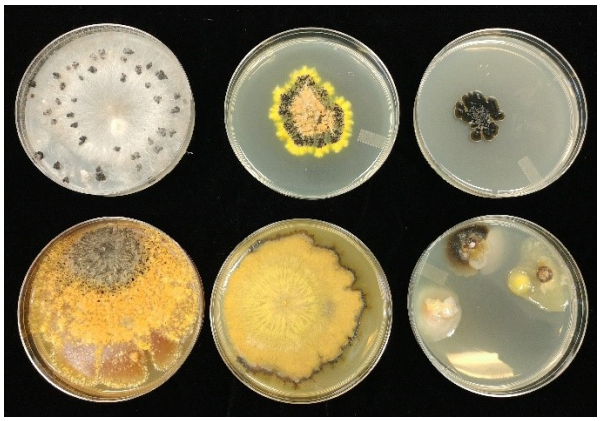
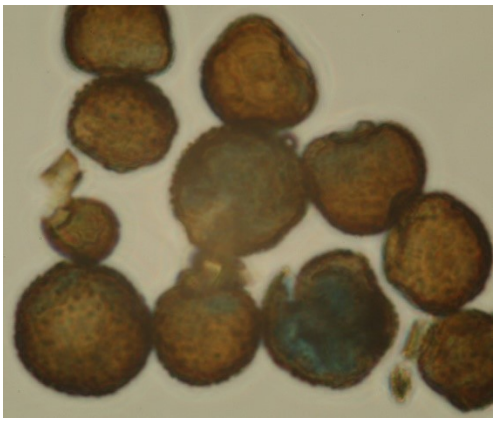
FUNGI ASSOCIATED WITH THE EMERALD ASH BORER ON URBAN GREEN ASH

By

Erin Kielt





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FUNGI ASSOCIATED WITH THE EMERALD ASH BORER ON URBAN GREEN ASH

By

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of the Requirements for the Degree of

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Major Advisor

Second Reader

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ABSTRACT

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Keywords: *Agrilus planipennis*, emerald ash borer, *Fraxinus pennsylvanica*, green ash, insect-fungal associations, urban forest.

The emerald ash borer (*Agrilus planipennis*) is an invasive beetle recently introduced from Asia. It has quickly spread throughout much of eastern North America causing death of millions of ash (*Fraxinus* spp.) in both natural forests and in urban landscapes. Since 2016, this insect has been found in Thunder Bay, where approximately 25% of the urban street trees are ash. The larvae produce serpentine galleries beneath the bark which girdle the tree within a few years. It has always been assumed that death of the ash is due to this girdling and no studies to date have investigated fungi associated with the emerald ash borer. This study attempts to investigate whether such fungal associations exist, and whether the fungi, if present, are involved in the decline of the trees. During the autumn a total of three green ash (*Fraxinus pennsylvanica*) infested with emerald ash borer were removed from a local park in Thunder Bay and cut into small logs, bagged and brought to Lakehead University. Isolations were made directly from galleries, and from larval frass found in these galleries. In addition, washings were made from larvae when these were found. A total of 178 fungal isolates were obtained, including species of *Cytospora* (26.9%), species of *Phoma* (10.6%), and species of *Cladosporium* (9.0%). All fungi found were either typical inhabitants of woody plants or else are cosmopolitan in their substrate preference. None of the species found are thought to be closely associated with the emerald ash borer, but rather have an accidental association with the insect. Further studies are needed to verify this finding.

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INTRODUCTION

The urban forest has the potential to make positive contributions to a community through a wide range of benefits (McPherson and Simpson 2002). When individual trees are planted to help mitigate environmental degradation, vast social, economic and environmental advantages can be achieved. By modifying microclimates, urban forests can contribute to the health and wealth of a community, improve the comfort of humans and lower energy costs of cooling a house (Miller *et al.* 2015). The urban forest also plays a key role in reducing the rate and volume of flooding due to storm water, resulting in less money spent by municipalities for wastewater handling (Dwyer *et al.* 1992). Trees provide habitat for wildlife that would otherwise be absent in an urban landscape (Dwyer *et al.* 1992). Trees intake oxides and small particles caused by pollution and produce oxygen resulting in an improvement in air quality (Wolf 1998). In addition, urban street trees sequester large amounts of carbon, which helps reduce the levels of atmospheric carbon dioxide (Nowak and Crane 2002).

Trees in an urban landscape make the urban environment a more pleasant place to live and work. The urban forest provides sites for recreation, which positively affects both physical and mental health (Dwyer *et al.* 1992). One study found that the presence of a forest canopy in an urban setting was linked to lower rates of obesity for citizens living the neighbourhood (Pereira1 *et al.* 2013). Other effects of a forest canopy are improving worker attitudes on the job and decreasing feelings such as fatigue, anger, depression and anxiety. These physical and mental affects are important for happiness within a community (Tyrväinen 2005).

Trees are resources that appreciate over time, increasing their potential for benefits as they get larger (McPherson 2003). This reveals how important the health of a tree is in an urban setting. A healthy tree will maximize its growth potential, resulting in an increase in its benefit producing ability and therefore an escalation in the positive contribution to a community (Miller *et al*. 2015).

There are varieties of abiotic and biotic stresses that may inhibit a tree from growing to its full potential in an urban landscape. Abiotic stresses include soil compaction, limited soil volume, salinity and lower air quality (Alvey 2006). Biotic stresses are dependent on the respective tree species and may include insect, pathogen or human vandalism damage. Trees being subject to these stresses leave few species capable of successfully growing in urban environments.

GREEN ASH

One genus that is capable of growing very well in an urban environment is the ash tree (*Fraxinus* spp.) (Burns and Honkala 1990). Ash trees are a significant portion of the urban forest in Thunder Bay. It is the most common city street tree, making up about 29% of the entire composition (DRG 2011). Green ash (*Fraxinus pennsylvanica* Marsh.) is the most widely distributed ash tree in North America (Burns and Hokala 1990) and it is the most common species of ash planted in Thunder Bay (DRG 2011). While green ash trees have the potential of growing to over 50 years and up to 15 meters, most do not live that long in Thunder Bay because they are currently subject to a variety of stresses.

A common problem with green ash is ash anthracnose, which is produced by the causal agent *Gnomoniella fraxini* Redlin and Stack. Leaves of the ash trees become distorted with small to large blotches of necrosis. Severe infections can result in significant defoliation and branches may be cankered and die. Several years of this disease can cause ash trees to decline and become susceptible to other pathogens or pests (GOC 2015). Ash anthracnose is a common disease infecting ash trees in Thunder Bay, but not causing mortality (DRG 2011).

One pest which has recently arrived in Thunder Bay, and is threatening green ash, is the emerald ash borer (*Agrilus planipennis* Fairemain). An ash tree could be subject to minimal abiotic stresses, be perfectly healthy and still be infested and killed by the emerald ash borer. The emerald ash borer (EAB) is an invasive insect from Asia that has devastated both urban and natural populations of ash trees in Eastern North America. So far it has infected five species of ash trees; green ash, white ash, black ash, pumpkin ash and blue ash. Ash trees have been widely planted in municipalities throughout North America and these trees are all susceptible to EAB (NRC 2016b). Notable cities which it has affected are Toronto, Ottawa and Windsor. It has killed tens of millions of ash trees and is continuing to spread to new areas in Canada and the United States. The economic impacts are tragic. Thunder Bay alone has agreed to spend $6.3 million to manage for EAB (Baxter and Thompson 2017). The Canadian Forest Service estimates that Canadian municipalities will spend over $2 billion throughout a 30-year period managing for EAB (NRC 2016a).

Adult beetles feed on the foliage of ash trees, but that has little effect on the health of the tree. The ash tree dies by the larvae of the beetle feeding on phloem in the cambium,

between the bark and the sapwood. While they feed, they create serpentine galleries (Figure 1). These galleries interrupt the tree's transportation of nutrients throughout the tree, and water up from the roots. This eventually girdles the tree in approximately 1-3 years, depending on how many larvae are creating galleries beneath the bark of the tree (NRC 2016b).

Figure 1. Serpentine gallery chewed by larvae of EAB

Emerald ash borer was initially found in Detroit, Michigan, USA in 2002 and was likely introduced through shipping containers containing solid wood pallets and crating. Since 1990, at least 10 non-indigenous forest insects associated with solid wood materials (in addition to EAB) have been discovered in the USA or Canada (Poland and McCullough 2006). This is an enormous concern, as non-indigenous forest insects will likely find a suitable host within the same genus in North America that they infected in their indigenous land. The insect is capable of becoming established in North America and can spread, potentially devastating forest and urban landscapes. The EAB has accomplished this, as have other insects in the past, for example the Asian longhorned beetle (Poland and McCullough 2006).

In Asia, indigenous species of Asian ash trees are the only host to the EAB (MES 2002). Due to coevolutionary relationships between Asian ash species and the EAB,

Asian ash trees are more resistant to the beetle than North American species of ash. In Asia, EAB mostly attacks ash trees growing in an open space, or ash trees along a forest's edge. Entire stands are only killed during cyclic outbreaks. (Rebek *et al.* 2007)

LIFE CYCLE OF THE EMERALD ASH BORER

The lifecycle of the EAB starts with adults emerging by chewing a distinct 'D' shaped exit hole through the bark of the tree (NRC 2016b). A 'D' shaped exit hole from a sample included in this study is illustrated in Figure 2. The adult feeds on ash foliage for approximately two weeks and then mate. The females lay eggs in bark crevices or under bark scales of the tree. Larvae emerge from the disk-shaped eggs after one to two weeks of incubation. The new larva tunnels directly into the bark until it reaches the interface of the bark and the wood. Here it starts feeding and excavates a distinct serpentine gallery (Figure 1). The larva goes through five molts in the gallery and then chews another tunnel into either the wood or the bark to pupate. The larva doubles over becoming a J-shaped prepupa to overwinter in. This position of the larvae is demonstrated in Figure 3. Approximately 20% of larvae do not reach maturity in the 1st summer and require a 2nd winter to complete development. In the spring, prepupae transform into pupae and eventually turn into adults in these chambers (NRC 2016b).

Figure 2. D-shaped exit hole

Figure 3. J-shaped prepupa

INSECT-FUNGAL ASSOCIATIONS

Insect-fungal relationships that negatively affect trees occur around the globe. They are important to study because their damage can have immense economic and environmental implications. There is a vast variety of insect-fungal associations. Many large groups of insects owe their success to fungi as a food source, and the fungi benefit from dispersal of spores and providing habitat (Wheeler and Blackwell 1984). The number of associations that have existed throughout history and evolution are essentially innumerable. Large outbreaks of insect-fungal associations that have occurred in the past include the mountain pine beetle and associated blue stain fungi, and Dutch elm disease involving the vascular wilt pathogen *Ophiostoma ulmi* (Buisman) Melin & Nannefeldt and bark beetles. It is important to understand the role of both the fungi and the insect to effectively control the outbreak.

In the case of the mountain pine beetle, it is dependent on blue-stain fungi to successfully colonize lodgepole pines trees. The fungi aid in overwhelming the host

tree's defences (Raffa and Berryman 1983) and provide nutrition for the larvae throughout the beetle's life cycle (Adams and Six 2007). Two species of fungi that are commonly associated with the mountain pine beetle are *Ophiostoma clavigerum* (Rob.-Jeffr. & R.W. Davidson) Harrington and *Ophiostoma montium* (Rumbold) von Arx (Lee *et al.* 2006). The adult Mountain pine beetle transports spores of blue-stain fungi in a specialized sac known as a mycangium. When the adult beetle chews through the bark of a new host tree and lays its eggs, the spores are inoculated into the new host tree (Safranyik *et al.* 2010). While the blue-stained wood from lodgepole pine trees has been sold as a value-added wood product in North America, the presence of the fungi affects some of the physical properties of the wood, decreasing the potential profitability from lodgepole pine trees (Safranyik and Wilson 2006). The fungus responsible for Dutch elm disease is vectored by elm bark beetles, the primary species being *Scolytus multistriatus*. The causal agents are *Ophiostoma ulmi* and the more lethal strain *Ophiostoma novo-ulmi* Brasier. Starting in the 1930s, this insect-fungal association began overwhelming forest and urban elm trees across North America (McLeod *et al.* 2005). More notably the effects were seen in urban landscapes, as elm trees were commonly used as street trees. Both of these insect-fungal associations are responsible for the death of millions of trees across North America. In current times, it is the emerald ash borer that is overwhelming urban landscapes, as ash was planted to replace a lot of the elm trees that were lost to Dutch elm disease (Poland and McCullough 2006).

OBJECTIVE

The emerald ash borer has already wreaked havoc in many cities and is continuing to spread throughout Eastern North America. However, to my knowledge, there appears to be a lack of research conducted regarding the fungal communities present in the galleries of infected ash trees. Knowing the species of wood-inhabiting fungi present would help determine if there is an insect-fungal association. This thesis would not prove that there is or is not a definite relationship present, but would examine the potential for an insect-fungal association to exist with the emerald ash borer.

METHODS AND MATERIALS

On October 17th, 2017, three ash trees infested with the emerald ash borer were cut down. The trees were located in the small park adjacent to the corner of Third Avenue and High Street, next to the Thunder Bay Transit Garage in Thunder Bay, Ontario. Figure 4 shows the location of this park as indicated with the red diamond. The trees were discovered to be infested by the EAB first by the Canadian Food Inspection Agency. All three trees appeared outwardly healthy; only upon inspection and removing the bark was it known the trees were infected by the EAB. The trees were cut by employees of Rutter Urban Forestry who were contracted out by the City of Thunder Bay. They cut sections of the trunks for use in this thesis and to the Ontario Ministry of Natural Resources and Forestry (OMNRF). The OMNRF required samples including the base of the stem for further research. Therefore samples included in this study were limited to the upper portion of the trunk of the ash trees. Samples were bagged and labelled and were immediately taken to Lakehead University's campus to be stored in the forestry freezer.

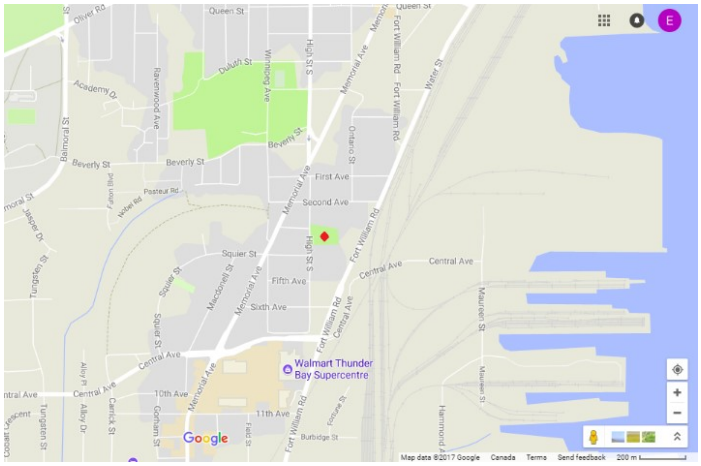

Figure 4. Park containing the three ash trees used in this study

Photos of tree 1, tree 2 and tree 3 are illustrated in figures 5, 6 and 7, respectively. The samples of logs used in this study did not possess evidence of damage by any other cause, other than by the emerald ash borer. Therefore, fungi isolated from the galleries, frass and larvae could be associated with the emerald ash borer, as opposed to have infected the tree through other wounds.

Figure 5. Tree 1

Figure 6. Tree 2

Figure 7. Tree 3

For isolation, each sample was taken out of the freezer the night before they were worked on to allow time to thaw. This made it a lot easier for the bark to separate from the cambium. Since the larvae of emerald ash borer feed on the cambium, that is where the larvae, frass and galleries were located. The bark was chiseled off each ash sample using a hammer and chisel, which were sterilized with 70% alcohol. Figure 8 is a photo of one sample of ash with the bark partially peeled off. For one sample in which the bark was very thick, a draw-knife was used to peel off the bark, layer by layer until galleries were exposed. This tool was also sterilized with 70% alcohol. The bark was a lot thicker than the rest of the samples because it was cut from lower down the stem. The first isolations were completed on October 31st, 2017. Isolations were placed on modified 2% malt extract agar, which is a standard medium used for wood-inhabiting fungi. The medium was comprised of 20g malt extract, 1g yeast extract, 15g agar and 1000 mL of distilled water, all mixed in Erlenmeyer flasks. The flasks were placed in the lab's autoclave for 1 hour at 121 degrees Celsius and 1.7kg/cm^2 chamber pressure. Once removed from the autoclave the flasks containing the molten medium were placed in a water bath set at 45 degrees Celsius until the medium was cool enough to handle. The medium was then distributed into sterile plastic Petri dishes (90 mm diameter) in a transfer hood previously sterilized with 70% alcohol. The dishes were left overnight to allow the agar to harden and allow condensation to dissipate inside the dish. The plates were then wrapped with parafilm and stored to prevent drying out over the next few months until needed.

Figure 8. A sample of ash after some of the bark was been chiseled off

Prior to the isolations from the samples, the table counter was sterilized with 70% ethanol and tools used were flamed to kill any fungal or bacterial spores that may be present. Isolations were made from a variety of areas of the sample of ash trees. A code system was used to record the isolations and labelled directly on the Petri dish. 'T' stood for tree (1-3), and 'S' for sample number from the tree (1-6). The last letter from the code was either 'F' for frass, 'G' for galleries or 'L' for larvae. The last letter was dependent on which part of the log isolations were made from. Figure 9 and 10 show examples of Petri dishes with the code and date written on them. Antibiotics were added to each Petri dish to reduce the number of bacteria growing from the isolations, or any bacteria from the air contaminating the agar dish. Antibiotics were comprised of a mixture of streptomycin sulphate and penicillin-G, which were aseptically added to the agar surface as a small pinch placed at the tip of a sterile needle. When isolating from larvae, each larva was placed in a 2mL sterile plastic centrifuge tube containing water and the tube was shaken to remove spores that were present on the larva. That water was poured onto the agar surface in the Petri dishes. After the larval washing, the larva was stored in the plastic centrifuge tube with 70% ethanol. For each frass sample, 4

clumps of frass were placed, evenly spaced, onto the agar surface of a Petri dish. When isolating from the galleries, a sterilized Q-tip™ was used to remove any existing frass from the gallery. Most frass was removed, but some may have remained present on the wood chips. Next, a flame sterilized scalpel blade was used to cut four chips from the gallery, then placed, equally spaced, on the agar surface in the Petri dish. After each isolation from a gallery, frass or larva the dish was re-wrapped with parafilm and stored in an incubator, in the dark at 20 degrees Celsius.

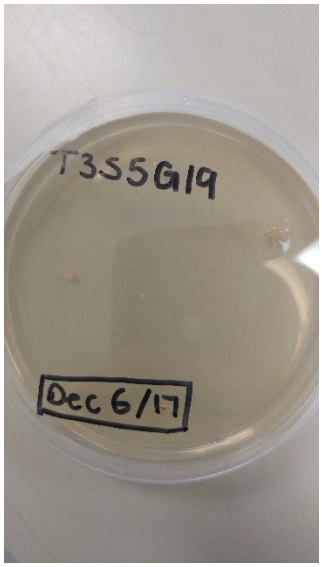

Figure 9. Agar dish with code and date of isolation

Figure 10. Isolation from gallery of tree 3

After allowing time for the fungal colonies in the Petri dishes to grow out (three to six weeks) in the incubator, individual colonies were transferred to fresh Petri dishes. The same code was used in the transfers, with the addition a letter of the alphabet (i.e. ‘a’) depending on how many different colonies grew out of a single isolation. This allowed for the colony to grow as a pure culture on the agar in hopes of it sporulating. If fruiting bodies or other sporulating structures were present, identification of the fungi

could be completed. Glass microscope slides were prepared for each identification using 1% Phloxine (in some cases Melzer's solution) and examined through a compound microscope for distinguishable morphological features. Most commonly, spores were used to confirm the identification of the fungus. The fungi were identified to genus, and when possible to species. If a fungus was not producing distinct morphological features, the Petri dishes were placed under fluorescent lights in hopes of stimulating production of fruiting bodies and/or spores.

Once all of the cultures could be identified down to genus and/or species, a modification of Good's Hypothesis (Good 1953) was used to determine if enough samples had been isolated from the logs (Moore and Holdeman 1974). Good's Hypothesis tests the sampling efficiency of the experiment. The calculation divides the number of species that were isolated once, by the total number of fungi that were isolated. The more species that only occurred one time, decreased the sampling efficiency. The results from Good's Hypothesis were used to determine if more samples should have been taken from the logs.

RESULTS

A total of 178 cultures of fungi were isolated from the three green ash trees that were utilized in this study. A list of all isolations organized by tree and isolation code is included in the Appendix. A summarization of the taxa at the genus level, and their frequency is included in Table 1. Out of the 178 isolates obtained, 143 were allocated to 24 different genera or recognizable taxa. A further 25 isolates could not be identified and are also summarized in Table 1. These latter fungi were either sterile, or lacked distinctive morphological features which prevented them from being identified. Nineteen sterile isolates were divided up into eight groups based on colour and morphology of the colonies and are listed as Sterile sp. 1 – Sterile sp. 8 in the Appendix. Several of the isolates were grouped together as "black unknown", as these cultures possessed similar features, but were not distinct enough to name down to a genus. In addition, two isolates were listed as unknown Hymenomycetes. These decay fungi could only be identified based on their fruiting bodies.

The most common genus isolated was *Cytospora*. Isolates of this genus were found in all three trees and made up 26.9% of the total (48 out of 178) (Figure 11). The species of *Cytospora* were not determined but based on morphology of the colonies, three different species were recognized and are listed as *Cytospora* sp. 1, *Cytospora* sp. 2, and *Cytospora* sp. 3 in the Appendix. The colonies observed were varying shades of orange, with dark coloured pycnidia (asexual fruiting bodies) scattered over the surface of the colonies. From these fruiting bodies, conidia (asexual spores) were produced in sticky masses that were colourless, one-celled and sausage-shaped (Figure 12). For all three species, the size of the conidia were very small, approximately 1.5 x 4 μm.

Table 1. Common genera of fungi isolated from the three infested trees

Genus	Composition	Frequency
Cytospora	48	26.9%
Phoma	19	10.7%
Cladosporium	16	9.0%
Penicillium	14	7.9%
Pycnidia-forming	8	4.5%
Lecythophora	7	3.9%
Coniothyrium	6	3.4%
Mucor	6	3.4%
Acremonium	4	2.2%
Epicoccum	5	2.8%
Stigmina	4	2.2%
Alternaria	3	1.7%
Aureobasidium	2	1.1%
Botrytis	1	0.6%
Camarosporium	1	0.6%
Exophiala	1	0.6%
Libertella	1	0.6%
Microsphaeropsis	1	0.6%
Pithomyces	1	0.6%
Rhinocladiella	1	0.6%
Rhizomucor	1	0.6%
Sporodochium-forming	1	0.6%
Thysanophora	1	0.6%
Trichoderma	1	0.6%
Sterile	19	10.7%
Black unknown	4	2.2%
Unknown Hymenomycete	2	1.1%

The next most common genus isolated was *Phoma* at 10.6%. None of the *Phoma* isolates were identified to species, as *Phoma* has a simple morphology, consisting of flask-shaped fruiting bodies called pycnidia which produce one-celled colourless spores (Domsch *et al.* 1980). Over 2,000 species have been described and no modern taxonomic monograph exists for their identification (Sutton 1980). However, six different species were recognized based on morphology of cultures, and spore characteristics and are listed in the Appendix as *Phoma* sp.1 to *Phoma* sp.6.

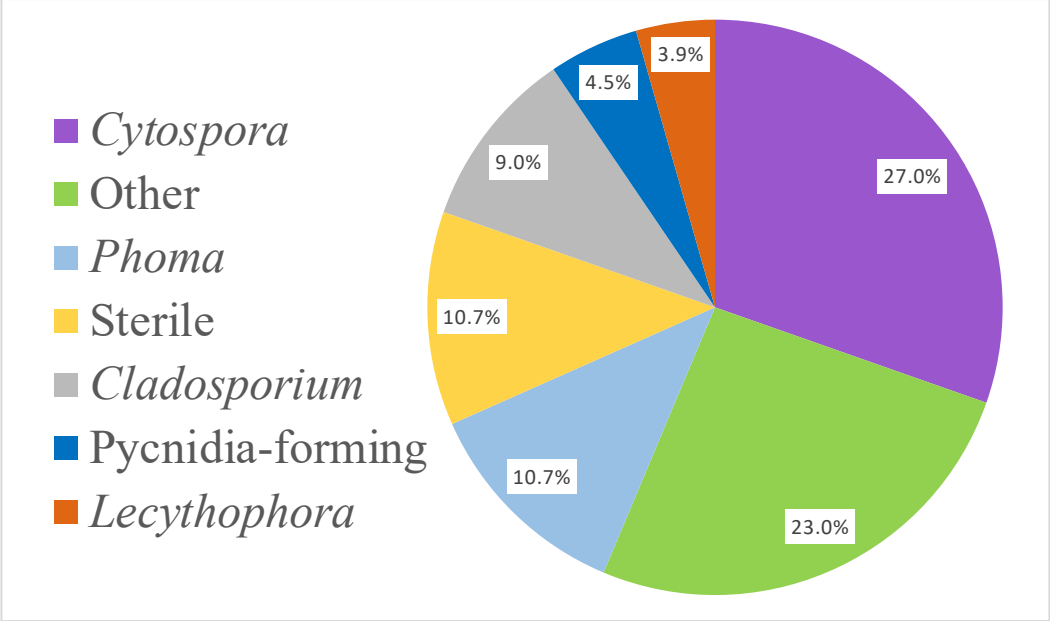

Figure 11. Composition of common genera

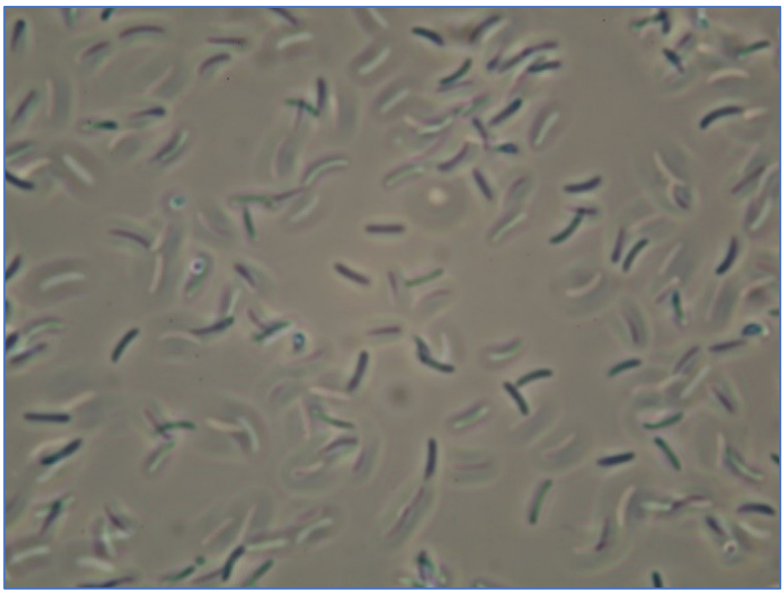

Figure 12. Conidia of *Cytospora* sp.

The common and ubiquitous mould genera *Cladosporium*, *Penicillium*, and *Epicoccum* provided 9.0%, 7.8%, and 2.8% of the isolates, respectively. Most of the remaining isolates were found fewer than a few times each, or else only once. Two taxa that were distinctive but could not be identified to genus were listed as pycnidium-

forming and sporodochium-forming, based on the type of asexual reproductive structures they produced, and represented 4.5% and 0.5% of the total. Interesting species that were found include *Camarosporium orni* Hennings (Figure 13) and an unidentified *Stigmina* (Figure 14).

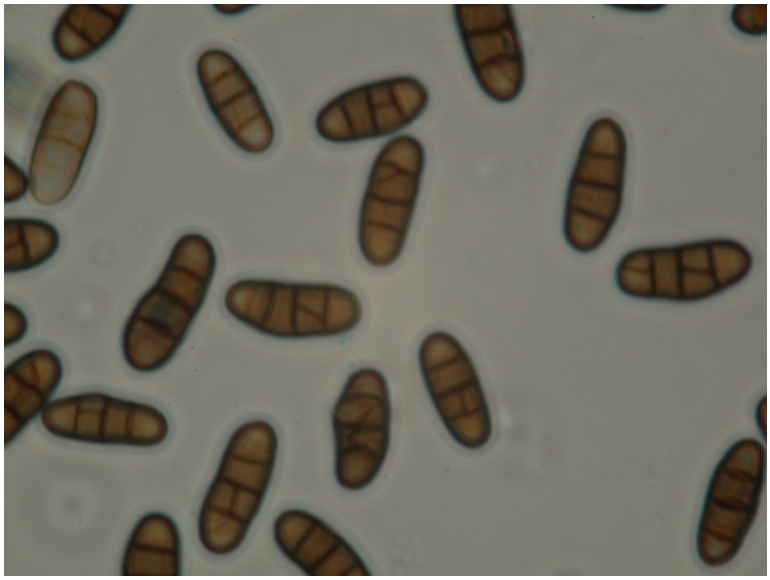

Figure 13. Conidia of *Camarosporium orni*

Figure 14. Conidia of *Stigmina* sp.

DISCUSSION

There has not been any published papers on fungal associations of emerald ash borer. This is quite a surprise since the insect has caused significant impacts to municipalities across North America. One would think scientists would be curious about all aspects of the insect, in case any new knowledge could contribute to its management. Since there is an innumerable amount of insect-fungal associations, not all potential relationships have been studied to date. However, insects that are widespread, or have caused significant impacts to industries or municipalities have been investigated for associated fungi, such as the mountain pine beetle and elm bark beetles. Their associations with a fungus or a few species of fungi have been confirmed by the repeated presence of the fungus/fungi when isolating fungi from an infected tree. A study by Lee *et al*. (2006) found that three species of *Ophiostoma* (which cause blue-staining) made up 92% of the fungal isolations from the galleries and wood of lodgepole pine that was infested with the mountain pine beetle. The other 8% was comprised of *Entomocorticium* sp., *Ambrosiella* sp. and 2 species of *Leptographium* (Lee *et al.* 2006).

In the study by Lee *et al.* (2006), there was also a consistent and repeated presence of a genus of wood-decaying fungi (*Entomocorticium* sp.). In the present study, there were only two species of unidentifiable wood decaying fungi (listed under species codes T3S3G2a and T3S3G6c in the Appendix). This suggests that no wood-decay fungi were vectored consistently by the emerald ash borer.

Species in the genus *Cytospora* were the most common isolates in this study comprising 26.9% of the composition. *Cytospora* species are generally opportunistic canker-causing fungi (Sinclair *et al.* 1987) usually infecting trees that have been subject

to other stresses such as drought, heat, winter damage, mechanical injuries, poor nutrition, insect damage or other diseases. However, there was no observable damage to the ash samples used in this study. The high frequency of *Cytospora* could suggest that this genus of fungi is benefitting from the emerald ash borer colonizing the ash trees. However, it is unlikely that a strict insect-fungal relationship between *Cytospora* and the emerald ash borer exists because species of *Cytospora* are very widespread (Farr *et al*. 1989) and inhabit a wider geographic range than the emerald ash borer. *Cytospora* species can exist as harmless endophytes in trees until the tree is weakened (Sinclair *et al*. 1987). Perhaps the high frequency of *Cytospora* occurred as the tree began to decline due to the EAB presence. As the tree was weakened by the EAB, the presence of *Cytospora* could have increased, occupying a larger space in the tree. However, the biological role of *Cytospora* species in ash trees is not clearly understood, and fungi associated with the emerald ash borer have not been researched prior to this study, so it is inconclusive. Kowalski *et al*. (2016) and Bakys *et al*. (2008) found low numbers of *Cytospora* in healthy shoots of European ash (*Fraxinus excelsior* L.). Therefore, before a conclusion could be made, more research must be completed regarding the ecology of *Cytospora* and the emerald ash borer in green ash trees.

Phoma was also commonly isolated in this study, covering 10.6% of the composition. Since it is such a common fungus to inhabit woody plants (Farr *et al*. 1989), it is not surprising that it was found in all three of the ash trees used in this study. Species of *Phoma* tend to be opportunistic pathogens that commonly form cankers on the host's tissues, but none were present on the ash samples. The ash appeared to be outwardly healthy. Perhaps if the trees were not cut down and the health of the trees

started to decline due to the EAB, cankers would be formed by the *Phoma* species present.

Species of *Penicillium* were commonly isolated in the study. *Penicillium* is a dry-spored genus of ubiquitous and cosmopolitan moulds (Domsch *et al*. 1989). In trees, it is frequently found to colonize sapwood by growing through the ray cells and exploiting the nutrients present (Garcia and Morrell 1999). However, little is known about *Penicillium*'s relationship to beetle biology (Kerrigan and Rogers 2003).

Cladosporium, *Epicoccum*, *Mucor*, *Alternaria*, *Aureobasidium*, and *Pithomyces* being present is not surprising since they are all cosmopolitan fungi that have a wide substrate range (Domsch *et al*. 1980, Farr *et al*. 1989). Other fungi found include species of *Coniothyrium*, *Lecythophora*, *Libertella*, *Microsphaeropsis*, and *Rhinocladiella*. These fungi are also cosmopolitan but tend to be more specific to woody plants (Farr *et al*. 1989). Only one fungus, *Camarosporium orni*, is known to be specific to ash (Ellis and Ellis 1997). It was only isolated once so did not make up a significant portion of the fungal composition found.

Insects and fungi have co-evolved in an innumerable number of associations around the globe, most of these being parasitic relationships, and some mutual relationships (Steinhaus 1967). Many large groups of insects owe their success to having a mutual relationship with fungi. This is usually exhibited by fungi being a food source and providing habitat for the insect, while the fungi benefit from the dispersal of their spores (Wheeler and Blackwell 1984).

In the case of beetles which occur in the jewel or metallic beetle family (Buprestidae) (Paiero *et al*. 2012), it is known that the adult beetle oviposits eggs in cracks along the bark of the host tree (Garcia and Morrell 1999). At this point, spores of fungi could be directly inoculated into the tree at the same crack in the bark. This deposition of eggs and spores at the same time gives insect-vectored wood-inhabiting fungi a competitive advantage to be established into the tree when compared to fungi that are only dependant on wind dispersal. Garcia and Morrell (1999) explain that the adult beetles pick up spores and hyphal fragments as they exit the larval galleries in the wood and crawl over the bark's surface. The spores and hyphal fragments stick to their bodies and are vectored to the trees they oviposit their eggs in. Interestingly enough, many species of fungi isolated in this experiment possess wet spore masses that could easily stick to bodies of beetles. So, some of the species of fungi could have indeed be vectored by the adult emerald ash borer into the tree. However, this would just be a coincidental relationship, as neither the EAB nor the fungi are reliant on each other since there was such a diverse number of species of fungi isolated. Since *Penicillium* does not possess sticky spore masses, it is unlikely that adult EABs vectored spores of species of this genus into the ash trees. This study did not research the fungi that were associated with the adult beetles of EAB. A comparative study should be completed examining the fungi isolated from the bodies of adult beetles, and the fungi isolated from the galleries, frass, and larvae. This would confirm that spores are successfully sticking to the adult beetle and being inoculated into the host tree.

Another method for insects vectoring fungi is by using a mycangium. It is an organ attached to the beetle’s cuticle and the purpose is to collect spores for transport

(Crowson 1984). The mountain pine beetle possesses this organ. When the beetle chews through the bark of a new host tree and lays its eggs, the spores contained in the mycangium are inoculated into the new host tree (Safranyik *et al*. 2010). However, the EAB is within the family Buprestidae and this group generally lacks a mycangium. It is believed that when an insect successfully vectors a fungus to the host without utilizing a mycangium, it is coincidental and not the result of a mutual association (Berryman 1987). No article confirms or denies the presence of a mycangium on EAB so it is inconclusive if there is actually one on the EAB.

The sampling efficiency of this study using Good's Hypothesis (Good 1953) as modified by Moore and Holdeman (1974) was only 48% which is low. In total, there were only 178 fungal cultures isolated from the three sample trees. Within these 178 cultures, 49 distinct taxa were found. The sampling efficiency was reduced due to many colonies not being identified to genus or species. The most significant group of cultures that reduces the sampling efficiency were the 19 cultures that were sterile. These cultures were grouped into Sterile sp.1 to Sterile sp.8 based on morphological features such as colour of the colony and its growth pattern. Since the cultures were sterile there were no fruiting bodies or other unique features present to provide more specific classifications.

After reflecting on the process of this study, there is room for some improvement if it were to be repeated. First, the isolation of fungi from more sample trees from different locations in Thunder Bay, as opposed to only three trees from the same parkette. This would result in a more well-rounded composition of the fungal communities associated with the galleries, frass, and larvae of the emerald ash borer. It

also would have been beneficial to take into consideration exactly how long the trees were infested with EAB, and the degree to which they were infested. The galleries from EAB girdle the tree in approximately one to three years (NRC 2016b), so it would be interesting to see the composition of fungi after the initial infestation versus in three years when the tree is near death. A succession of different fungi could occur in an ash tree infested with EAB. It is well known this occurs in other trees (Dix and Webster 1995). If fungal community succession does occur with infested EAB trees, then depending on the timing of the study, the results could change. The three trees used in this study were all from the same parkette, and it is likely they became subject to an EAB infestation in the same season. All three trees contained the most commonly isolated genera (*Cytospora*, *Phoma*, and *Penicillium*).

In addition to affecting the composition of fungi, the timing of cutting the infested ash trees could affect the number of tree samples containing larvae. There were a very low number of larvae in the galleries of the infested samples. Tree 1 only contained two larvae and Tree 2 only contained four larvae. The samples of the infested trees were from the upper stem and branches because the Ministry of Natural Resources and Forestry required the lower portion of the stem. If this study were to be completed again, I would advise using samples from all over the tree to get an even representation of fungi that may be present in the tree.

In addition to isolating fungi from the adult emerald ash borers and comparing the composition to fungi isolated from the larvae, frass and galleries, isolating fungi from healthy ash trees in the City of Thunder Bay would be valuable. It would be interesting to compare the differences in the fungi communities present in the wood of

healthy ash trees versus the galleries, frass, and larvae of infested ash trees. The trees selected should be from the same relative area, so that they would be growing in similar environments. There have been a few studies that examined healthy ash trees in Europe such as Kowalski *et al*. (2016) and Bakys *et al*. (2008) but none on healthy ash trees in Canada. A statistical analysis could be completed on the fungal biodiversity to see if any fungus was only present when the emerald ash borer was present. Since trees in an urban setting offer many social, economic and environmental benefits, and given the risk ash trees are facing due to the EAB infestation, it is unlikely that healthy ash trees would be removed to complete a study. However, healthy trees that are at risk of being infested with EAB and do not meet the criteria to be treated with the insecticide TreeAzin™ are being removed in the City of Thunder Bay. This could provide an opportunity to obtain healthy ash trees for research.

Regarding the experimental design, there were several inconsistencies. First, each tree did not have the same number of logs cut from it, and each log did not have the same number of cultures isolated. It was difficult to keep the number and location of the isolations from each tree consistent because each tree contained varying amounts of galleries due to different severities of infestation. The least number of cultures were isolated from Tree 2. This is because it was the least infested and therefore, fewer galleries were present compared to Trees 1 and 3, even after taking off all of the bark and exposing the wood where the galleries would be present.

Based on the results of this study, it is highly unlikely that a fungal association exists with the emerald ash borer. While there was a repeated presence of *Cytospora* species, it only comprised 26.9% of the total composition of fungi that were isolated,

which does not indicate a consistent and repeated presence of the fungus. While *Cytospora*, is known to cause cankers, there were no visible cankers present on the samples of ash used in this study, therefore the fungus was not contributing to the decline of the ash tree. *Cytospora*, along with most of the fungi isolated, possess sticky spore masses. This attribute means that some of the fungi present in the galleries, frass, and larvae could have been vectored by spores sticking to the adult beetle. At the time the EAB oviposits its eggs into the tree, it could also inoculate the tree with fungal spores that were stuck to the body of the insect. However, neither the EAB or any of the fungi are reliant on each other in this process, thus it is a coincidental association. Only two species of wood decay fungi were isolated, therefore, it is confident to say the fungi present in the galleries, frass or larvae are not contributing to the decline of the ash tree. Since *Cytospora* was the most frequently found on all three ash trees, it would be the genus that would most likely form an association. More research needs to be done.

CONCLUSION

To be able to determine if a fungal association exists with the emerald ash borer, certain aspects must be researched to complement this study. First, isolations should be made from healthy ash trees in the same localities as EAB infested ash trees. This would determine the composition of fungi that are already existing before the presence of the EAB. Another requirement is that additional EAB infested trees should be studied that are at the same level of infestation to account for fungal community successional stages. Lastly, adult EAB must be studied to determine whether the fungi that are present on the adults are the same fungi present in the frass, galleries, and larvae. This can be completed by doing body washings (using the same method as larval washings used in this study) or allowing the beetle to walk over an agar medium. This is an important topic given the social, economic and environmental devastation the emerald ash borer has caused to Eastern North America. More research should be completed regarding the associated fungi with the emerald ash borer to achieve a definitive answer if an association does not or does exist.

LITERATURE CITED

Adams, A.S. and Six, D.L. 2007. Temporal variation in mycophagy and prevalence of fungi associated with developmental stages of *Dendroctonus ponderosae* (Coleoptera: Curculonidae). Environmental Entomology. 36:64–72.

Alvey, A. 2006. Promoting and preserving biodiversity in the urban forest. Urban Forestry & Urban Greening. 5:191-201

Bakys, R., Vasaitis, R. and Barklund, P., Thomsen, I.M, and Stenlid, J. 2009. Occurrence and pathogenicity of fungi in necrotic and non-symptomatic shoots of declining common ash (*Fraxinus excelsior*) in Sweden. European Journal of Forest Resources. 128:51–60.

Barryman, A.A. 1987. Resistance of conifers to invasion by bark beetle-fungus associations. Bioscience. 22(10): 598-602.

Baxter, M and Thompson, J. 2017. Across Ontario, the emerald ash borer is eating up trees - and municipal budgets. The Ontario Educational Communications Authority https://tvo.org/article/current-affairs/across-ontario-the-emerald-ash-borer-is-eating-up-trees--and-municipal-budgets

Burns, R.M and Honkala, B.H. 1990. Silvics of North America. USDA Forest Service, Washington, D.C. Agriculture Handbook No 654.

Crowson, R.A. 1984. The Associations of Coleoptera with Ascomycetes. Pp. 256-285. *in* Wheeler, Q. and Blackwell, M. Fungus-Insect Relationships. Perspectives in Ecology and Evolution. Columbia University Press. New York. 514 Pp.

Davey Resource Group (DRG). 2011. Urban Forest Management Plan City of Thunder Bay. City of Thunder Bay. (online)

Dix, N.J. and J. Webster. 1995. Fungal Ecology. Chapman and Hall, New York. 549 Pp.

Domsch, K.H., Gams, W. and Anderson, T-H. 1980. Compendium of Soil Fungi. Volume 1. Academic Press Inc. New York, New York. 859 Pp.

Dwyer, J.F., McPherson, E.G., Schroeder, H.W. and Rowntree, R.A. 1992. Assessing the Benefits and Costs of the Urban Forest. Journal of Arboriculture. 18(5):228-229.

Ellis, M.B. and J.P.Ellis. 1997. Microfungi on Land Plants: An Identification Handbook. Richmond Publishers, Slough, U.K. 868 Pp.

Farr, D.D., Bills, G.F., Chamuris, G.P. and Rossman, A.Y. 1989. Fungi on Plants and Plant Products in the United States. APS Press.

Garcia, C.M. and J.J. Morrell. 1999. Fungal associates of *Buprestis aurulenta* in Western Oregon. Canadian Journal of Forestry Research. 29:517–520.

Good, I.J. 1953. The population frequencies of species and the estimation of population parameters. Biometrika 40:237-264

Government of Canada (GOC) 2015. Anthracnose. Agriculture and Agri-Food Canada. Government of Canada. http://www.agr.gc.ca/eng/science-and-Inovation/agricultural-practices/agroforestry/diseases-and-pests/anthracnose/?id=1366992332570 (Decmber 10, 2017)

Kowalski, T., Kraj, W., and Bednarz, B. 2016. Fungi on stems and twigs in initial and advanced stages of dieback of European ash (*Fraxinus excelsior*) in Poland. European Journal of Forest Resources. 135:565–579.

Lee, S., Kim, J. and Breuil, C. 2006. Diversity of fungi associated with mountain pine beetle, *Dendroctonus ponderosae*, and infested lodgepole pines in British Columbia. Canadian Forest Service. Canadian Forest Service.

McPherson, E.G. 2003. A Benefit-Cost Analysis of Ten Street Tree Species in Modesto, California, U.S. Journal of Arboriculture. 29:1-8.

McPherson, E.G. and Simpson, J.R. 2002. A Comparison of Municipal Forest Benefits and Costs in Modesto and Santa Monica, California, USA. Urban Forestry & Urban Greening. 2:61-74.

McLeod, G., Gries, R., von Reuß, S.H., Rahe, J.E., McIntosh, R., König, W.A. and Gries, G. 2005. The pathogen causing Dutch elm disease makes host trees attract insect vectors. Proceedings of the Royal Society B: Biological Sciences. 272(1580):2499-2503.

Michigan Entomological Society (MES). 2002. The Emerald Ash Borer: A New Exotic Pest in North America. Newsletter of the Michigan Entomological Society. 47(3&4):1-2

Miller, R.W., Hauer, R.J., and Werner, L.P. 2015. Urban Forestry: Planning and Managing Urban Greenspaces. Third Edition. Waveland Press Inc. Long Grove Illinois. 560Pp.

Moore, W.E.C. and L.V. Holdeman. 1974. Human fecal flora: The normal flora of 20 Japanese-Hawaiians. Appl. Microbiol. 27:961-979.

Natural Resources Canada (NRC). 2016a. Emerald Ash Borer. Government of Canada. http://www.nrcan.gc.ca/forests/fire-insects-disturbances/top-insects/133777 (November 10, 2017)

Natural Resources Canada (NRC). 2016b. Emerald Ash Borer (Factsheet). Government of Canada. http://www.nrcan.gc.ca/forests/fire-insects-disturbances/top-insects/13395 (November 10, 2017)

Nowak, D.J. and Crane, D.E. 2002. Carbon storage and sequestration by urban trees in the USA. Environmental Pollution. 116:381-389.

Paiero, S.M., M.D.Jackson, A. Jewiss-Gaines, T.Kimoto, B.D.Gill, and S.A.Marshall. 2012. Field Guide to the Jewel Beetles (Coleoptera: Buprestidae) of Northeastern North America. Canadian Food Inspection Agency, Ottawa. 411 Pp.

Pereira1, G., Christian, H., Foster, S., Boruff, B., Bull, F., Knuiman, M. and Giles-Corti. 2013. The association between neighborhood greenness and weight status: an observational study in Perth Western Australia. Environmental Health. 12(49). 9Pp.http://europepmc.org/backend/ptpmcrender.fcgi?accid=PMC3710261&blobtype=pdf

Poland, T.M. and McCullough, D.G. 2006. Emerald ash borer: Invasion of the urban forest and the threat to North America's ash resource. Journal of Forestry. 104(3):118-124

Raffa, K.F. and Berryman, A.A. 1983. Physiological aspects of lodgepole pine wound response to a fungal symbiont of the mountain pine beetle *Dendroctonus ponderosae*. Canadian Journal of. Entomology. 115:723– 734

Rebek, E.J., Herms, D.A. and Smitley, D.R. 2007. Interspecific variation in resistance to emerald ash borer (Coleoptera: Buprestidae) among North American and Asian ash (*Fraxinus* spp.) Environmental Entomology. 37(1):242-246.

Safranyik, L. and Wilson, W.B. 2006. The Mountain Pine Beetle A Synthesis of Biology, Management, and Impacts on Lodgepole Pine. Natural Resources Canada. Pacific Forestry Centre, Victoria, B.C. 304 Pp.

Safranyik, L., Carroll, A.L., Régnière, J., Langor, D.W., Riel, W.G., Shore, T.L., Peter, B., Cooke, B.J., Nealis, V.G. and Taylor, S.W. 2010. Potential for range expansion of mountain pine beetle into the boreal forest of North America. The Canadian Entomologist. 142(5):415-442.

Sinclair, W.A., Lyon, H.H. and Johnson, W.T. 1987. Diseases of Trees and Shrubs. Cornell University Press, Ithaca, New York. 574 Pp.

Steinhaus, E.A. 1967. Insect Microbiology. Hafner Publishing Company. New York and London. 763pp.

Sutton, B.C. 1980. The Coelomycetes: Fungi Imperfecti with Pycnidia, Acervuli and Stromata. Commonwealth Mycological Institute, Kew, U.K. 696 Pp.

Tryvainen, L., Pauleit, S., Seeland, K. and de Vries, S. 2005. Benefits and uses of urban forests and trees. Pp. 81-114 in: Konijnendijk, C.C. (Ed.) Urban Forests and Trees. Springer, Berlin. 520 Pp.

Wheeler, Q. and Blackwell, M. 1984. Fungus-Insect Relationships. Perspectives in Ecology and Evolution. Columbia University Press. New York. 514 Pp.

Wolf, K.L. 1998. Urban Forest Values: Economic Benefits of Trees in Cities Center for Urban Horticulture. University of Washington. College of Forest Resources. Washington.3. http://www.naturewithin.info/Policy/EconBens-FS3.pdf

APPENDIX: RAW DATA

Specimen Code	Genus and Species
T1S2F11a	*Phoma* sp.1
T1S2F11b	*Cytospora* sp.1
T1S2G11a	*Cladosporium cladosporioides*
T1S2G11b	*Phoma* sp.1
T1S2G11c	*Epicoccum purpurascens*
T1S3F33	*Cytospora* sp.1
T1S3F34a	*Phoma* sp.6
T1S3F34b	*Cytospora* sp.1
T1S3F35	Bacteria
T1S3F36	*Cytospora* sp.1
T1S3F37	*Cytospora* sp.1
T1S3F38a	Sterile sp.8
T1S3F38b	*Phoma* sp.6
T1S3G33a	*Cytospora* sp.1
T1S3G33b	*Phoma* sp.5
T1S3G34	*Cytospora* sp.1
T1S3G35a	*Alternaria alternata*
T1S3G35b	*Cladosporium cladosporioides*
T1S3G35c	*Aureobasidium pullulans*
T1S3G36	Bacteria
T1S3G37	Bacteria
T1S3G38a	*Alternaria alternata*
T1S3G38b	*Penicillium* sp.
T1S3G38c	*Coniothyrium fuckelii*
T1S3L3	*Penicillium* sp.
T1S3L4a	*Cytospora* sp.1
T1S3L4b	*Mucor hiemalis*
T1S3L4c	*Cladosporium* sp.
T1S3L4d	*Cladosporium sphaerospermum*
T1S4F20a	*Cytospora* sp.1
T1S4F21a	*Coniothyrium fuckelii*
T1S4F21b	*Coniothyrium fuckelii*
T1S4F21c	*Epicoccum purpurascens*
T1S4F22a	*Botrytis cinerea*
T1S4F22b	*Epicoccum purpurascens*
T1S4F23a	*Stigmina* sp.
T1S4G20a	*Penicillium thomii*

Specimen Code	Genus and Species
T1S4G20b	*Penicillium* sp.
T1S4G20c	*Coniothyrium fuckelii*
T1S4G20d	*Cytospora* sp.1
T1S4G21a	*Phoma* sp.1
T1S4G21b	*Penicillium thomii*
T1S4G22a	*Cytospora* sp.1
T1S4G23a	*Phoma* sp.6
T1S4G23b	*Cladosporium sphaerospermum*
T1S4G23c	Sporodochium-forming sp.
T2S1F45a	*Cytospora* sp.1
T2S1F45b	black unknown
T2S1F45c	*Cladosporium* sp.
T2S1F46	*Cladosporium cladosporioides*
T2S1F47	Sterile sp.8
T2S1F48a	*Penicillium* sp.
T2S1F48b	Sterile sp.2
T2S1F48c	Pycnidia-forming sp.1
T2S1G45a	*Penicillium* sp.
T2S1G45b	black unknown
T2S1G45c	*Aureobasidium pullulans*
T2S1G46	Sterile sp.9
T2S1G47a	*Exophiala jeanselmei*
T2S1G47b	Pycnidia-forming sp.1
T2S1G47c	*Stigmina sp.*
T2S1G48	*Epicoccum purpurascens*
T2S2F49	Sterile sp.8
T2S2F50a	Pycnidia-forming sp.1
T2S2F50b	*Stigmina sp.*
T2S2G49	Sterile sp.6
T2S2G50	Sterile sp.6
T2S2L5	Sterile sp.8
T2S2L6a	Pycnidia-forming sp.1
T2S2L6b	*Stigmina sp.*
T2S3F24a	*Penicillium* sp.
T2S3F24b	*Coniothyrium* sp. 1
T2S3F24c	Pycnidia-forming sp.1
T2S3F24d	Sterile sp.7
T2S3F25a	black unknown
T2S3F25b	*Pithomyces chatarum*

Specimen Code	Genus and Species
T2S3F25c	*Cytospora* sp.3
T2S3F25d	*Penicillium sp.*
T2S3G24a	*Thysanophora penicillioides*
T2S3G24b	Sterile sp.9
T2S3G25	Bacteria
T2S3L1a	Sterile sp.2
T2S3L2a	Pycnidia-forming sp.1
T2S3L2b	Pycnidia-forming sp.2
T2S3L2c	*Cytospora* sp.1
T2S3L2d	Pycnidia-forming sp.1
T3S2F39	*Cytospora* sp.1
T3S2F40a	*Phoma* sp.6
T3S2F40b	*Cytospora* sp.1
T3S2F41	*Cytospora* sp.1
T3S2F42	*Cytospora* sp.1
T3S2F43a	*Cladosporium sphaerospermum*
T3S2F43b	*Alternaria alternata*
T3S2F44a	*Lecythophora* sp. 1
T3S2F44b	*Cladosporium sphaerospermum*
T3S2F44b	*Cytospora* sp.1
T3S2G39	*Cytospora* sp.1
T3S2G40	*Phoma* sp.4
T3S2G41	*Cytospora* sp.1
T3S2G42	*Cytospora* sp.1
T3S2G43a	*Cladosporium cladosporioides*
T3S2G43b	Sterile sp.8
T3S2G43c	Sterile sp.8
T3S2G44	*Cytospora* sp.1
T3S3F1ai	*Trichoderma* sp.
T3S3F2a	*Phoma* sp.2
T3S3F2b	*Lecythophora* sp. 1
T3S3F3a	*Penicillium* sp.
T3S3F3b	*Cladosporium cladosporioides*
T3S3F4a	*Phoma* sp.4
T3S3F4b	*Lecythophora* sp. 1
T3S3F5a	*Acremonium* sp.
T3S3F5b	*Acremonium* sp.
T3S3F5c	*Cytospora* sp.1
T3S3F6a	*Phoma* sp. 3

Specimen Code	Genus and Species
T3S3F6b	Sterile sp.5
T3S3F7a	*Mucor hiemalis*
T3S3F7b	*Cytospora* sp.1
T3S3F8a	*Phoma* sp.2
T3S3F9a	*Penicillium* sp.
T3S3F9b	*Cytospora* sp.1
T3S3F10	Bacteria
T3S3G1a	*Phoma* sp.2
T3S3G1b	*Camarosporium orni*
T3S3G1c	*Cladosporium herbarum*
T3S3G2a	Unknown Hymenomycete sp. 2
T3S3G2b	Sterile sp.5
T3S3G3a	Sterile sp.1
T3S3G4a	*Lecythophora* sp. 1
T3S3G4b	*Acremonium* sp.
T3S3G4c	*Cytospora* sp.1
T3S3G4d	*Lecythophora* sp. 1
T3S3G5a	*Cytospora* sp.1
T3S3G6a	*Phoma* sp.1
T3S3G6b	*Lecythophora* sp. 1
T3S3G6c	Unknown Hymenomycete sp. 1
T3S3G7a	*Phoma* sp.2
T3S3G7b	*Cytospora* sp.1
T3S3G7c	*Cytospora* sp.1
T3S3G8a	*Lecythophora* sp. 1
T3S3G9a	*Libertella* sp.
T3S3G9b	*Penicillium* sp.
T3S3G10	Bacteria
T3S4F27	*Cytospora* sp.1
T3S4F28a	*Cladosporium sphaerospermum*
T3S4F28b	*Cytospora* sp.1
T3S4F29	Sterile sp.8
T3S4F30a	*Phoma* sp.6
T3S4F30b	*Cladosporium cladosporioides*
T3S4F31	*Cytospora* sp.1
T3S4F32a	*Mucor* sp.
T3S4F32b	*Cytospora* sp.1
T3S4G26	*Cytospora* sp.1
T3S4G27	*Cytospora* sp.1
T3S4G28	*Cytospora* sp.1

Specimen Code	Genus and Species
T3S4G29a	*Cladosporium spaerospermum*
T3S4G29b	Sterile sp.4
T3S4G30a	*Phoma* sp.6
T3S4G30b	Bacteria
T3S4G31	*Cytospora* sp.1
T3S4G32	*Cytospora* sp.1
T3S5F12a	Sterile sp.3
T3S5F13a	*Cytospora* sp.1
T3S5F14a	*Cytospora* sp.2
T3S5F15a	*Cytospora* sp.1
T3S5F15bi	black unknown
T3S5F16a	*Cytospora* sp.1
T3S5F16b	*Phoma* sp.1
T3S5F17a	*Cladosporium sphaerospermum*
T3S5F17b	*Penicillium thomii*
T3S5F18a	*Cytospora* sp.1
T3S5F18b	*Cytospora* sp.1
T3S5F19a	*Penicillium* sp.
T3S5F19b	*Mucor circinelloides*
T3S5F19c	*Acremonium* sp.
T3S5G12a	*Rhizomucor* sp.
T3S5G12b	*Epicoccum purpurascens*
T3S5G13a	*Cytospora* sp.1
T3S5G14a	*Mucor circinelloides*
T3S5G14b	*Microsphaeropsis olivacea*
T3S5G15a	*Cytospora* sp.1
T3S5G16	*Rhinocladiella* sp.
T3S5G17a	*Coniothyrium fuckelii*
T3S5G18	*Cytospora* sp.1
T3S5G19a	*Mucor circinelloides*