1H-T10-E2	i	Prosthemium sp.
		T1H-T11-E1		Sterile #25
		T1H-T11-E2		Sterile #16
		T1H-T11-E2		Coniothyrium sp. 1
		T1H-T11-E2	i	Geniculosporium sp. 1
		T1H-T12-E1		Sterile #25
		T1H-T12-E2		Bothrodiscus berenice
		T1H-T12-E2		Foveostroma abietinum
		T1H-T12-E2		Sterile #24
		T1H-T12-E2		Sterile #28
		T1H-T13-E1		Sterile #14
		T1H-T13-E1		Sterile #22
		T1H-T13-E2		Foveostroma abietinum
		T1H-T13-E2		Sterile #3
		T1H-T13-E2		Sterile #24
		T1H-T14-E1		Sterile #25
		T1H-T14-E1	i	Phoma sp. 1
		T1H-T14-E1	ii	Geotrichum-like sp.
		T1H-T14-E2		Bacteria
		T1H-T15-E1		Sterile #3
		T1H-T15-E1		Sterile #22
		T1H-T15-E1		Sterile #24
		T1H-T15-E2		Geniculosporium sp. 1
		T1H-T16-E1		Sterile #16
		T1H-T16-E2		Sterile #14
		T1H-T16-E2		Sterile #12
1	BROOM	T1B-T1-E1	i	Alternaria alternata
		T1B-T1-E1	ii	Sterile #23
		T1B-T1-E2	i	Sterile #6

		T1B-T1-E2	ii	Sterile #3
		T1B-T1-E2	iii	Sterile #27
		T1B-T2-E1	i	Sterile #6
		T1B-T2-E1	ii	Cladosporium cladosporioides
		T1B-T2-E1	iii	Penicillium sp.
		T1B-T2-E2	i	Coniothyrium fuckelii
		T1B-T3-E1		Lecythophora sp. 3
		T1B-T3-E2		Phoma sp. 1
		T1B-T3-E2	i	Sterile #10
		T1B-T3-E2	ii	Phoma sp. 1
		T1B-T4-E1		Sterile #9
		T1B-T4-E2		Ulocladium chartarum
		T1B-T4-E2	i	Ulocladium chartarum
		T1B-T4-E2	ii	Sterile #10
		T1B-T5-E1		Lecythophora sp. 1
		T1B-T5-E1	i	Lecythophora sp. 1
		T1B-T5-E2		Sterile #6
		T1B-T6-E1		Sterile #3
		T1B-T6-E2		N/A
		T1B-T7-E1		N/A
		T1B-T7-E2		Phoma sp. 1
		T1B-T8-E1		N/A
		T1B-T8-E2		Sterile #16
		T1B-T9-E1		Sterile #26
		T1B-T9-E2	i	Sterile #26
		T1B-T9-E2	ii	Mortierella sp. 1
		T1B-T9-E2	iii	Mortierella sp. 1
2	HEALTHY	T2H-T1-E1	i	Overgrown
		T2H-T1-E1	ii	Lecythophora sp. 2
		T2H-T1-E1	ia	Sterile #2
		T2H-T1-E1	ib	Sterile #6
		T2H-T1-E1	iii	Sterile #3
		T2H-T1-E2		Sterile #12
		T2H-T2-E1	i	Sterile #6
		T2H-T2-E1	ii	Sterile #12
		T2H-T2-E2		Sterile #15
		T2H-T3-E1	i	Geniculosporium sp. 1
		T2H-T3-E1	ii	Sterile #12
		T2H-T3-E2		Sterile #3
		T2H-T4-E1	i	Sterile #3

T2H-T4-E1	ii	Sterile #3
T2H-T4-E1	iii	Sterile #4
T2H-T4-E1	iv	Sterile #2
T2H-T4-E1	v	Coniothyrium sp. 2
T2H-T4-E2	i	Sterile #8
T2H-T4-E2	ii	Sterile #3
T2H-T4-E2	iii	Sterile #6
T2H-T4-E2	iv	Sterile #3
T2H-T5-E1	i	Sterile #10
T2H-T5-E1	ii	Geniculosporium sp. 1
T2H-T5-E1	iii	Geniculosporium sp. 1
T2H-T5-E2	i	Sterile #7
T2H-T5-E2	ii	Sterile #10
T2H-T5-E2	iii	Sterile #10
T2H-T6-E1		Unknown Sporulating sp.
T2H-T6-E2		Phoma-like sp.
T2H-T7-E1	i	Foveostroma abietinum
T2H-T7-E1	ii	Sterile #1
T2H-T7-E1	iii	Sterile #1
T2H-T7-E2		N/A
T2H-T8-E1		Foveostroma abietinum
T2H-T8-E2		Sterile #6
T2H-T9-E1	i	Gloeosporidiella-like sp.
T2H-T9-E1	ii	Thelebolus caninus
T2H-T9-E2	i	Sterile #2
T2H-T10-E1		Sterile #22
T2H-T10-E2		Zythiostroma pinastri
T2H-T11-E1		Sterile #12
T2H-T11-E2	i	Sterile #12
T2H-T11-E2	ii	Sterile #17
T2H-T12-E1		Sterile #9
T2H-T12-E2		Sterile #3
T2H-T13-E1		N/A
T2H-T13-E2		Sterile #4
T2H-T14-E1		Zythiostroma pinastri
T2H-T14-E2	i	Sterile #6
T2H-T14-E2	ii	Foveostroma abietinum
T2H-T15-E1		Sterile #6
T2H-T15-E2		Sterile #6
T2H-T16-E1		Sterile #24
T2H-T16-E1		Sterile #9

		T2H-T16-E2	i	Sterile #12
		T2H-T16-E2	ii	Zignoella sp.
		T2H-T17-E1		Phoma-like sp.
		T2H-T17-E1		Sterile #12
		T2H-T17-E1		Sterile #9
		T2H-T17-E2	i	Lecythophora sp. 2
		T2H-T17-E2	ii	Sterile #13
		T2H-T18-E1	i	Geniculosporium sp. 2
		T2H-T18-E1	ii	Foveostroma abietinum
		T2H-T18-E1	iii	Geniculosporium sp. 2
		T2H-T18-E2	i	Zythiostroma pinastri
		T2H-T18-E2	ii	Sterile #5
		T2H-T18-E2	iii	Phoma-like sp.
		T2H-T18-E2	iv	Phoma-like sp.
		T2H-T19-E1	i	Foveostroma abietinum
		T2H-T19-E1	ii	Zythiostroma pinastri
		T2H-T19-E2	i	Foveostroma abietinum
		T2H-T19-E2	ii	Yeast-like
		T2H-T20-E1	i	Sterile #29
		T2H-T20-E1	ii	Sterile #4
		T2H-T20-E1	iii	Sterile #4
		T2H-T20-E1	iv	Foveostroma abietinum
		T2H-T20-E2	i	Nigrospora sphaerica
		T2H-T20-E2	ii	Sterile #21
2	BROOM	T2B-T1-E1	i	Gloeosporidiella-like sp.
		T2B-T1-E2	i	Papulospora sp.
		T2B-T1-E2	ii	Phoma sp. 1
		T2B-T1-E2	iii	Phoma sp. 2
		T2B-T2-E1	i	Thelebolus caninus
		T2B-T2-E1	ii	Cladosporium sphaerospermum
		T2B-T2-E1	iii	Sterile #6
		T2B-T2-E2		Penicillium frequentans
		T2B-T3-E1		Sterile #6
		T2B-T3-E2		Trichoderma koningii
		T2B-T4-E1		Sterile #24
		T2B-T4-E1		Sterile #12
		T2B-T4-E2	i	Thelebolus caninus
		T2B-T4-E2	ii	Thelebolus caninus
		T2B-T5-E1		Sterile #9
		T2B-T5-E2		Sterile #25

T2B-T6-E1		Mortierella sp. 2
T2B-T6-E2		N/A
T2B-T7-E1		Trichoderma koningii
T2B-T7-E2	i	Trichoderma koningii
T2B-T7-E2	ii	Gloeosporidiella-like sp.
T2B-T8-E1		Chaetomium cochlioides
T2B-T8-E2	i	N/A
T2B-T8-E2	ii	Sterile #20
T2B-T9-E1		Sterile #25
T2B-T9-E2	i	Sterile #6
T2B-T9-E2	ii	Foveostroma abietinum
T2B-T9-E2	iii	Sterile #10
T2B-T10-E1	i	Sterile #3
T2B-T10-E1	ii	Sterile #11
T2B-T10-E1	iii	Zignoella sp.
T2B-T10-E1	iv	Sterile #6
T2B-T10-E1	v	Zignoella sp.
T2B-T10-E2	i	Cladosporium cladosporioides
T2B-T10-E2	ii	Sterile #9
T2B-T10-E2	iii	Sterile #25
T2B-T11-E1	i	Thelebolus caninus
T2B-T11-E1	ii	Sterile #10
T2B-T11-E2	i	Lecythophora sp. 3
T2B-T11-E2	ii	Phoma sp. 1
T2B-T11-E2	iii	Sterile #10
T2B-T12-E1		Sterile #10
T2B-T12-E2		Foveostroma abietinum
T2B-T13-E1		Zythiostroma pinastri
T2B-T13-E1		Sterile #24
T2B-T13-E1		Sterile #22
T2B-T13-E2		Sterile #9
T2B-T14-E1		Foveostroma abietinum
T2B-T14-E1		Sterile #9
T2B-T14-E1		Sterile #22
T2B-T14-E2		Foveostroma abietinum
T2B-T14-E2		Sterile #24
T2B-T14-E2		Sterile #6
T2B-T15-E1		Sterile #23
T2B-T15-E1		Sterile #22
T2B-T15-E1		Sterile #24

T2B-T15-E1	Sterile #25
T2B-T15-E2	Sterile #25
T2B-T16-E1	Coniothyrium sp. 1
T2B-T16-E2	Sterile #9