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Atkins, Georgina

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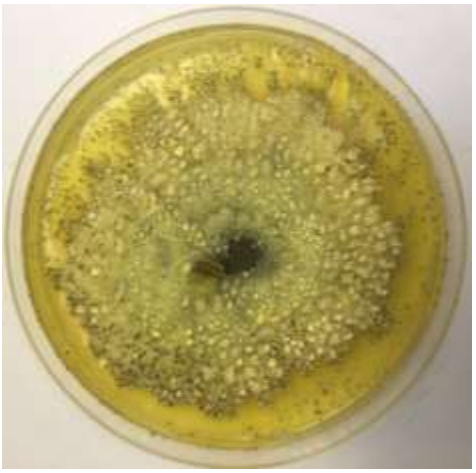

Faculty of Natural Resources Management

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AN INQUIRY INTO THE FUNGAL DIVERSITY ASSOCIATED WITH THE GALLS FORMED BY *PEMPHIGUS BETAE* DOANE

By

Georgina Atkins

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Dr. Leonard Hutchison	Dr. Don Henne
Major Advisor	Second Reader

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ABSTRACT

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Keywords: aphid, *Cladosporium*, entomopathogenic fungi, *Fusarium*, galls, *Pemphigus*, *Penicillium,* phylloplane fungi, *Populus, Verticillium*

Leaves affected by galls induced by the aphid *Pemphigus betae* were collected from a *Populus balsamifera* tree located near the community garden on the campus of Lakehead University Thunder Bay. Out of the galls that were collected, 100 were selected to examine the fungal diversity found within. Inner tissue samples were taken from each gall and inoculated onto 2% malt extract agar in Petri dishes. After pure cultures were obtained through isolation, the fungal taxa were identified. The fungal diversity found within the galls sampled, displayed an ecological and taxonomic pattern that varied from gall to gall. One hundred and ninety three isolates representing 43 different taxa were found. The most common species isolated from the galls were *Cladosporium cladosporioides* (from 54% of galls), *Verticillium lecanii* (from 26% of galls), *Penicillium simplicissimum* (from 21% of galls), *Fusarium* sp. 1 (from 20% of galls) and *Penicillium brevicompactum* (from 18% of galls). Ecologically, most fungi represented typical saprophytic and phylloplane species which tend to be ubiquitous and cosmopolitan, while a few represented entomopathogenic species, and some represented plant pathogenic species. Further analysis will be needed to understand the full diversity found within the galls. This can be done through an expansion in the number of samples collected and isolated from, and from multiple host trees over a larger geographic area.

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ACKNOWLEDGMENTS

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1.0 INTRODUCTION

1.1 CANADA'S BOREAL FOREST

Canada contains many diverse forest types including the Great Lakes-St. Lawrence, Acadian/maritime, montane, etc. (Canadian Council of Forest Ministers 2018). However, there is one forest type that spans the largest amount of area when compared to all others: the boreal forest. This forest type ranges from the Yukon Territory to Newfoundland and Labrador and makes up approximately 30% of the global boreal forest cover (Brandt *et al.* 2013; CCFM 2018). The boreal forest type, also known as 'taiga', is not unique to Canada and can be found in the state of Alaska, Russia, and the Scandinavian countries. The boreal forest makes up approximately one third of the world's forest cover which equates to approximately 16.6 million square kilometres. Vegetation found within this circumpolar ecoregion is dominated by conifer species, with limited deciduous species diversity. Genera of the most common vegetation found here include: *Picea, Abies, Larix, Pinus, Populus* and *Betula* (Brandt 2009; Brandt *et al.* 2013). The growing conditions for these species include: limited nutrients, a short growing season, cold temperatures, periodic fire, and snow cover. The cold-tolerant vegetation has adapted to the many large-scale disturbances that occur periodically in the boreal forest such as fires, insect infestation and disease. Flora and fauna have evolved naturally with these disturbances over time, forming an interlinked relationship (FAO 1999). In Ontario, the boreal forest makes up approximately 46.7 million hectares, in which half of this forested area is allocated for harvest. In general, the forest products sector within the province is valued at $14 billion, making the boreal forest very economically significant for this region (MNDMF 2010; OMNR 2010).

1.2 *POPULUS BALSAMIFERA*

Balsam poplar, *Populus balsamifera* L., is a deciduous tree found in North America that has a widespread range that expands transcontinentally (Keller *et al.* 2012; Farrar 2014). In its postglacial history, the population of *P. balsamifera* migrated out of the southern refugia where its ancient population receded during the period of warming. As the population expanded it moved into regional clusters of genetic diversity: the northern, central and eastern region clusters (Keller *et al.* 2010; Keller *et al.* 2011). *Populus balsamifera* is the northernmost hardwood species in North America and its northern region cluster can be found in Alaska and Canada's northern territories. The most widespread region is the central cluster, which is located from the prairie provinces to the Great Lakes. Finally, the eastern region cluster is located in both Quebec and the Maritime provinces (Keller *et al.* 2010; Keller *et al.* 2011; Farrar 2014). When comparing these regional clusters, a significant amount of adaptive variation is observed in the different populations' ecophysiology and phenology. This can be seen in the northern subpopulation as it has specifically adapted to the shorter, drier growing seasons found within this region (Keller *et al.* 2011).

Within the boreal forest *P. balsamifera* is an ecological keystone species. It grows within riparian habitats and on moist upland soils found throughout the boreal ecoregion, but it prefers river flood plains (Zasada and Phipps 1990; Keller *et al.* 2012). This species is fast growing, short lived in comparison to other tree species (up to 200 years old) and can be considered hardy. It can grow to be 90-180 cm across and 23-30 m in height. *Populus balsamifera* produces large seed crops after reaching the ages 8-10 (Zasada and Phipps 1990; Farrar 2014). The flower clusters known as catkins (on female

clones) flower before the leaf flush between April and May, and June or July in the northernmost ranges. Pollination occurs through male flowers, which occur on the male clones. The male to female ratio for clones is typically 1:1 so pollination is often successful. After flowering, seed dispersal occurs from May to June and the last week of July in the northernmost ranges. Despite prolific seeding, germination needs to occur on suitable substrate, and the majority of germinated seeds die within several weeks. Dormancy does not occur as the seeds only remain viable for 4-5 weeks after first being dispersed. *Populus balsamifera* also uses vegetative reproduction as new stems can arise from both intact and broken roots, preformed or adventitious buds on stumps, at the base of the stem, and buried stems or branches. This species is considered an early successional species due to its low shade tolerance, rapid growth, large seed production, short life span, and self-pruning ability. As the trees reach maturity, they are often replaced with conifer species (Zasada and Phipps 1990).

As well, some individuals of *P. balsamifera* do not reach maturity due to damaging agents found naturally within the environment (Zasada and Phipps 1990). Mature trees of this species are often able to withstand mild to moderate fire intensities, however, forests in the earlier stages of succession often only burn as low-intensity fires until the later stages (often mixed balsam poplar-conifer stands) (Zasada and Phipps 1990; Gom and Rood 1999). Another damaging factor are the river areas where this species is often found. Erosion on river banks can lead to exposed roots and to the loss of area in established poplar stands (Stromberg and Patten 1992). Browsing on the stem material by species such as moose, deer, and elk is another issue. However, this does not always negatively affect the *P. balsamifera* trees as the browse can stimulate an increase

in twig biomass. Other fauna damaging agents include girdling caused by hares or rodents which can lead to the death of both saplings and smaller trees (Szaro and Pase 1983; Zasada and Phipps 1990).

Populus balsamifera trees are affected by a multitude of insect pests including: the poplar and willow wood borer, *Cryptorhynchus lapathi* L., bronze poplar borer, *Agrilus granulatus liragus* Barter and Brown, the poplar borer, *Saperda calcarata* Say, the forest tent caterpillar, *Malacosoma disstria* Hübner, satin moth, *Stilpnotia salicis* L., gray willow leaf beetle, *Pyrrhalta decora* LeConte, and aspen leaf beetle, *Chrysomela crotchi* Brown (Zasada and Phipps 1990).

There are also many fungal species that can be considered damaging agents to this species. Species that cause significant decay, most commonly in mature trees, include *Ganoderma applanatum* (Pers.) Pat. (artist's conk), *Fomes fomentarius* (L.) Fr. (tinder conk), *Armillaria mellea* (Vahl) P. Kumm. (honey mushroom), *Chondrostereum purpureum* (Pers.) Pouzar (silver leaf), and *Uncinula adunca* (Wallr.) Lév. (powdery mildew). Other less significant fungi that cause damage include *Neofabraea populi* G.E. Thomps. (causes canker), *Rhytidiella moroformis* Zalasky (causes roughing of bark), *Melampsora occidentalis* H.S. Jacks. (causes leaf rust), *Linospora tetraspora* G.E. Thompson., (causes leaf blight), and *Venturia populina* (Vuill.) Fabric. (causes leaf and twig blight) (Callan 1998). These damaging agents can be economically significant as *P. balsamifera* is a valuable commercial wood product (Zasada and Phipps 1990).

Within the wood products industry, *P. balsamifera* is specifically chosen to produce waferboard due to its mechanical qualities. Some parts of the tree including the

buds have volatile compounds which can be turned into a fragrance to be used in products such as oils. *P. balsamifera* is a widespread ecologically and economically significant boreal species, therefore noting its growth characteristics and damaging agents is important to understand (Zasada and Phipps 1990).

1.3 BIOLOGY OF GALLS

A disease can be defined as the disruption to normal life processes or abnormal physiological conditions in an organism. These diseases are caused by parasitic, living and non-parasitic agents, which include fungi, insects, and chemical toxins. The presence of these agents can be determined through visible signs and symptoms. The formation of galls within the leaves or respective petioles is considered a disease symptom from a living agent (OMNR 1991).

Cecidology is the study of plant galls, and it requires an interdisciplinary background in subjects such as: cytology, biochemistry, entomology and pathology, among others. Galls are the representation of the interaction that takes place between a host plant's tissue and another living organism. Galls are found on a wide range of host species including the majority of the plant kingdom and some branches within the kingdom of the fungi. However, these galls do not occur on every species within the plant kingdom as no insect-induced galls have ever been recorded on algae. Gall formation on a host species is typically seasonal, and usually occurs in spring. This can also occur later in the growing season during a second flush when the fruits, flowers and cones are formed (Shorthouse and Rohfritsch 1992).

During the gall formation process there are four main stages of development: initiation, growth and differentiation, maturation, and dehiscence (Shorthouse and Rohfritsch 1992). Gall-forming organisms cause reactions in their hosts through irritation or stimuli within the plant tissues that create abnormal growths. Galls are formed by a multitude of species including insects, mites, bacteria, fungi, and nematodes (Shorthouse and Rohfritsch 1992; Harper *et al.* 1997). These reactions are often the result of activities such as feeding, stinging, the laying of eggs, or the injection of toxins. Galls are unique as their general characteristics including shape and location upon the host can change between galls. Galls have been found to form on buds, stems, flowers, leaves, petioles and roots of various plant species (Harper *et al.* 1997). While they are easily noticeable on their hosts, they are merely a cosmetic issue and do not affect the overall health of their plant host (OMNR 1991).

Globally, there have been 13,000 cecidogenous (gall-inducing) insect species identified, which equates to approximately 2% of the total global insect population (Shorthouse and Rohfritsch 1992).The gall formation process when induced by insects begins with the stimulation of the host plant which causes the deformation of cells within the plant tissue in either size or quantity. After the formation of the gall, the insect is provided with an adequate food source and shelter from predation and exposure. Some gall forming insects include: aphids, wasps, sawflies, midges, beetles, moths and scales. Each gall formed has unique characteristics specific to the gall forming insect species (Harper *et al.* 1997). The gall structures formed by insect stimulation can be classified into two categories: true galls and pseudogalls. True galls have chambers and will not have openings until maturity is reached, whereas

pseudogalls do not have chambers and are permanently open (Alleyne and Morrison 1977). Galls typically associated with insects are produced by members from sap-sucking orders such as the *Asphondylia* and *Lasioptera (Diptera: Itonidinae)* which often form fleshy and soft galls (Batra and Lichtwardt 1963).

1.3.1 Fungi associated with insect galls

As galls are created through the interaction of an outside agent and plant tissue, many fungi are capable of producing galls on plants as well. Often vectored by insects, the presence of these fungus species can either be random or a transient association in others. Once inside, the gall specific fungus may have either direct or indirect effects on the plant host or the gall inducing insect species (Wilson 1995). However, many fungi typically associated with galls are often airborne, and can grow on a variety of substrata, and are ubiquitous. Typically, interactions between these fungi and their insect vectors are not codependent (Batra and Lichtwardt 1963; Wilson 1995). These fungi can be introduced into the plant gall by being carried on the external parts of the insect, and the use of specialized storage mechanisms. Some of the fungi often recorded in association with these insect-induced galls belong to the Phyla: *Zygomycota, Ascomycota, Basidiomycota, or Deuteromycota* (anamorphs of Ascomycota). The fungi recorded within these galls fall into two general categories with regards to their ecological patterns: those associated with sap-sucking insects, and those that are saprophytic (Batra and Lichtwardt 1963).

Fungi are introduced in the beginning stages of gall development where they line the inner surface of the gall with hyphae. The fungi found within these galls can be

broken down into two ecological patterns: benign saprophytes or fungal inquilines. The fungi that are considered to be inquiline will often dominate the surface area within the gall, making them the only species present (Wilson 1995). Fungal inquilines are typically associated with a specific insect species, but the fungi can often be isolated from other substrata as well. A representation of this ecological group can be seen with species of *Cladosporium* which are found in the galls formed by *Asphondylia capparidis* Rübs. on the host *Capparis spinosa* L. (Batra and Lichtwardt 1963).

The fungi that are considered to be benign saprophytes are often associated with galls that are soft and fleshy. However, these galls can be hard and woody when sclerenchyma is present in the tissues. The mycelium of these fungi, when found within the inner tissue of the gall are often sparse and non-uniform in growth. This is suggested to be the reason why several fungus species can be found within a single gall (Batra and Lichtwardt 1963). The benign saprophytes are typically not introduced into the gall tissue until the later stages of development have begun. Many mature galls will often contain a multitude of fungus species. One method of gall inoculation can be through the parasites and predators of the gall-inducing insects themselves (Batra and Lichtwardt 1963; Wilson 1995).

1.4 BIOLOGY OF APHIDS

A group of insects, in which some of the species are considered to be cecidogenous, are the aphids which are also known commonly as plant lice (Cerezke and Kusch 1991; Shorthouse and Rohfritsch 1992). These insects are considered to be serious plant pathogen vectors as they attack nearly all species of plants (Shorthouse and Rohfritsch 1992). They have the ability to stunt plant growth, induce plant galls, vector

plant viral diseases, and create deformations on the leaves, buds, and flowers of their host. Aphid species are in the family *Aphididae* and can be described as small (approximately the size of a pinhead), pear-shaped, soft-bodied, sap-sucking insects. Most species possess a pair of tube-like projections known as cornicles on their abdomen that secrete defensive fluid (Cerezke and Kusch 1991; Shorthouse and Rohfritsch 1992). Between different species, their bodies can have a large variation in colour including translucent, and various shades of green, brown, yellow, and white (Encyclopaedia Britannica 2020). The adult form of the aphid may or may not be winged depending on their stage of development, the current season, and species (Shorthouse and Rohfritsch 1992).

Most aphid species have a very unusual and complex life cycle that can vary greatly between species (Cerezke and Kusch 1991; Encyclopaedia Britannica 2020). Most aphid species will feed passively on the sap found within the phloem vessels in their host plant. Once this vessel is punctured using sucking mouthparts known as stylets, the phloem is forced into the aphid's food canal via pressure differences. Some aphid species will also feed on the sap found within the xylem vessels (Cerezke and Kusch 1991; Shorthouse and Rohfritsch 1992). Many aphid species have been observed to secrete honeydew from their anus which is composed of excess ingested sap, sugars, and waste materials. After being deposited onto a surface (*e.g.* foliage, cars, etc.), over time a black sooty mould will grow onto the honey dew deposits. Honeydew attracts many other insect species due to its sweetness (high glucose) such as flies, wasps, and ants. Some aphid species have a mutualistic association with certain ant species due to their production of honeydew, and the ants will protect the aphids from predation in

order to maintain the honeydew production (Cerezke and Kusch 1991; Encyclopaedia Britannica 2020).

Aphids have multiple natural enemies including: predatory ladybugs, hoverfly larvae, parasitic wasps, aphid midge larvae, crab spiders, lacewing larvae, and entomopathogenic fungi. However, aphids are typically found in large groups due to the nature of their reproduction (*e.g.* rapid asexual reproduction), known as parthenogenesis. Therefore, minimal to moderate predation is not always significant (Cerezke and Kusch 1991).

Approximately one in ten aphid species require multiple hosts within their life cycle (Kundu and Dixon 1995). These species practice seasonal movements between the primary and secondary host species, where the primary host is generally a woody plant and the secondary host is typically a herbaceous plant (Cerezke and Kusch. 1991; Kundu and Dixon 1995). However, many aphid species have evolved over time to require only a single herbaceous host plant that tends to be more nutritionally favourable. The transition from one host to another can also be more costly in terms of population numbers due to the predation of individuals during the transition (Kundu and Dixon 1995). The purpose of having multiple host plants has been studied by Kundu and Dixon (1995), and they provide two explanations. The first hypothesis is each stage of the life cycle has specialized individuals (particular morphology) depending on the current host, which has been built into the reproductive cycle. Therefore, this specialization prevents the aphid species whole life cycle from moving to one host (the secondary). This is a maladaptive consequence but remains due to maintenance by constraint, despite it not being favoured by natural selection (Kundu and Dixon 1995).

The second hypothesis deals with optimal host use. Aphid species have the ability to utilize the growth patterns of both the primary and secondary host species in order to compliment their population growth rates throughout the year. Aphid species transfer to the secondary host during the summer when the primary host has matured and the foliage is nutritionally poor. Within aphid species that have the multiple host adaptation, the success of their specific life cycle is dependent on the number of host transfers, the success of each transfer, and the total number of generations that have the ability to complete on the secondary host (Kundu and Dixon 1995).

Most aphid species are capable of both sexual reproduction and asexual reproduction (cyclical parthenogenesis), while others are completely asexual (obligate parthenogenesis) (Simon *et al.* 2002). Cyclical parthenogenesis predominates within the different aphid species, and is beneficial as it combines the advantages of sexual reproduction (*e.g.* the ability to generate progeny that are genetically diverse, and eliminate some deleterious mutations) with the benefits of asexual reproduction (*e.g.* high demographic increase potential). Aphid species that practice cyclical parthenogenesis are typically able to produce diapausing eggs that are cold-resistant which is a sexual benefit for those who have hosts within a colder climate (Simon *et al.* 2002; Wool 2004). A limited percentage of aphid species have evolved to lose their sexual phase and instead only use obligate parthenogenesis. A possible variation in this type of reproduction is the ability to retain the capacity for male production (Shorthouse and Rohfritsch 1992; Simon *et al.* 2002).

Only about 10% of all described aphid species have the ability to induce gall formation, excluding “pseudogall” formers and leaf rollers (Wool 2004). *Pemphigus*

betae Doane, a gall-inducing insect, has a life cycle that is considered to be a holocycle (Floate 2010; Wenninger 2011). This lifecycle begins when a gall is induced through the stimulation from one aphid individual. Galls are initiated in the spring after the nymph emerges from its overwintering egg. This is timed with the phenology of the host as galls must be formed on young, growing plant tissues (*e.g.* bud burst) (Wool 2004). After the gall is induced, the aphid reproduces parthenogenetically creating clones that are now found within the gall (Simon *et al.* 2002; Wool 2004). The individuals within the gall remain wingless until June-July when they become winged in order to disperse to the ground below the host. These individuals (crawlers) then move to the secondary host in their lifecycle and colonize the roots in order to feed, but they do not induce galls on this host. Winged adults are formed during the autumn season on the roots of the secondary host. The individuals then fly back to the primary host, where sexual males and females are produced. These individuals form mouthparts as they do not feed, and instead molt, mature, and mate. After mating, a single egg is formed by each female that has the ability to overwinter until spring for the cycle to repeat the following year (Wool 2004; Floate 2010; Wenninger 2011). This lifecycle is an example of one specific gall-inducing aphid species, but each species is unique due to many natural variations (Wool 2004).

Gall-inducing aphid species typically have the same geographic distribution as their primary host, with the exception of anholocyclic individuals which are found outside of this range. In order for a gall to be successfully induced on the host a close interaction must be achieved between the two organisms. This often requires the host choice to be specialized down to species instead of genus. Aphid species specialize even

further to the specific site the gall is induced on the host (Wool 2004; Pretorius *et al.* 2016).

Galls can be induced using multiple methods including: the injection of a cecidogenic substance from the aphid saliva into the plant tissue, injury of the plant tissue to simulate growth and division, and hormonal changes to the plant tissue induced by the aphid (Shorthouse and Rohfritsch 1992; Rose and Lindquist 1997; Wool 2004). Similar to gall placement, gall shape is also species specific as little variation is seen between individuals of the same species. Different aphid species on the same host plant will induce different gall shapes as the insect, not the plant host, is responsible for regulating the plant shape. The specific gall each species induces is considered an extended phenotype of that insect (Wool 2004). Some examples of different aphid species that induce galls varying in both structure and cycle complexity on a variety of different hosts include aphids from the genus *Pemphigus*. Members of this genus induces complex gall structures such as the twisted-spiral galls formed by *Pemphigus spirotheca* Pass. on the host *Populus*. The species *Melaphis rhois* Fitch. forms the largest aphid induced galls in North America on the host *Rhus*. These gall-inducing aphid species can also have different life cycles on multiple hosts. In the case of *Hormaphis hamamelidis* Fitch., one stage of the life cycle takes place on *Hamamelis virginiana* L. in which galls are induced on the host, and another stage occurs on *Betula* in which galls are not formed (Shorthouse and Rohfritsch 1992).

A specialization that is unique to gall-inducing aphids is the presence of sterile morphs that are called soldiers (or defenders). These soldiers display an attack

behaviour and respond to colony disturbance such as the introduction of artificial larvae or eggs, and potential predators (Wool 2004).

During the colonization of the primary host, differential herbivory can be observed. Within a sample area a single host tree can be colonized multiple times, while adjacent trees of the same species are never colonized. Explanations for this phenomenon include: tree age, genetic resistance to galling, resistance mechanisms (through hybridization), and variation in both premature abscission of galled leaves and bud burst (Wool 2004).

1.5 *PEMPHIGUS BETAE*

The genus *Pemphigus* contains over 70 described species, with 21 of these species being present in North America (Pretorius *et al*. 2016). While many species within this genus have been described there is still a high level of uncertainty in the true identity of some of these 'identified' species, and in regards to the undescribed species within the genus *Pemphigus*. Accurate identification through morphological characteristics is often difficult or impossible especially when aphids are in their subterranean morph stage on their secondary host. Through molecular analysis species can be more accurately identified using mitochondrial DNA cytochrome c oxidase subunit 1 and nuclear microsatellite flanking region sequences (Pretorius *et al*. 2016). Through these methods, species such as *P. betae* have been identified to have co-occurring genetic "types" (three in total). Without testing, these genetic types cannot be identified through morphological means, and this is significant as multiple genetic types can be observed on a single host tree. More molecular analysis studies are required to determine if these types are sibling species, and which mechanisms are utilized in order

to maintain reproductive isolation (Floate 2010; Pretorius *et al.* 2016). A study by Serikawa (2007) observed *P. betae* populations from different regions in North America including: Montana, Colorado, Idaho, Nebraska, and Alberta. These populations have been isolated and observed to have high genetic variability within populations rather than between them. High and variable levels of gene flow have also been recorded. The *P. betae* population observed in Michigan was found to be genetically distinct compared to the other populations studied. Due to its cryptic biology, more studies into the molecular analysis of the populations, co-occurring genetic types, and the general identification of *P. betae* are needed in order to create a clearer understanding of this species (Pretorius *et al.* 2016).

Pemphigus betae Doane (syn. *P. populivenae* Fitch; syn. *P. balsamiferae* Williams) commonly known as the sugarbeet root aphid belongs to the Order Hemiptera, and Family Aphididae. Their lifecycle is classified as holocyclic, as it is both complex and varied and includes an overwintering generation (produced asexually), host alteration (two hosts), and sexual and asexual generations (Harveson *et al.* 2009; Wenninger 2011; Pretorius *et al.* 2016).

Pemphigus betae has *Populus* spp. as its primary host, and has been recorded inducing galls on the foliage of Balsam poplar, *Populus balsamifera* L., black cottonwood, *Populus trichocarpa* Torr. & A. Gray ex. Hook and Narrowleaf cottonwood, *Populus angustifolia* James. Its main secondary hosts are comprised of a multitude of herbaceous species including common lambsquarters (*Chenopodium album* L.) and pigweed (*Amaranthus* spp.), beet root (*Beta vulgaris* L.), also several cultivated relatives of sugar beets such as Swiss chard (*B. vulgaris* var. *cicla* L.), spinach (*Spinacia*

oleracea L.), and alfalfa (*Medicago sativa* L.). Other possible secondary hosts that have been observed but not confirmed include green foxtail (*Setaria viridis* (L.) P. Beauv.), prostrate knotweed (*Polygonum aviculare* L.), and dock (*Rumex* spp.). The geographic range of *P. betae* is limited to the same distribution of its primary host *Populus* spp., even though its secondary host has more diverse ranges (Alleyne and Morrison 1977; Harveson *et al.* 2009; Floate 2010; Wenninger 2011; Pretorius *et al.* 2016).

In the early spring (late April to early May) *P. betae* hatches into a wingless, parthenogenetic adult female from its overwintering egg. Through feeding, a pouch-shaped gall is stimulated to form on the upper leaf surface at the base and/or the midrib on the leaf of its primary host *Populus*. These galls are classified as complex and are approximately 10mm in length and have a depth of around 5 mm (extends below the leaf surface). As galls develop, they appear to be green in colour but, after maturity many galls will turn reddish. Typically, only one gall will be formed per leaf, however, multiple galls per leaf is also not uncommon (*e.g.* five or more per leaf) (Harper 1959; Harveson *et al.* 2009; Floate 2010; Wenninger 2011; Pretorius *et al.* 2016).

After the asexual female aphid births a colony of winged aphids (summer migrants) within the gall, they remain inside until mature (Floate 2010; Wenninger 2011). Colony sizes within the gall ranges from 1-180 individuals, with multiple averages reported in different studies (41, 74 and 163 individuals) (Harper 1959; Floate 2010). Within the gall these winged aphids are often found in a woolly waxy mass (Wenninger 2011).

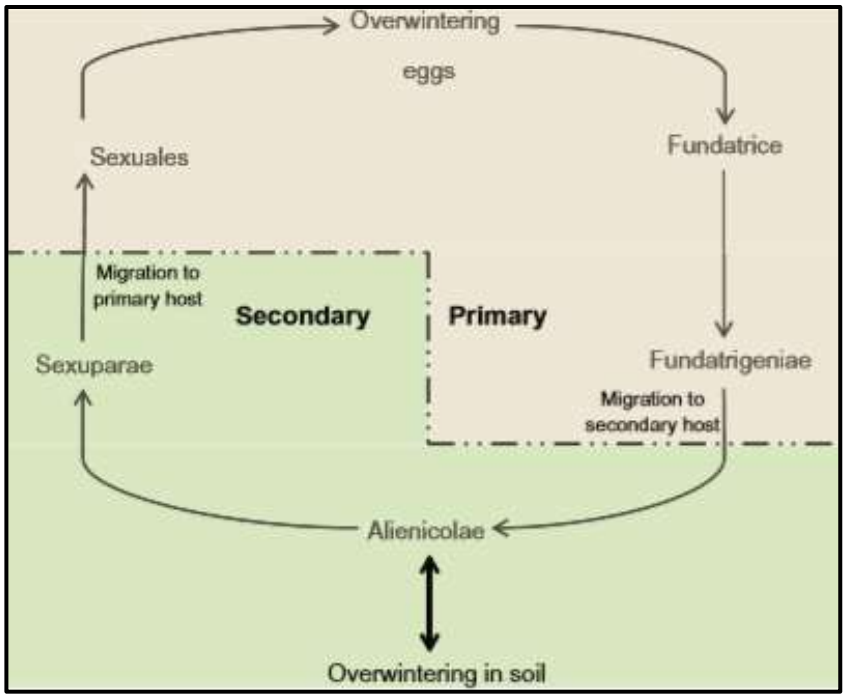
After maturity is reached in the summer season, the aphids travel to their secondary host, in a mass migration that is on occasion over a large distance. Migration

start dates vary depending on geographical factors such as latitude and elevation, but in general it occurs from mid June to mid July. Once the secondary host is reached the aphids will establish colonies on the roots (mainly the fibrous roots rather than the main storage roots) of the sugar beets (*B. vulgaris*) in the agricultural fields. This colonization can negatively impact the host through repeated sap sucking from multiple aphids as they feed. This feeding can lead to decreased nutrient and water uptake as the roots become flaccid and rubbery, severe cases lead to stress through drought, leaf yellowing (chlorosis) and wilting. Additional stress caused by additional factors can increase the overall impact of aphid colonization. Colonization is mainly limited to the roots, but abundant infestations have also been observed on the surface of the sugar beet (Harveson *et al.* 2009; Floate 2010; Wenninger 2011; Pretorius *et al*. 2016). Travel between plants is not uncommon after the host plant becomes depleted, the aphids spread typically occurs in elliptically shaped damage patterns (Alleyne and Morrison 1977). Depending on conditions such as host health and climatic factors, large colonies can develop on the roots. After migration when colonization begins, all new progeny formed for several generations (up to seven) will be wingless, born live, parthenogenetic, yellowish white, with a body shape that is broadly oval and approximately 2 mm in length (Harper 1959; Wenninger 2011; Pretorius *et al*. 2016).

Once on the secondary host each individual female aphid has an average reproduction capacity of 13.3 aphids, with optimal reproduction rates occurring at a soil temperature of 25-27^{o}C. The aphids in these generations have the ability to secrete a material that is white and waxy, which gives the colonies their distinctive white appearance. This waxy substance is believed to have the ability to repel moisture and the

honey dew that the aphids produce from their bodies. The total abundance of waxy substance on the roots of the sugar beet is often used to determine the infestation level within the field (Harveson *et al.* 2009; Floate 2010; Wenninger 2011; Pretorius *et al*. 2016).

After the population peak within the colony is reached in late August to early September, new parthenogenetic progeny that are winged and born live will be created (fall migrants). These progenies then migrate back to the primary host in order to reproduce sexually to create overwintering eggs (one per female individual) which are deposited into the bark of the tree (protection from ambient conditions). Over the winter season this egg will turn from white (due to waxy secretion) to a darker colour, before completing obligatory diapause (Harveson *et al.* 2009; Floate 2010; Wenninger 2011; Pretorius *et al*. 2016). The lifecycle of *P. betae* can be seen visually in Figure 1 (Wenninger 2011). Some populations of *P. betae* have been observed remaining in the soil through autumn in order to overwinter. These populations remain wingless and therefore have limited movement, they will often not travel between fields. This population is easily controlled through the crop rotation of non-host species (Harveson *et al.* 2009; Wenninger 2011; Pretorius *et al*. 2016).



Source: Wenninger 2011

Figure 1. The lifecycle of *Pemphigus betae*

Pemphigus betae has the ability to be controlled through biological measures such as using predatory fly species. The fly species *Thaumatomyia glabra* Meigen in its larval stage and some species of predatory ground beetles will attack the subterranean morph of *P. betae*. The fly species *Syrphus bigelowi* Curran, and *Leucopis pemphigae* Malloch predate upon the aphids when they are in the galls on the primary host. Generalist predators have been observed predating upon *P. betae,* but their overall significance on aphid population numbers is not known (Harveson *et al.* 2009; Wenninger 2011; Pretorius *et al*. 2016). Another method of biocontrol is through the use of the fungus *Entomophthora aphidis* Hoffmann which has the ability to drastically reduce aphid populations (Wenninger 2011). While these organisms have the ability to reduce population numbers, they have not been used commercially as they are not a

preferred method compared to the use of cultural practices and insecticide applications which are much less expensive. However, conventional insecticides are ineffective in their ability to reduce aphid populations on *B. vulgaris*. This is because the systemic insecticides cannot be transported downward to the root system where the aphid colonies reside. The use of these insecticides can actually reduce the predator numbers, which can lead to an increase in the aphid population (Harveson *et al.* 2009; Wenninger 2011; Pretorius *et al*. 2016).

Other methods of pest control include the use of cultural practices. Strategies including sanitation, crop rotation, planting resistant varieties (main method), managing alternate hosts (primary and alternate secondaries), and maintaining adequate soil moisture regimes to improve the health of *B. vulgaris*. Proper sanitation on farm equipment between fields is particularly important as *P. betae* has been known to travel in the contaminated soil. In regards to the management of *P. betae*, overall chemical control is not a viable management strategy, so cultural practices are currently the main method to reduce infestations. Biological control, while not commercially used, will most likely play a larger role in the prevention of economic loss in the agriculture of sugar beets in the future (Harveson *et al.* 2009; Wenninger 2011; Pretorius *et al*. 2016).

1.6 STUDY OBJECTIVE AND HYPOTHESIS

1.6.1 Objective

The purpose of this study was to make an inquiry into the diversity of the fungal communities found within the inner tissues of the galls induced by the aphid *Pemphigus betae* on the foliage of the host tree *Populus balsamifera*. Once fungi were identified, the secondary goal of this study was to determine if the fungal diversity found within the

galls sampled, with regards to their ecological patterns (*e.g*. benign saprophytes, fungal inquilines both), were consistent, from gall to gall or varied from gall to gall.

2.0 METHODS AND MATERIALS

2.1 STUDY AREA AND SAMPLE COLLECTION

All samples collected were taken from within the study area as outlined in Figure 2. The study area is located on the Lakehead University campus in Thunder Bay, Ontario and is adjacent to the university's community garden; the tree's location is outlined in white. The sample tree is a balsam poplar, *Populus balsamifera*, and it is the only tree in the study area that has been affected by the aphid induced galls. The leaf material from a single sample tree was collected, and leaves were chosen for the presence of aphid induced galls, as can be seen in Figure 3.

Source: Google Imagery, 2020

Figure 2. Location of sample area, Lakehead University Campus, Thunder Bay, Ontario

Source: Georgina Atkins

Figure 3. Leaf material collected from sample tree

Leaf material was collected on September 10th and 17th 2020, using two different methods depending on the branch height above the ground from the sample tree. Leaves that were within reach were collected by hand and placed into labeled Ziplock brand freezer bags. Leaves that were out of reach were collected using a pole pruner to cut sections off branches, and were then removed by hand and placed into a freezer bag. After the leaves were collected and placed into labelled bags they were stored in a freezer until the proceeding lab work could begin.

2.2 LAB PROCEDURE

2.2.1 Inoculation

After being removed from the freezer, each sample was soaked in 70% alcohol for 30 seconds. They were then removed and placed on a paper towel to dry. After all the alcohol had evaporated off the surface of the sample, a flame-sterilized scalpel was used to cut the galls in half. Three to four pieces from the exposed inner tissue were

removed and inoculated onto a sterile plastic 90 mm Petri dish containing 2% malt extract agar (See section 2.2.3. for recipe). The tip of a wooden-handled needle was used to apply a small quantity of antibiotics (streptomycin sulphate and Penicillin G) onto the dish to prevent bacterial contamination. The Petri dish was then sealed with Parafilm ® to prevent drying out and contamination, and labeled with the corresponding sample number (see section 2.2.4.) This was repeated until isolations from 100 galls had been made.

2.2.2. Transfers

After growth had occurred and unique fungal isolates were observed in a single sample plate, a transfer occurred. Transfers were conducted until a pure culture of each unique fungal species was obtained. Unique fungal species were removed using a flame sterilized scalpel to cut a small square from the surface of the agar containing the hyphae of the fungus. The square was then inoculated onto a new Petri dish, antibiotics were applied, and the dish was sealed with Parafilm ®. The dish was then labeled with the transfer number for each corresponding sample (See section 2.2.4).

2.2.3. 2% Malt Extract Agar Recipe Procedure

The 2% malt extract Agar recipe is as follows: malt extract (10.0g), yeast extract (0.5g), agar (7.5g) and water (500ml). Eight 1 litre flasks were filled with all the above listed ingredients. The mouths of the flasks were then wrapped in aluminum foil and the contents mixed by shaking and swirling movements. The flasks were then sterilized in an autoclave for twenty minutes at 121 degrees Celsius. Once removed from the autoclave, each flask was placed in a water bath until slightly cool. The molten agar

from each flask was then poured into sterile plastic Petri dishes (90 mm diam.) until around 100-130 dishes were filled. These dishes were left in the transfer hood for two days to reduce the condensation build up under the lids. After two days, each plate was wrapped with Parafilm ® to prevent drying out. After one week plates were checked for accidental contamination, and disposed of appropriately when found.

2.2.4. Sample labeling and numbering system

Every inoculated and transferred sample dish was labeled and given a unique identifying number. Each dish was labeled with the date of inoculation or transfer, and initials to identify ownership of the plate. When a sample dish was first inoculated with the inner gall tissue it was given the designation S#. The number associated with the specific sample dish was dependent on how many original inoculations had occurred up until that point, it was given the next number in the sequence, *e.g*. S1, S2, S3, etc. Transferred samples were given additional identifying markers (*i.e*. letters and Roman numerals) beyond the S# designation. Letters were added in sequence to the S# designation for each unique fungal isolate in the original sample dish. For reference, if three unique fungal isolates were found in S1, the transferred cultures would be labeled S1-A, S1-B and S1-C. If additional transfers were needed, Roman numerals were added to the designation. For reference, if sample S1-A had two unique fungal isolates present the newly transferred cultures would be labeled S1-A-I and S-A-II. The numbering system was not expanded on after the Roman numeral addition as it was not necessary.

2.2.5. Identification

Once a sample dish contained a pure culture it was set aside for identification. Identification of the fungal species was done using the following equipment: dissecting microscope, compound microscope, microscope slides, glass cover slips, 1% phloxine, alcohol burner, wooden handled needles, and scalpel. Dr. Hutchison was able to visually identify some samples to genus and/or species using the dissecting microscope. Other cultures were identified to genus and/or species by creating a slide from the pure culture to be identified under the compound microscope. Taxonomic literature was used to assist in the identification process. Not every culture was identified to the species level, and sterile cultures were present in some cases; each unique sterile culture was given a unique designation *i.e.* Sterile #1, etc. Once identified, the genus and/or the species was written on the lid of the sample dish as well as was entered into a master list on excel.

3.0 RESULTS

One hundred gall samples were obtained from a single *Populus balsamifera* tree that was located on Lakehead University campus, near the community garden. A complete list of all the isolates and their sample numbers is provided in Appendix I. This list includes all originals, transfers, and other observations (*e.g*. no growth).

Table 1 summarizes the different fungal taxa found within the samples and their frequency, with the isolates identified down to species when possible. Twelve isolates could not be identified as they were either sterile or lacked distinctive morphological features, but they were still recorded in Table 1. The eleven sterile samples were separated using the colour and morphology of each colony. In some cases, multiple fungal isolates were found in one gall sample, hence the total number of recorded observations (193) is over one hundred. Of the 193 isolates taken from the 100 galls, these could be allocated to 43 different taxa (See Table 1). The genera *Fusarium* and *Phoma* could not be identified down to the species level, but were instead divided by colony colour and morphological characteristics. No fungi grew out of four of the gall samples, although, two of these samples yielded bacteria (See Table 2).

Table 1. Taxa isolated from the gall samples

Genus	Species	Composition	# of galls found in	Total Frequency (%)
Alternaria	alternata	5	5	2.6
Aspergillus	sydowii	2	2	1.0
Aspergillus	ustus	1	1	0.5
Aureobasidium	pullulans	4	4	2.1
Basidiomycota	(sterile)	1	1	0.5
Chaetomium	cochliodes	1	1	0.5
Cladosporium	cladosporioides	54	54	28.0
Cladosporium	sp.	1	1	0.5
Cytospora	chrysosperma	1	1	0.5
Fusarium	#1	20	20	10.4
Fusarium	#2	1	1	0.5
Fusarium	#3	2	2	1.0
Fusarium	#4	7	7	3.6
Hadrotrichum	globiferum	1	1	0.5
Lecythophora	sp.	1	1	0.5
Metarrhizium	sp.	1	1	0.5
Nigrospora	sphaerica	2	2	1.0
Penicillum	brevicompactum	18	18	9.3
Penicillium	frequentans	1	1	0.5
Penicillium	simplicissimum	21	21	10.9
Penicillium	thomii	1	1	0.5
Phialophora	sp.	1	1	0.5
Phoma	#1	1	1	0.5
Phoma	#2	1	1	0.5
Phoma	#3	1	1	0.5
Phoma	#4	1	1	0.5
Phoma	#5	1	1	0.5
Phoma	#6	1	1	0.5
Rhinocladiella	sp.	1	1	0.5
Sterile	#1	1	1	0.5
Sterile	#2	1	1	0.5
Sterile	#3	1	1	0.5
Sterile	#4	1	1	0.5
Sterile	#5	1	1	0.5
Sterile	#6	1	1	0.5
Sterile	#7	1	1	0.5
Sterile	#8	1	1	0.5
Sterile	#9	1	1	0.5
Sterile	#10	1	1	0.5
Sterile	#11	1	1	0.5
Trichoderma	viride	1	1	0.5
Verticillium	lecanii	26	26	13.5
Yeast	sp.	1	1	0.5
	Total	193		

There were five main species that made up the majority of occurrences. The most common species isolated was *Cladosporium cladosporioides* as it had a frequency of 54% out of 100 samples. This was followed by *Verticillium lecanii* (26%), then *Penicillium simplicissimum* (21%) (See Figure 4), *Fusarium* sp. 1 (20%) and finally *Penicillium brevicompactum* (18%) (See Figure 5). The most common genus isolated was *Cladosporium* as it was found in 55% of the galls. This was followed by *Penicillium* (41%), *Fusarium* (30%) and finally *Verticillium* (26%) (See Table 3).

Table 2. Other occurrences observed in gall samples

Other Occurrences	Number #
Bacteria contamination: no growth	2
No growth observed	2

Table 3. Genera isolated from the gall samples

Genus	Composition	# of galls found in	Total Frequency (%)
Alternaria	5	5	2.6
Aspergillus	3	3	1.6
Aureobasidium	4	4	2.1
Chaetomium	1	1	0.5
Cladosporium	55	55	28.5
Cytospora	1	1	0.5
Fusarium	30	30	15.5
Hadrotrichum	1	1	0.5
Lecythophora	1	1	0.5
Metarrhizium	1	1	0.5
Nigrospora	2	2	1.0
Penicillum	41	41	21.2
Phialophora	1	1	0.5
Phoma	6	6	3.1
Rhinocladiella	1	1	0.5
Trichoderma	1	1	0.5
Verticillium	26	26	13.5
Total	180		

Figure 2. Conidiophore of *Penicillium simplicissimum*

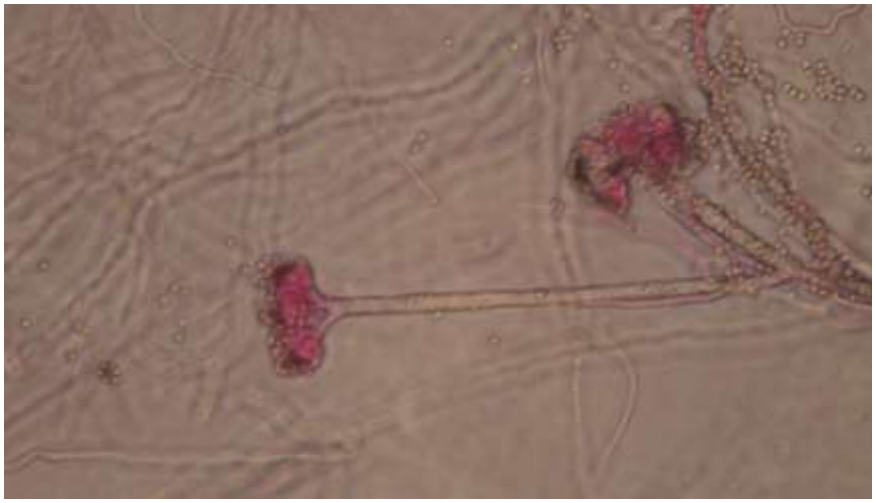


Figure 3. Conidiophore of *Penicillium brevicompactum*

As multiple fungal isolates were found in one gall for many of the samples, Table 4 displays the frequency of occurrence for the number of species found per gall. The majority of gall samples (38) only had one species, followed by two species (34), then three species (21). Only one gall had six species found in it, but three galls had a

total diversity of five. The total number of galls observed for their level of diversity was 98 as two gall samples did not have any growth occur.

Table 4. Number of Species found in the gall samples

Number of species found per gall	Frequency of occurrence
1	38
2	34
3	21
4	1
5	3
6	1
Total	98

3.1 GOOD'S HYPOTHESIS

The formula for Good's Hypothesis (Good 1953) as modified by Moore & Holleman (1974) was utilized to determine what proportion of taxa was likely isolated from the total proportion that could be expected. The sampling efficiency of this study using the modified Good's Hypothesis was:

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

Using this formula, the percentage of total biodiversity that was likely to be found in the galls isolated was:

$$1 - \left(\frac{32}{43}\right) \text{X } 100 = 25.59\ \%$$

The above formula determined that within this study 25.59 % was the percentage of taxa that were likely isolated from the total proportion that could be expected.

4.0 DISCUSSION

Within the literature, the origin of fungi within insect induced galls is often not investigated. This can be better understood through the ecological patterns associated with the fungi found within these galls. Ecological patterns can determine if the fungi were acting as pathogens (on the insect or plant host), saprophytes, fungal inquilines, parasites, phylloplane fungi, etc. The distribution and frequency of occurrence of a fungus is also important for understanding its origin within the galls. Fungi that are considered cosmopolitan and ubiquitous would be more likely to be inoculated within these galls by chance (Wilson 1995).

Presently there is not any literature that covers the fungal diversity associated with the galls induced by *Pemphigus betae*. The focus of this study was to identify the fungal communities within these galls and to understand if their ecological patterns varied or remained consistent. The ecology of each fungus can also aid in understanding how each of the fungi arrived in the gall originally and remained until the time of isolation.

4.1 DESCRIPTION AND ECOLOGICAL PATTERNS OF THE ISOLATED FUNGI

Alternaria alternata (Fr.) Keissler

Alternaria alternata is a very common cosmopolitan fungus with a wide host range. It has been isolated from a multitude of substrates, some of which include herbaceous surfaces, decaying wood, cultivated or forest soils, foodstuffs, seawater, freshwater, sewage and textiles (Domsch *et al.* 1993). *Alternaria alternata* has previously been isolated from a few of the same hosts used by *Pemphigus betae* such as

alfalfa, sugar beet and poplar (Domsch *et al.* 1993; Bashan 1994). Depending on what was grown in the Lakehead University community gardens, *P. betae* could have been a possible source of inoculation through a shared host. *Alternaria alternata* has been found in association with other insect species such as in the intestine and integuments of the *Dendrolimus sibiricus* Chetverikov caterpillar, and as a food source for springtail *Hypogastrura tullbergi* Schäffer (Domsch *et al.* 1993). Further research will need to be conducted to understand if there is a definitive association between *A. alternata* and *P. betae*. However, *A. alternata* only had a frequency of occurrence of 5% from galls, and so an association with *P. betae* is unlikely.

Aspergillus: *A. sydowii* (Bain. and Sart.) Thom and Church, *A. ustus* (Bain.) Thom and Church

Species within the genus *Aspergillus* are more commonly found in warmer climates. This genus has been isolated from a multitude of substrates including soils, compost, decaying plant matter, and stored grain, among others (Barron 1968; Domsch *et al.* 1993).

Aspergillus sydowii is distributed worldwide and is most commonly isolated as a soil fungus in a diverse variety of habitat types (Barron 1968; Domsch *et al.* 1993; Rypien 2008). *Aspergillus sydowii* has been isolated in both cultivated and forested soils, so its presence near the host tree is likely as the community garden contains cultivated soils (near forested land.). *Aspergillus sydowii* has also been observed on the rhizospheres of some cultivated plants such as corn, wheat, sorghum and soya (Domsch *et al.* 1993). More research is necessary to determine if any of the secondary hosts of *P.*

betae are also colonized by *A. sydowii*. Other substrates *A. sydowii* has been isolated from include: herbaceous substrates, decaying herbaceous matter, nests and feathers (free-living birds), foodstuffs, seawater and textiles (Domsch *et al.* 1993; Rypien 2008). *Aspergillus sydowii* is a saprophytic fungus that can occasionally act as a pathogenic fungus in humans and Caribbean gorgonian corals *Pterogorgia anceps* (Pallas) (Rypien 2008).

Aspergillus ustus is a ubiquitous soil-borne fungus that has a worldwide distribution (Domsch *et al.* 1993). As *A. ustus* is well documented, approximately 80% of the records place the tropics and subtropics as the main range for the species. However, exceptions have been observed in other ecotypes in specific localities at low frequencies. *Aspergillus ustus* is commonly found in cultivated soils, specifically in soils with crops such as wheat, beet, lupin, paddy, potatoes, and citrus (Domsch *et al.* 1993). *Aspergillus ustus* has been specifically isolated in soils containing alfalfa, which is a secondary host of *P. betae*. If alfalfa was grown in the Lakehead University community gardens, then *P. betae* could have been a possible source of inoculation through a shared host. *Aspergillus ustus* has also been isolated from forest soils under the tree species: *Pinus, Salix, Fagus* and *Tectona*, as well as from desert soil, maritime habitats, bat caves, uranium mines, sewage, and many other unusual habitats. *Aspergillus ustus* has also been found within the rhizospheres of poplar and the rhizoplane of the leaves of several vascular plants (Domsch *et al.* 1993). These occurrences could potentially be the source of inoculum for *A. ustus* within one of the galls isolated (S75-C). *Aspergillus ustus* acts as a pathogenic fungus in humans (transplant complications, and in nails), but

it has also been known to cause weight loss in poplar blocks when inoculated (Nolard *et al.* 1988; Domsch *et al.* 1993).

Aureobasidium pullulans (de Bary) Arnaud

Unlike other species in the *Aureobasidium* genus, *Aureobasidium pullulans* is a ubiquitous and cosmopolitan saprophyte (Barron 1968; Domsch *et al.* 1993). This fungus is most commonly isolated from the surface of plant leaves, in which it dominates the colonization of surface areas. While it commonly colonizes the surface of leaves as a phylloplane fungus, *A. pullulans* can also act as an invader on its hosts healthy leaf tissue. This ecological pattern explains its presence within four of the gall isolates. However, of the four isolates *A. pullulans* was only able to dominate the surface area of one of the galls completely. *Aureobasidium pullulans* has also been isolated as an endophyte from the bark, wood and twigs of multiple host genera such as *Acer, Fagus, Salix,* and *Pinus*. *Aureobasidium pullulans* has also been isolated from peat bogs, fresh water, marine sediments, sewage, and forest soils (especially under *Populus* and *Salix* stands) among other things. *Aureobasidium pullulans* is not tolerant to heat, meaning it is most commonly observed in temperate regions such as Britain, North America, Denmark, Germany, and Poland. However, this fungus has also occasionally been observed in arid and tropical environments such as Brazil, India, Egypt, Iran, Hawaii and Jamaica (Domsch *et al.* 1993).

Chaetomium cochliodes Pall.

Within the genus *Chaetomium* there are 160-180 recognized species (Ames 1961). Species within this genus are significant agents of decay on cotton and cellulosic

man-made materials. They have also been known to cause soft rot within wood (hardwoods and softwoods) and fruit rot in apples (Domsch *et al.* 1993).

Chaetomium cochliodes is a cosmopolitan species that is commonly isolated from soils. Other substrates *C. cochliodes* has been isolated from include: tobacco leaves, the crown and roots of strawberry plants, the roots of tomatoes, the dung of multiple other species (*e.g*. bat, rabbit, cow, etc.), on caterpillars, paper, wood, and the seeds of various crop plants, among others (Domsch *et al.* 1993). Some of these crop species could have been grown in the Lakehead University community gardens, making it a possible source of inoculum. This is a more plausible explanation as the outdoor spore concentration of *C. cochliodes* is not very high (Government of Québec 2016).

Cladosporium cladosporioides (Fres.) de Vries

Species within the *Cladosporium* genus are very common cosmopolitan air-borne fungi (Barron 1968; Domsch *et al.* 1993). Their sporulation is favoured in wet conditions as water droplets contribute to the transportation of their conidia. Along with one other species, *C. cladosporioides* is the most common species on plant material and in soil within the genus *Cladosporium*. *Cladosporium cladosporioides* is a very common phylloplane and soil fungus, which may explain its high frequency of occurrence (54%) in the gall isolations. In temperate regions, *C. cladosporioides* is frequently isolated in forest habitats and on the leaves of herbaceous plants (Domsch *et al.* 1993). It has been specifically isolated from the rhizosphere of poplar, but *C. cladosporioides* has been found on multiple substrates (*e.g*. stored grains, food products,

bird's nests, etc.). Notably *C. cladosporioides* can act as a suitable food source for many mite species, but an association with *P. betae* in this sense is not known at this time.

Cytospora chrysosperma (Pers.) Fr.

Cytospora chrysosperma has a large host range and is widely spread throughout the world in regions such as Asia, North and South America, Africa and Oceania (Farr *et al.* 1989). Species in the genus *Cytospora* have the potential to cause large losses in yield of economically significant crops such as peach (*Prunus persica* (L.) Batsch) crops in Iran (Bagherabadi *et al*. 2017). *Cytospora chrysosperma* is the pycnidial stage of the fungus *Valsa sordida* Nits. *Valsa sordida* is parasitic and is responsible for the syndrome "blackstem" which causes a stem canker on *Populus* among other genera. *Cytospora chrysosperma* is described as a facultative parasite that has the ability to kill the cambium of its host by spreading rapidly through the bark (Biggs *et* al. 1983). Symptoms of this pathogen include wood lesions, cankers (with and without fruiting bodies), and dieback (Bagherabadi *et al*. 2017). As *Populus* is one of the many hosts vulnerable to *C. chrysosperma,* this may explain its presence within one of the galls isolated (S55-A).

Fusarium Link ex. Fr.

The genus *Fusarium* is known for its fast-growing colonies that are pale or bright coloured (Barron 1968: Nelson and Toussoun 1968). The species within *Fusarium* are difficult to identify due to variability between isolates, and the lack of development of key features within the cultures. Most species within this genus are cosmopolitan soil fungi that have the ability to decompose cellulosic plant substrates.

There is a variation in the ecological patterns between species as some are plant parasites that cause disorders such as vascular wilt, stem and root rot, etc. (Domsch *et al.* 1993). Other patterns include host-specific pathogenic strains, saprophytic strains, and occasionally human pathogenic strains (Domsch *et al.* 1993).

Hadrotrichum globiferum Ellis and Everh.

The conidiophores of *Hadrotrichum* are both simple and pigmented, while also being produced in a dense stand on a stroma (sporodochium). These conidia are produced both singly and successively. This occurs as the blown out ends of new growing points on a sympodially extending conidiophore. Species of *Hadrotrichum* are often found as foliar parasites of higher plants, such as *Populus* (Barron 1968). This may explain the occurrence of *Hadrotrichum globiferum* in one the gall isolates (S97).

Lecythophora sp. Nannf.

There is limited information available with regards to the genus *Lecythophora*. However, species in this genus have previously been observed as being pathogenic to humans, and endophytic in certain plant species (Ahmad *et al.* 1985; Sugijanto *et al.* 2011). One study found that *Lecythophora* sp. displayed antifungal activity against specific strains of fungi (*e.g*. *Aspergillus fumigatus* Fresenius and *Candida krusei* (Castellani) Berkhout) (Sugijanto *et al.* 2011). This may explain why *Lecythophora* sp. was the only fungi found within the gall it was isolated from.

Metarrhizium sp. Sorok.

The genus *Metarrhizium* is known for containing entomopathogenic fungi, however, at this time species of *Metarrhizium* have no known association with *P. betae* (Barron 1968; Domsch *et al.* 1993; Lomer *et al.* 2001). Some species are considered significant insect pathogens that are highly specialized to the families Coleoptera, the Elateridae and Curculionidae (Domsch *et al.* 1993). In some cases, species of *Metarrhizium* have been used as a method of biocontrol to manage specific pest species such as the locust (Lomer *et al.* 2001). Species of *Metarrhizium* have also been isolated on substrates such as cysts on nematodes, cultivated soils (*e.g.* corn fields), forest soils, organic detritus, river sediments, bird nests, and healthy strawberry roots (Domsch *et al.* 1993).

Nigrospora sphaerica (Sacc.) Mason

Nigrospora sphaerica is often found in warmer regions such as the southern USA, the state of Ohio, Australia, Israel, India, and Italy among others (Domsch *et al.* 1993). While preferring the tropical and subtropical regions, *N. sphaerica* has been found around the world. *Nigrospora sphaerica* is often difficult to identify when in culture, as they tend to form smaller conidia when compared to growth on a natural substrate (Domsch *et al.* 1993). *Nigrospora sphaerica* has been found in the soil, air and on plants often as leaf pathogens. *Nigrospora sphaerica* can occur as an endophyte, but it also can have the ability to decompose cellulose (Domsch *et al.* 1993; Wright *et al.* 2008). This species has been isolated from many substrates including forest soils, cultivated soils (*e.g.* sugarcane and cotton), grasslands, sand, bat caves, mangrove

swamps, sewage, feathers and nests (free living birds), and the seeds and rhizosphere of many common crop species (Domsch *et al.* 1993). Notably *N. sphaerica* has been used as a suitable food source for many insect and mite species. An association between *N. sphaerica* and *P. betae* is not known at this time.

Penicillium: P. brevicompactum Dierckx*, P. frequentans* Westling*, P. simplicissimum* (Oudem.) Thom*, P. thomii* Maire

Species within the genus *Penicillium* are ubiquitous saprophytes, that have their conidia easily distributed through the atmosphere. *Penicillium* species are more commonly found in the soils of temperate regions (Barron 1968; Domsch *et al.* 1993).

Penicillium brevicompactum is a cosmopolitan species that is often found in soil. While being found in a variety of different soil habitat types, they do not have a high frequency of occurrence. *Penicillium brevicompactum* is more common in forest soils under mixed hardwood stands when compared to cultivated soils. Other habitats *P. brevicompactum* has been isolated from includes caves (including ice caves), sewage, uranium mine, the rhizospheres of wheat, groundnuts, and poplar, rabbit dung, bird nests, and some food products, among others (Domsch *et al.* 1993). *Penicillium brevicompactum* has been known to be antagonistic against *Fusarium sporotrichioides* Sherb. However, *P. brevicompactum* was isolated with *Fusarium* #1 & #3 in multiple galls, meaning these species may not be *F. sporotrichioides* (Domsch *et al.* 1993).

Penicillium frequentans occurs very frequently and has a worldwide distribution (Domsch *et al.* 1993). It is especially found in acidic forest soils but it can still be isolated from a diverse range of soil habitats. It is rarer in arable and cultivated soils, but

is still found in other substrates such as sewage, freshwater, organic detritus, seawater, caves (including ice caves), uranium mine, the rhizospheres of various forest and cultivated plants, rabbit dung, bird nests, and some food products, among others (Domsch *et al.* 1993). *Penicillium frequentans* is considered a phylloplane fungi on vascular plants, which may explain its presence in the galls (Domsch *et al.* 1993). *Penicillium frequentans* has been known to be antagonistic against *Fusarium sporotrichioides*. However, *P. frequentans* was isolated in gall S96 without the presence of any other fungi species, so no association with the isolated *Fusarium* species could be determined.

Penicillium simplicissimum is a cosmopolitan species that can be isolated from a range of different habitats. Some of these habitats include forest soils, floodplain communities containing *Populus* and *Salix*, grasslands, acid sand dunes, carst caves, mangrove swamp and uranium mines (Domsch *et al.* 1993). *Penicillium simplicissimum* is rarer in cultivated soils, but is considered a phylloplane fungi on vascular plants, which may explain its presence in the galls (Domsch *et al.* 1993).

Penicillium thomii is known for its characteristic production of hard pink sclerotia on the colony's surface. This species is more widely distributed in temperate regions when compared to tropical ones. *Penicillium thomii* has been isolated from a range of different habitats including forest soils, grasslands, acid sand dunes, seawater, sewage, the rhizospheres of multiple species (*e.g*. oat, tomatoes, etc.), foodstuffs, and the feathers and nests of birds (free living) (Domsch *et al.* 1993). *Penicillium thomii* has been found in association with some insect species such as in the integuments of the *Dendrolimus sibiricus* Chetverikov caterpillar, and as a food source for specific mite

species (Domsch *et al.* 1993). However, at this time *P. thomii* has no known association with *P. betae*.

Phialophora sp. Medlar

Colonies within the genus *Phialophora* are typically slow growing. While many species within this genus have been described, the delimitation of species is still very difficult (Domsch *et al.* 1993). *Phialophora* species have been observed as being parasitic to plants and occasionally humans (McColloch 1944; Barron 1968; Domsch *et al.* 1993).

Phoma Saccardo

Saccardo's taxonomic system refers to the genus *Phoma* as "pycnidia with one-celled hyaline conidia occurring on herbaceous stems" (Saccardo 1884). However, advancements in research have led to the understanding that the species in this genus are ubiquitous saprophytes that can appear and grow on a diverse range of substrata. Some substrata in this range include dead and dying herbaceous plants, woody plants, soils, water, milk, butter, paint, and paper among other things (Domsch *et al.* 1993). There are approximately 2,800 taxa of *Phoma* that have been recognized (Boerema *et al.* 2004). Most species within the *Phoma* taxa can be divided into one of two groups: pluirvorous fungi (saprobic or weakly parasitic) and specific plant pathogens (of cultivated plants). Different species within *Phoma* have a range of ecological patterns with many isolates from a range of hosts being described as saprophytes, opportunistic parasites and well-known pathogens (Boerema *et al.* 2004). As the six species of *Phoma* found within the

galls isolated were not identified down to species their specific ecological pattern cannot be determined at this time.

Species within the *Phoma* genus have spores that are colourless, unicellular, and are less then 15 micrometres (μm) (Bridge *et al.* 1990). Identification down to the species levels is difficult within this genus. Mature pycnidia within *Phoma* spp. will have their hyaline unicellular conidia arise from cells that are less differentiated and line the pycnidial cavity. This causes repetitive monopolar budding to occur, and that makes individuals difficult to identify even with the thickening that can occur on the top of the conidiogenous cells (Bridge *et al.* 1990; Boerema *et al.* 2004). Advancements in light microscopy and transmission electron microscopy have aided in identifying more details to distinguish the different species of *Phoma*. Other techniques for identification besides the use of pycnidia and conidia, is through culture identification. This is done through mycelial characteristics (*e.g*. development of chlamydospores) and biochemical properties (*e.g*. production of pigment and crystals) (Boerema *et al.* 2004).

Rhinocladiella sp. Nannf.

The genus *Rhinocladiella* is composed of mitosporic fungi that are ubiquitous, cosmopolitan and endophytic (de Hoog 1977). *Rhinocladiella* is composed of approximately 10 species, five of which are medically significant (Wagenaar *et al.* 2000). The species *Rhinocladiella mackenziei* (C.K. Campb. & Al-Hedaithy) Arzanlou & Crous is a fatal neurotropic organism that is almost exclusively found in the Middle East. These fungi are found in soils, herbaceous substrates, decaying wood, and occasionally on a variety of indoor substates (de Hoog 1977). Species in this genus produce dry spores that are wind dispersed. This form of dispersal could have led to

spores landing on the leaf surface before the gall was induced by the aphid. This may explain the occurrence of *Rhinocladiella* sp. in one of the galls that was isolated (S76-A).

Trichoderma viride Pers. ex Gray

Trichoderma viride is considered one of the most widely distributed soil fungi, as it is found in a range of habitats such as the forest, grasslands, alpine area, dunes and deserts, peat lands, sewage, freshwater and in the soil of a crater on the rim of volcanoes (Domsch *et al.* 1993). *Trichoderma viride* often occurs in cooler temperate regions, and can be isolated from other substrates besides soil. Some of these substrates include the rhizospheres of many woody plants, marine algae, rabbit dung, many common crop species (*e.g*. corn, sugarcane, rice, carrots), the mycorrhiza of certain forest trees, and stored grains (Domsch *et al.* 1993). *Trichoderma viride* has previously been isolated from the same secondary hosts (sugar beet) used by *Pemphigus betae*. Depending on what was grown in the Lakehead University community gardens, *P. betae* could have been a possible source of inoculation through a shared host. Notably *T. viride* can act as a suitable food source for certain mite species, but an association with *P. betae* in this sense is not known at this time. *Trichoderma viride* is often used as a biocontrol against other plant pathogenic fungi, due to their fungicidal activity (Li Destri Nicosia *et al.* 2015). This may explain why *T. viride* was the only fungi found within the gall it was isolated from.

Verticillium lecanii (Zimm.)

Within the genus *Verticillium* there are important plant-pathogenic species and numerous saprophytic species (Domsch *et al.* 1993; Gams and Zare 2001). However, *Verticillium lecanii* is both the most significant and common entomopathogenic species. *Verticillium lecanii* is found in all climatic regions, and specifically on coccids, aphids, thrips, Diptera, Homoptera, Hymenoptera, Lepidoptera, and mites (Domsch *et al.* 1993). While there are hundreds of entomopathogenic ascomycetes, very few are specific to aphids such as *V. lecanii*. This species of fungus is considered the most important entomopathogenic fungus of aphids, and is often used as a biocontrol for aphid pests (Montalvo *et al.* 2017). This species has also been isolated from other important substrates such as rusts and other fungi. *Verticillium lecanii* has the ability to decompose cellulose and chitin, as well as it can complete significant proteolytic activity (Domsch *et al.* 1993). Gams and Zare (2001) moved fifteen species within the *Verticillium* genus into the genus *Lecanicillium,* including *V. lecanii*. *Verticillium lecanii* is presently referred to as *Lecanicillium lecanii* (Zimm.) R. Zare & W. Gams (Kope and Leal 2005).

While there is not any specific literature documenting an association between *V. lecanii* and *P. betae,* there was a frequency of occurrence of 26% within the isolated galls. It is therefore likely that the presence of *V. lecanii* within the galls is not a random occurrence, but is in fact entomopathogenic in nature. However, further studies will be needed in order to prove this association.

5.0 CONCLUSION

This study helped to fill a gap in knowledge with regards to the fungal diversity associated with the galls induced by the aphid species *Pemphigus betae*. The study revealed that the fungal diversity found within the galls sampled displayed an ecological pattern that varied from gall to gall. Many of the species isolated in this study were ubiquitous and cosmopolitan, meaning their occurrence within the galls are of less significance. However, there were several notable species that share a possible association with *P. betae* such as *Verticillium lecanii*. Through additional research, these associations can be assessed to test their significance with regards to the diversity of fungi found within the gall isolates.

The LUSU community gardens are rented out to the public, students and facility of the university. However, no records of the species grown in the individual plots could be found. This study could be improved by observing the community garden during the summer to record which plant species are present. This could allow for the identification of secondary hosts that are being colonized by the aphid. This can add additional information to the study that was not possible to originally collect due to the start time (autumn start) of this study.

Another way to improve upon this study would be to examine the trees over the spring and summer. Once galls are first formed, samples can be collected in the spring and summer to see if there are changes in fungus diversity and composition over time.

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APPENDIX I

Sample #	Genus	Species
S1		
S1-A	Penicillium	simplicissimum
S1-B	Cladosporium	cladosporioides
S2		
S2-A	Cladosporium	cladosporioides
S2-B	Fusarium	#1
S3		
S3-A	Fusarium	#1
S4		
S4-A	Cladosporium	cladosporioides
S4-B-i	Penicillium	simplicissimum
S4-B-ii	Fusarium	#3
S4-B-iii	Aureobasidium	pullulans
S4-B-iv	Fusarium	#1
S5		
S5-A	Cladosporium	cladosporioides
S6		
S6-A	Fusarium	#1
S6-B	Cladosporium	cladosporioides
S6-C	Penicillum	brevicompactum
S7		
S7-A	Cladosporium	cladosporioides
S7-B	Fusarium	#4
S8		
S8-A	Fusarium	#1
S8-B	Penicillium	simplicissimum
S9		
S9	Fusarium	#1
S10		
S10-A	Sterile	#1
S10-B	Fusarium	#1
S11		
S11-A-i	Cladosporium	cladosporioides
S11-A-ii	Penicillum	brevicompactum
S11-A-iii	Fusarium	#1
S11-B	Penicillium	simplicissimum
S11-C	Fusarium	#3
S12		
S12-A	Alternaria	alternata
S12-B	Verticillium	lecanii
S12-C	Penicillium	simplicissimum
S13		
S13	Cladosporium	cladosporioides

S14		
S14-A-i	Fusarium	#1
S14-A-ii	Penicillum	brevicompactum
S14-B	Sterile	#2
S15		
S15-A	Fusarium	#1
S15-B	Fusarium	#4
S15-C	Cladosporium	cladosporioides
S16		
S16-A	Penicillium	simplicissimum
S16-B	Cladosporium	cladosporioides
S17		
S17-A	Verticillium	lecanii
S17-B	Penicillum	brevicompactum
S17-C	Cladosporium	cladosporioides
S18		
S18-A	Verticillium	lecanii
S18-B	Cladosporium	cladosporioides
S19		
S19-A	Cladosporium	cladosporioides
S19-B	Alternaria	alternata
S20		
S20-A	Verticillium	lecanii
S20-B	Cladosporium	cladosporioides
S21		
S21-A	Cladosporium	cladosporioides
S21-B	Verticillium	lecanii
S22		
S22-A	Penicillum	brevicompactum
S22-B	Chaetomium	cochlioides
S22-C	Alternaria	alternata
S23		
S23-A	Aureobasidium	pullulans
S23-B	Cladosporium	cladosporioides
S24		
S24	Cladosporium	cladosporioides
S25		
S25	Verticillium	lecanii
S26		
S26	Verticillium	lecanii
S27		
S27-A	Cladosporium	cladosporioides
S27-B	Verticillium	lecanii

S28		
S28-A	Penicillium	brevicompactum
S28-B	Verticillium	lecanii
S29		
S29	Basidiomycota	(sterile)
S30		
S30-A	Verticillium	lecanii
S30-B	Penicillium	brevicompactum
S30-C	Cladosporium	cladosporioides
S31		
S31-A	Verticillium	lecanii
S31-B	Cladosporium	cladosporioides
S31-C	Phoma	sp. #1
S32		
S32-A	Penicillium	brevicompactum
S32-B	Verticillium	lecanii
S33		
S33-A	Penicillium	brevicompactum
S33-B	Cladosporium	cladosporioides
S33-C	Verticillium	lecanii
S34		
S34	Fusarium	#1
S35		
S35	Fusarium	#4
S36		
S36-A	Penicillium	simplicissimum
S36-B	Fusarium	#4
S37		
S37	Penicillium	simplicissimum
S38		
S38-A	Fusarium	#4
S38-B	Fusarium	#2
S38-C	Penicillium	simplicissimum
S39		
S39-A	Penicillium	simplicissimum
S39-B	Fusarium	#1
S40		
S40-A	Fusarium	#1
S40-B	Penicillium	simplicissimum
S41		
S41-A	Penicillium	simplicissimum
S41-B	Sterile	#3
S41-C	Cladosporium	cladosporioides

S42		
S42-A	Alternaria	alternata
S42-B	Fusarium	#4
S43		
S43-A	Fusarium	#1
S43-B	Verticillium	lecanii
S44		
S44-A	Cladosporium	cladosporioides
S44-B	Verticillium	lecanii
S44-C	Penicillium	brevicompactum
S45		
S45-A	Penicillium	simplicissimum
S45-B	Verticillium	lecanii
S45-C	Fusarium	#1
S46		
S46-A	Penicillium	simplicissimum
S46-B	Verticillium	lecanii
S46-C	Penicillium	thomii
S47		
S47-A	Cladosporium	cladosporioides
S47-B	Alternaria	alternata
S47-C	Aureobasidium	pullulans
S48		
S48-A	Phoma	sp. #5
S48-B	Nigrospora	sphaerica
S48-C	Verticillium	lecanii
S49		
S49	Cladosporium	cladosporioides
S50		
S50-A	Phoma	sp. #4
S50-B	Sterile	#10
S51		
S51	Fusarium	#1
S52		
S52-A	Fusarium	#1
S52-B	Verticillium	lecanii
S53		
S53-A	Cladosporium	cladosporioides
S53-B	Verticillium	lecanii
S54		
S54-A	Cladosporium	cladosporioides
S54-B	Verticillium	lecanii

S55		
S55-A	Cytospora	chrysosperma
S55-B-i	Cladosporium	cladosporioides
S55-B-ii	Sterile	#4
S55-C	Verticillium	lecanii
S55-D	Aspergillus	sydowii
S55-E	Yeast	sp.
S56		
S56	Cladosporium	cladosporioides
S57		
S57-A	Penicillium	simplicissimum
S57-B	Cladosporium	cladosporioides
S58		
S58-A	Cladosporium	cladosporioides
S58-B	Aspergillus	sydowii
S59		
S59	Cladosporium	cladosporioides
S60		
S60-A	Cladosporium	cladosporioides
S60-B	Penicillium	brevicompactum
S60-C	Bacteria contamination	no growth
S61		
S61-A	Bacteria contamination	no growth
S61-B	Cladosporium	cladosporioides
S62		
S62	No growth observed	
S63		
S63-A	Sterile	#5
S63-B	Cladosporium	cladosporioides
S64		
S64	Nigrospora	sphaerica
S65		
S65	Cladosporium	cladosporioides
S66		
S66-A	Fusarium	#4
S66-B	Cladosporium	cladosporioides
S67		
S67-A	Verticillium	lecanii
S67-B	Cladosporium	cladosporioides
S68		
S68-A	Penicillium	simplicissimum
S68-B	Cladosporium	cladosporioides
S69		
S69	Trichoderma	viride

S70		
S70	Penicillium	simplicissimum
S71		
S71-A	Phoma	sp. #2
S71-B	Cladosporium	cladosporioides
S72		
S72-A	Cladosporium	cladosporioides
S72-B	Phialophora	sp.
S73		
S73	Penicillium	brevicompactum
S74		
S74-B	Penicillium	brevicompactum
S74-A	Cladosporium	cladosporiorides
S75		
S75-A	Penicillium	brevicompactum
S75-B	Verticillium	lecanii
S75-C	Aspergillus	ustus
S76		
S76-A	Rhinocladiella	sp.
S76-B	Penicillium	simplicissimum
S76-C	Penicillium	brevicompactum
S77		
S77-A	Fusarium	#1
S77-B	Cladosporium	cladosporioides
S78		
S78-A	Cladosporium	cladosporioides
S78-B	Verticillium	lecanii
S78-C	Penicillium	brevicompactum
S78-D	Fusarium	#1
S78-E	Penicillium	simplicissimum
S79		
S79-A	Penicillium	brevicompactum
S79-B	Cladosporium	cladosporioides
S79-C	Fusarium	#1
S79-D	Verticillium	lecanii
S80		
S80-A	Penicillium	brevicompactum
S80-B	Cladosporium	cladosporioides
S80-C	Sterile	#6
S81		
S81	Penicillium	simplicissimum

S82		
S82-A	Phoma	sp. #3
S82-B	Cladosporium	cladosporioides
S82-C	Sterile	#9
S83		
S83	Penicillium	simplicissimum
S84		
S84	Lecythophora	sp.
S85		
S85	Cladosporium	cladosporioides
S86		
S86	Cladosporium	cladosporioides
S87		
S87	Cladosporium	cladosporioides
S88		
S88	Aureobasidium	pullulans
S89		
S89	Cladosporium	cladosporioides
S90		
S90	Sterile	#7
S91		
S91	Cladosporium	cladosporioides
S92		
S92	Cladosporium	cladosporioides
S93		
S93-A	Phoma	sp. #6
S93-B	Sterile	#11
S94		
S94	No growth observed	
S95		
S95	Cladosporium	cladosporonides
S96		
S96	Penicillium	frequentans
S97		
S97	Hadrotrichum	globiferum
S98		
S98	Metarrhizium	sp.
S99		
S99	Cladosporium	sp.
S100		
S100	Sterile	#8