A SURVEY OF FUNGAL ENDOPHYTES FROM HEALTHY BRANCHES OF *GINKGO BILOBA*

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Dr. Leonard Hutchison Thesis Supervisor	Dr. Leni Meyer Second Reader

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ABSTRACT

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Keywords: cambium, fungal endophytes, *Gingko biloba,* healthy branches, opportunistic, isolates, ubiquitous.

Ginkgo biloba L. is known for being highly resistant to bacterial, insect, and fungal infestations and thus ginkgo trees are commonly cultivated and planted in urban landscapes. The objective of this study was to determine whether or not fungal endophytes are living within the healthy branches of ginkgo, and if present, do they form a structured recognizable community? Eleven healthy gingko trees were sampled across eleven different localities in Canada from sites that ranged from Nova Scotia to British Columbia. Each tree sampled had 1 to 11 branch samples taken. Each branch sample had four pieces of cambium placed into each petri dish containing agar media. In total there were 183 isolations made. Out of these, 102 fungal colonies arose representing 35 distinct species in 17 genera. In addition, 43 samples did not yield any fungi. *Phoma* represented the most significant number of fungal isolates at 29 percent, with seven distinct, but unidentified species being recognized. The fungal endophytes identified are considered to be opportunistic and are ubiquitous across many woody plants and trees. The principal genera of fungi identified include *Phoma*, *Aureobasidium*, *Cladosporium*, *Coniothyrium*, *Epicoccum*, and *Stigmina*. This study shows that diverse communities of fungal endophytes are living in the healthy branches of *Ginkgo biloba* and that each tree sampled contained a unique assemblage.

TABLE OF CONTENTS

TABLES

Table Page

FIGURES

Figure Page

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INTRODUCTION

TAXONOMY OF GINKGO BILOBA

Ginkgo (*Ginkgo biloba* L.), commonly known as the ginkgo tree or maidenhair tree, is the sole survivor of the order Ginkgoales. There was once a total of 16 genera of the Ginkgophyta division (Zhiyan 2003). Fossil evidence of ginkgo has been dated back to over 200 million years. Thus it is commonly referred to as a living fossil. It had a worldwide distribution, but it is currently only native to China (Gong *et al*. 2008). The ginkgo is considered by plant taxonomists to be a conifer because it's fruits are the soft outer seed coat also known as the sarcotesta, indicative of gymnosperms (Crane 2013).

SILVICS OF GINKGO BILOBA

Ginkgo are large slow-growing trees that achieve an average height of approximately 20-30 m with some reaching heights of 50 m. Some living specimens have been recorded at being 2,500 years old. They are considered to be a shade intolerant species and prefer well-drained, well-watered areas. Ginkgo are capable of producing shoots on their trunks in disturbed areas and during crown damage (Royer *et al*. 2003). During the Pleistocene era, the ginkgo succeeded in surviving global glaciation and associated climate change (Taberlet 1998). The current natural distribution of this species is generally restricted to the temperate regions of eastern China, with the exception of cultivated urban street trees found throughout Europe and North America. Ginkgo trees have a very long reproductive period and they do not start seed production until after 20 years of age, after which they will stay reproductive for

over 1000 years (Major 1967). This adaptation is thought to be the success behind their longevity and persistence throughout history. This genus is dioecious and has separate sexes, with male trees producing pollen cones and female trees producing fruits. These Gingko fruits are regarded by many as undesirable due to a disagreeable smell. The fruits are about 1.5- 2 cm long and tend to have a yellowish brown outer layer. The branching of ginkgo is quite sparse with branches growing as shoots. After adequate growth is achieved, branches with larger diameters lead the way for new shoots to produce the leafing structures (Dallimore *et al*. 1966). These leafing structures are found alternately spaced among the branches and clustered at the end of the shoots. The leaves are two-lobed and have radiating veins. These leaves are highly prized for their aesthetics due to a bright yellow colour during the autumn season and a unique shape.

GINKGO AS AN URBAN TREE

Ginkgo is known for its long lifespan, large canopy, and aesthetic appeal which makes it a highly regarded urban tree (Plotnik 2000). This species is well known for its resistance to viruses, bacteria, insects, and disease, and thus ginkgo trees are commonly cultivated and planted as urban shade trees (Major 1967). Due to the foul-smelling fruits only male trees are planted in urban environments. They are resistant to urban pollutants such as high smoke levels, soil compaction, exhaust, street salt, and mechanical damage (He *et al*. 2007). The leaves act as a repellent towards insects through toxins produced, and this is also said to occur within the bark and root systems of the tree (Major 1967). Because this species has been known for being quite tolerant to a wide range of climate zones, it has been introduced to many North American cities, ranging from New Orleans, Louisiana in the south to as far north as Edmonton, Alberta.

USE OF GINKGO

Ginkgo has been widely used as a medicinal tree, particularly the seeds in traditional eastern medicines dating back centuries (Chan *et al*. 2007; Huh and Staba 1992; Pang *et al*. 1996; Qiu *et al*. 2010; Smith and Luo 2004). In more recent years, it is used more widely as a dietary supplement as well as a herbal medicine, with extracts from the leaves and the bark being used in addition to the seeds (McKenna *et al*. 2001). It is said to aid in the suppression of Alzheimer's disease, memory disorders, age-related disorders, as well as many other ailments (Mahadevan and Park 2008). Some of the chemical properties found within the ginkgo include flavonoid glycosides, diterpenes, and ginkgolides that are considered essential for platelet aggregation (Qin *et al*. 2009). There have been numerous studies conducted on the side effects on the use of ginkgo in medicinal practices. Some of the side effects on the users included internal bleeding, intracerebral hemorrhage, parietal hemorrhage, and bilateral subdural hematomas (McKenna *et al*. 2001). However, most of the stated symptoms coincided with previous surgeries, and health problems, as well as combining the following pharmaceuticals with the ginkgo supplements: acetylsalicylic acid, warfarin, ticlopidine, clopidogrel, and dipyridamole (Cupp 1999; McKenna *et al*. 2001). The validity of the use of ginkgo as a treatment for many disorders, diseases, and cognitive functions is still yet to be determined. The significance of medical abstracts may lead to more studies in the future of nutraceuticals to treat certain cancers, blood diseases, and viruses (Mahadevan and Park 2008).

FUNGI ASSOCIATED WITH GINKGO

There have been few studies conducted on fungi that are associated with ginkgo trees. Connors (1967) does not list any fungi found on ginkgo in Canada. As listed in the Index of Plant Diseases in the United States (USDA 1960), only six species were recorded to occur in living ginkgo trees: *Glomerella cingulata*, *Phyllostica ginkgo*, *Fomes conchatus*, *Oxyporus populinus*, *Bartheletia paradoxa,* and *Polyporus* sp. (USDA 1960). However, Farr *et al*. (1989) expanded the list to a total of 12 species of fungi on ginkgo in the United States. A more recent study by Adamčíková & Hrubík (2015) on ginkgo in the Czech Republic and Slovakia found *Epicoccum nigrum*, *Alternaria alternata*, *Fusarium* sp., *Phomopsis occulta* var. *ginkgoina*, *Cylindrosporium* sp., *Phyllosticta* sp., and *Cladosporium* sp. These were mostly found on leaves, with *Phomopsis* and *Alternaria* species showing up on the fruit of the trees and *Fusarium* on a branch sample (Adamčíková & Hrubík 2015). There have been analyses conducted on the bioactive metabolites produced by endophytic fungi (*Chaetomium globosum*) isolated from ginkgo leaves. A good example is the study conducted by Qin *et al*. (2009) on the effects of bioactive metabolites which were tested on brine shrimp and the fungus *Mucor miehei,* resulting in growth inhibitor activity in both the shrimp and fungi. It has been noted that specific single-chained proteins found within the seeds of ginkgo that act as an antifungal and antibacterial defence are similar to embryo-abundant proteins found in white spruce, *Picea glauca* (Moench)Voss. The protein referred to as ginkbilobin was said to produce a highly anti-fungal resistance to *Botrytis cinerea*, *Mycosphaerella arachidicola*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Coprinus*

comatus (Wang and Ng 2000). These proteins exhibited antibacterial tendencies against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Wang and Ng 2000). Because ginkgo is known to be highly resistant to insect outbreak, diseases, and viruses, it is not yet known whether or not these associated fungi play a vital role in the protective effect on this tree species. Most of the fungi found within the previously mentioned studies were found to be commonly distributed amongst all localities of ginkgo grown in urban settings, as well as semi-urban areas (Adamčíková & Hrubík 2015).

The purpose of this thesis is to examine if any fungi are growing as endophytes in the healthy branches of ginkgo collected from different localities across Canada. As ginkgo is very resistant to fungal infection, the following objectives were to be tested.

1) Are fungi living as endophytes in healthy branches of ginkgo?
2) If present, do they form a structured recognizable community?

METHODS & MATERIALS

Dr. Leonard Hutchison collected branches in 2016 and 2017 from 11 healthy ginkgo trees growing in various urban localities across Canada (Figure 1; Tables 1 & 2). These branches were approximately the size of pencils and were removed using handheld secateurs. All samples had their leaves removed before being placed in clear plastic bags, which were labeled with the collection date, the location, and the living situation (*i.e.,* street tree, lawn tree). All samples were placed in a portable icebox until samples could be transported back to Lakehead University. The samples were then stored in a freezer (-4°C) located in the Forest Pathology Teaching Lab (BB1050) until it was time to do isolations.

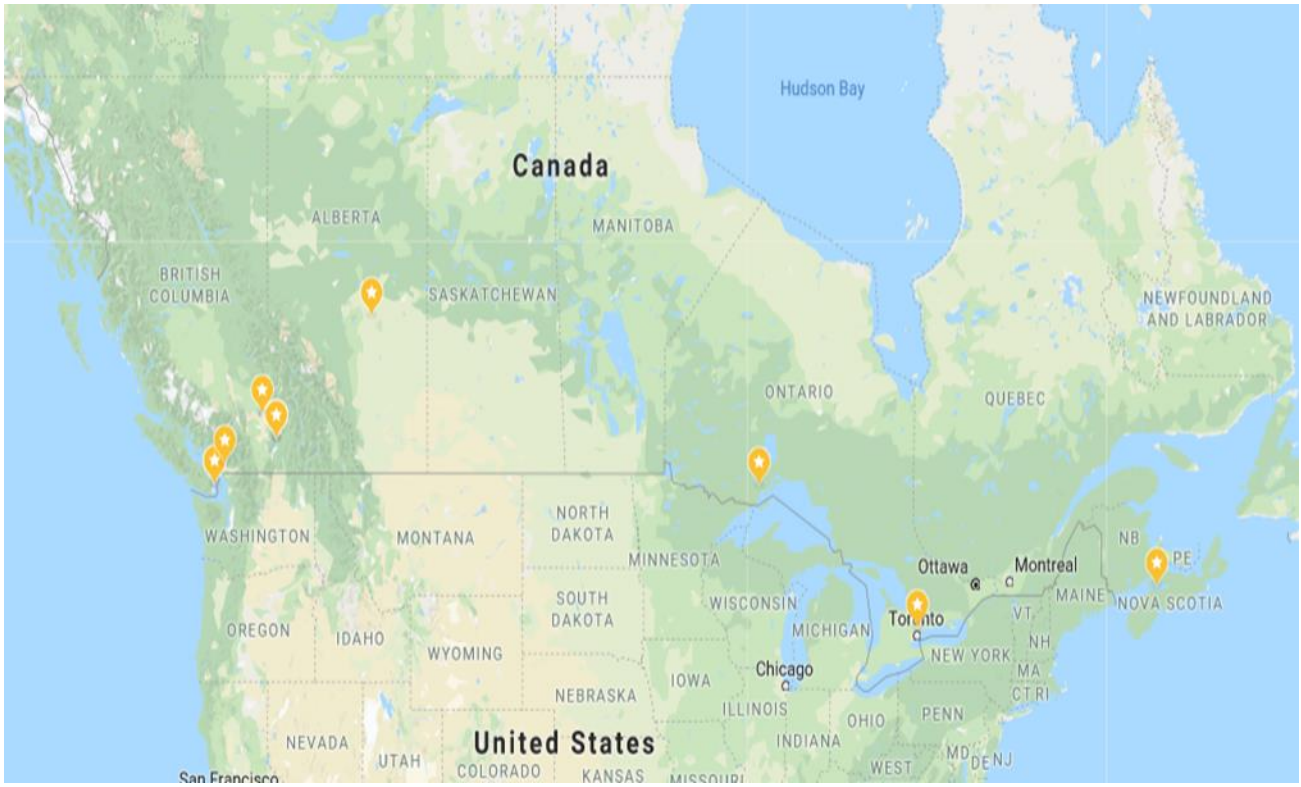

Figure 1. Locality of samples collected (Google Maps 2019)

The agar media used in the isolation process was modified 2 % Malt Extract Agar (20 g malt extract, 15 g agar, 1 g yeast extract, 1 L distilled water). All agar media was made in 500 mL batches and placed in 1 L sized Ehrlenmeyer flasks, covered with aluminum foil and sterilized for 30 minutes at 121°C in an autoclave. Once the flasks of media were sterilized, they were placed in a water bath and cooled to approximately 45°C before pouring the cooled molten agar into sterile plastic Petri dishes (90 mm diameter) in a sterile transfer hood. The Petri dishes were allowed to set overnight and were wrapped with Parafilm® when condensation dissipated from under the lids and then stored until ready to be used.

Isolations took place from October 2017 through January 2018. Bags containing the branch samples were allowed to thaw before isolation attempts were made. Each branch sample was placed in a glass tray containing 70 percent ethanol for one minute in order to surface sterilize the outer bark. Then each branch was removed with flame-sterilized forceps and placed on a sterile surface where the alcohol was allowed to evaporate throughout several minutes. Flame-sterilized pliers were used to hold each branch while flame-sterilized secateurs were used to remove approximately 1 cm from each end of the branch. A flame-sterilized surgical scalpel was used to shave off the outer bark to expose the cambium at each end. Four pieces of cambium were chipped from each end of each branch and placed into separate Petri dishes of agar media (four per dish). Then a small amount of antibiotics (a mixture of Streptomycin sulphate and Penicillin G) were added to each dish with the use of a flame sterilized dissecting needle in order to mitigate potential bacterial contamination. The Petri dishes were labeled based on tree number, branch number, dish number, and year that sample was collected

(*e.g.,* T2-B1-D1-17). The dishes were incubated in the dark at 20°C. Colonies of fungi growing out from each cambium chip were subsequently transferred to a new Petri dish of agar to keep each colony separate from each other if it was apparent that different species were growing out from each of the four chips per plate.

Initially, many colonies remained sterile, and it was necessary to place those under fluorescent lights in order to stimulate sporulation. Colonies were examined weekly from December through March. Mounts in 1 percent Phloxine were made on microscope slides and examined with the use of a bright field compound microscope. Standard taxonomic keys were used to identify each isolate based on micromorphological characteristics. The list of identified fungi, along with their associated tree, branch number and dish number can be found in Appendix I. Representatives were photographed utilizing an Olympus digital camera mounted on a Nikkon Eclipse 400 phase contrast microscope.

In order to ensure sampling efficiency, Good's Hypothesis (Good 1953) as modified by Moore and Holdeman (1974) was applied to the data. This equation is calculated by subtracting the total number of species observed once, over the total number of species observed from 1, and then multiplying the difference by 100 in order to obtain a percentage as seen in equation 1 (Appendix II). The percentage calculated is indicative of the efficiency of the sampling methods. If this number is close to 100 percent, then the sampling method is considered efficient.

Table 1. Locations of ginkgo trees sampled in 2016

Tree number	date	location	setting
1	6/14/2016	University of Toronto Toronto ON	lawn
2	6/6/2016	Acadia University Wolfville NS	lawn

Source: Dr. Hutchison 2018

Table 2. Location of ginkgo trees sampled in 2017

Tree number	date	location	setting
1	6/23/2017	Belmont Street Victoria BC	boulevard
2	6/24/2017	University of Victoria Campus Victoria BC	lawn
3	6/28/2017	Highway #10 Surrey BC	planter beside parking lot
4	6/29/2017	City Park Kelowna BC	lawn
5	6/29/2017	College Campus Kelowna BC	lawn
6	6/30/2017	Guisachan Road Kelowna BC	boulevard
7	7/1/2017	Three Rivers Campus Kamloops BC	lawn
8	4/7/2017	University of Alberta Campus Edmonton AB	lawn
9	12/13/2017	Lakehead University Thunder Bay ON	behind building

Source Dr. Hutchison 2018

RESULTS

Overall, there were a total of 183 isolation attempts made, including originals and transfers. One hundred isolates representing 17 genera were found, including some sterile isolates representing ten distinct morphological types. Twenty nine percent of the isolates occurred among seven distinct, but unidentified species of *Phoma*. Table 3 shows the total number of identified fungi and their percentage of occurrence. The table arranges them from most abundant such as *Phoma* at 28.4 percent followed by sterile isolates at 16.7 percent. Some species identified based on the appearance of conidia included *Epicoccum purpurascens* (Figure 2), *Diplodia* sp. (Figure 3), *Stigmina* sp. (Figure 4), and *Microsphaeropsis olivacea* (Figure 5).

Table 3. Percentage of occurrence of genera found on ginkgo branches

Genus	Total	Percentage
Phoma	29	28.4%
Sterile	17	16.7%
Aureobasidium	14	13.7%
Cladosporium	6	5.8%
Coniothyrium	5	4.9%
Epicoccum	5	4.9%
Sooty moulds	5	4.9%
Stigmina	5	4.9%
Rhizopus	3	2.9%
Diplodia	2	1.9%
Microsphaeropsis	2	1.9%
Trichoderma	2	1.9%
Penicillium	2	1.9%
Chaetomium	1	1%
Fusarium	1	1%
Hormonema	1	1%
Lecythophora	1	1%
Alternaria	1	1%
Total	102	99.7%

Source: Dr. Hutchison 2018

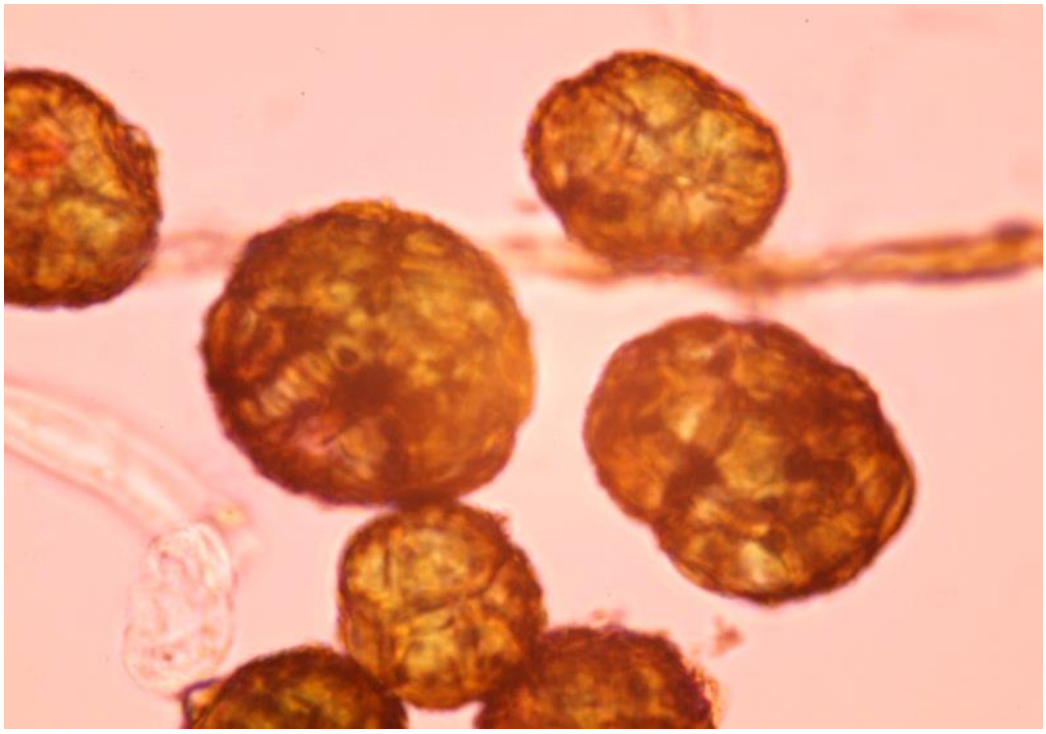

Figure 2. Conidia of *Epicoccum purpurascens*

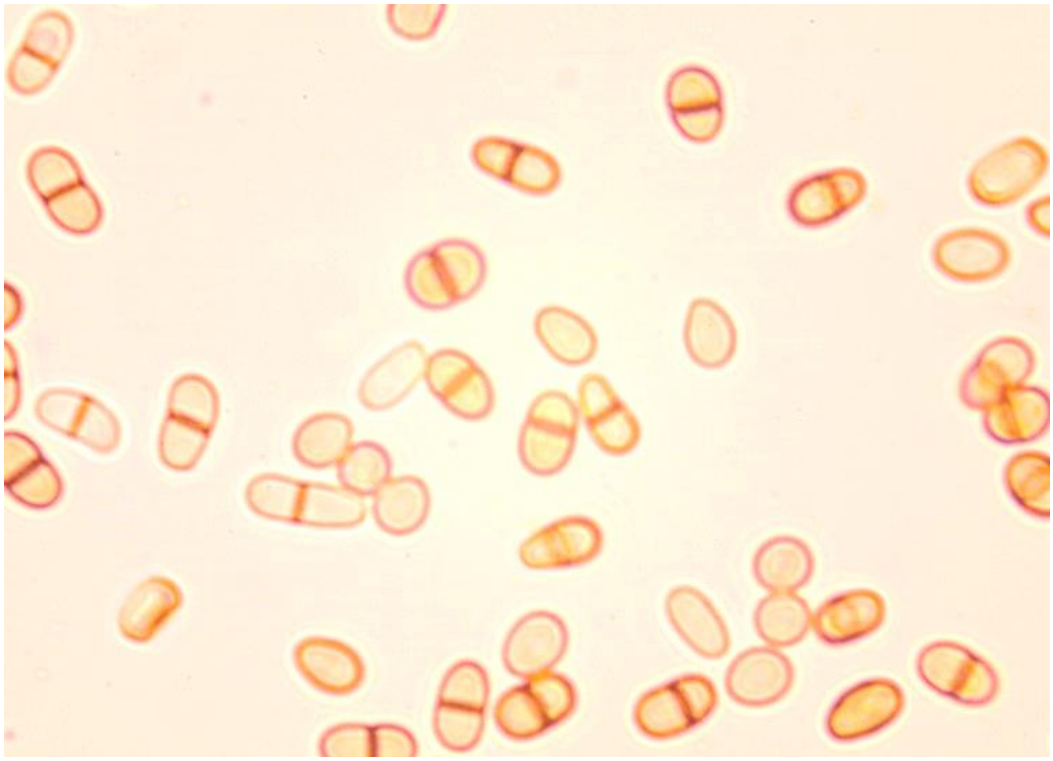

Figure 3. Conidia of *Diplodia* sp.

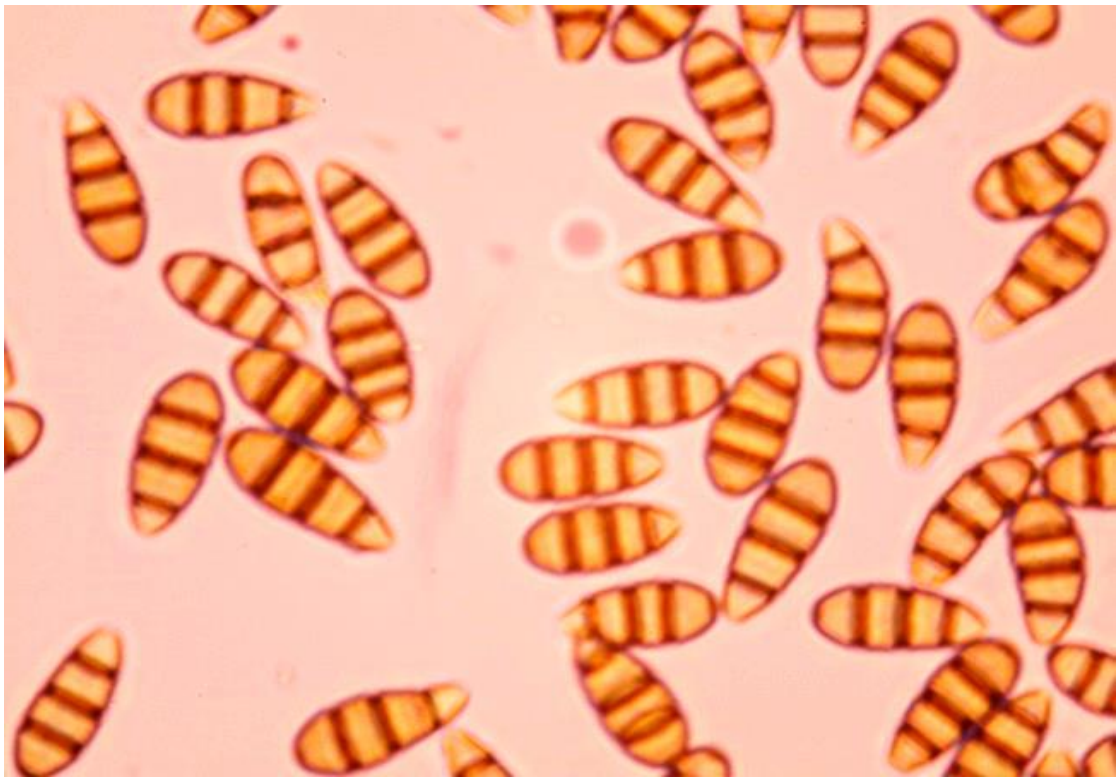

Figure 4. Conidia of *Stigmina* sp.

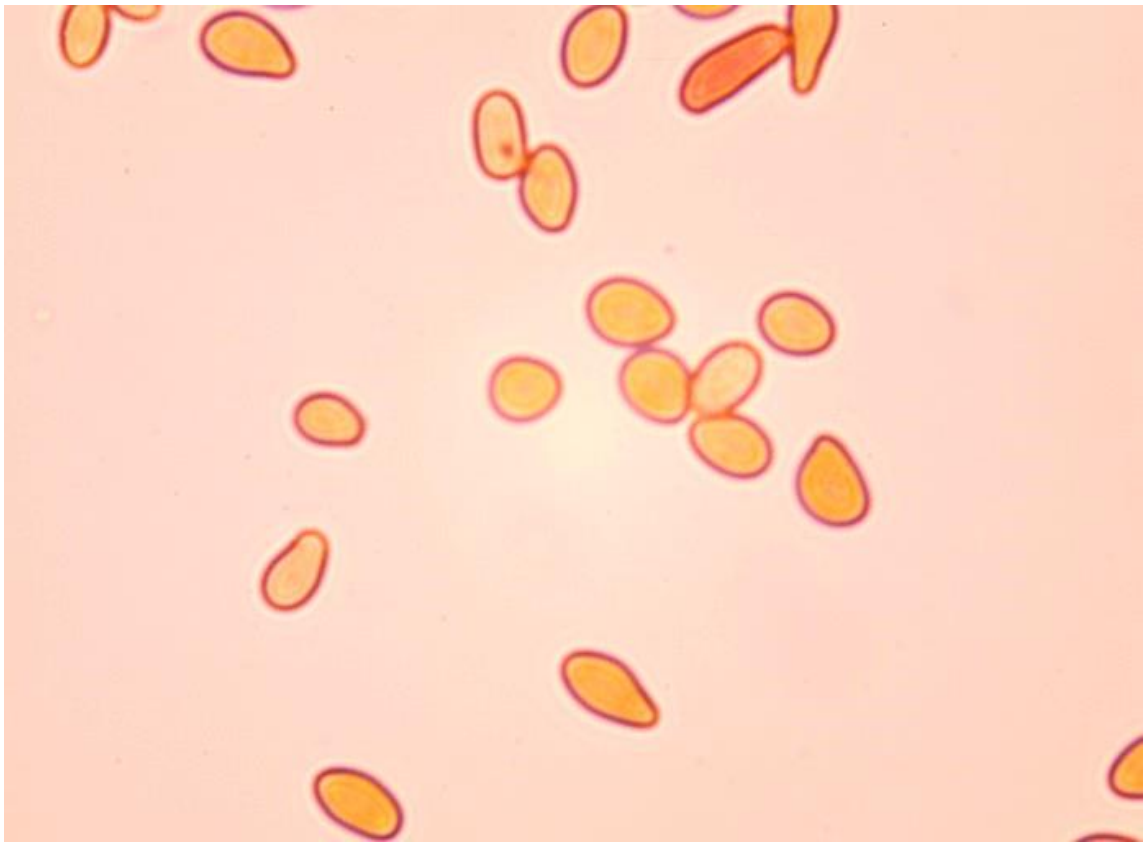

Figure 5. Conidia of *Microsphaeropsis olivacea*

DISCUSSION

A previous study by Adamčíková & Hrubík (2015) only found seven genera of endophytes from 54 trees sampled from 17 different Slovak and Czech localities. Their study used fruit and leaf samples taken from trees whose health ranged from poor to excellent. The following genera of fungi were detected on their samples: *Epicoccum*, *Fusarium*, *Alternaria*, *Phomopsis*, *Cylindrosporium*, *Phyllosticta*, and *Cladosporium*. Their study was the first scientific report on fungal endophytes associated with ginkgo trees. Unfortunately, research on the endophytic relationships between fungi and species of trees found within Canada are limited. It is essential to conduct this research around the world, to distinguish the different geographic endophytic relationships that fungi have with ginkgo trees and account for spatial variation. In comparison to the previous studies conducted, this study used a total of 183 samples from branches of eleven healthy ginkgo, resulting in a total of 102 fungi identifications. This information can aid researchers, urban planners, and greenhouses by identifying and consequently understanding how these fungi can potentially affect tree physiology and propagation.

Most of the genera of fungal endophytes found within the healthy branches of ginkgo are ubiquitous and reside within woody plants, leaf material, and bark (Farr *et al*. 1989). Some of these genera are classified as opportunistic necrotrophs, such as *Coniothyrium*, *Microsphaeropsis*, *Phoma*, and *Diplodia* (Sinclair and Lyon 2005). The fungal endophytes that have been identified and described are very widespread throughout the globe, occurring in tropical, temperate, and taiga regions (Farr *et al*. 1989). These fungi are often introduced to trees through exposed wounds in the bark

which can result from freezing conditions, weather events, mechanical damage, and insect pests. The other fungi found within the healthy branches are classified as common saprophytes that occur across many woody plants and trees. These include *Alternaria alternata*, *Aureobasidium pullulans*, *Epicoccum purpurascens*, *Cladosporium cladosporioides*, and *Hormonema* sp. There have not been any recent studies showing that these fungi have either a positive or an adverse effect on healthy gingko trees. Compared to Basidiomycetes which commonly fruit outside of woody tissue, endophytes are difficult to identify due to their internal nature. Further studies would be required on the other fungal endophytes that were unidentifiable due to the lack of fruiting bodies and spores.

In order to ensure sampling efficiency, a modified version of Good's Hypothesis was applied to the data (Appendix II). It was calculated at 65.7 percent suggesting that only a moderate sampling efficiency was achieved. More trees sampled and more branches sampled from, would undoubtedly result in a greater percentage of fungi recovered.

CONCLUSION

The objectives of this study were to determine whether or not fungi were living as endophytes in the healthy branches of ginkgo. This study determined that diverse communities of fungal endophytes are living within healthy ginkgo trees. Each tree that was sampled had a unique community of fungi living within the cambium (Appendix III). The genera of fungi that were identified in this study are ubiquitous and widely spread across many regions. There were no noticeable trends across all specimens sampled. It would be interesting to determine whether or not these fungal endophytes produce metabolites that protect against pathogens and insect pests. Future studies would be essential to understand the global trends of fungal communities of sampled ginkgo trees from a wider geographic area.

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APPENDICES

APPENDIX I

Table 4. Total Observed Fungal Species

Tree #	Observed fungal species	Branch	Dish
1a	*Aureobasidium pullulans*	2	3
1a	*Coniothyrium* sp. 1	3	6
1a	*Coniothyrium sporulosum*	1	1
1a	*Coniothyrium sporulosum*	1	2
1a	*Epicoccum purpurascens*	2	4
1a	*Phoma* sp. 4	3	5
2a	Bacteria	1	1
2a	Bacteria	4	7
2a	Bacteria	4	8
2a	Bacteria	1	2
2a	*Coniothyrium* sp.	3	6
2a	*Phoma* sp.4	4	7
2a	*Phoma* sp.6	3	5
2a	Sterile 9	2	4
1	*Alternaria alternata*	6	12
1	*Alternaria alternata*	6	12
1	*Aureobasidium pullulans*	6	11
1	*Aureobasidium pullulans*	7	14
1	*Aureobasidium pullulans*	2	3
1	*Aureobasidium pullulans*	6	11
1	*Cladosporium cladosporioides*	5	9
1	*Coniothyrium fuckelii*	5	9
1	*Coniothyrium fuckelii*	5	9
1	*Coniothyrium* sp. 1	3	6
1	*Coniothyrium sporulosum*	1	1
1	*Coniothyrium sporulosum*	1	2
1	*Epicoccum purpurascens*	4	7
1	*Epicoccum purpurascens*	2	4
1	*Epicoccum purpurascens*	4	7
1	*Penicillium sp.*	1	2
1	*Penicillium sp.*	1	2
1	sterile 2	1	1
1	sterile 2	5	10
1	sterile 2	4	8
1	sterile 2	4	7
1	sterile 2	1	1

1	*Stigmina* sp.	5	10
1	*Stigmina* sp.	7	13
1	*Stigmina* sp.	7	13
1	*Stigmina* sp.	5	10
1	*Stigmina* sp.	5	9
2	*Aureobasidium pullulans*	5	10
2	Bacteria	1	1
2	*Epicoccum purpurascens*	2	3
2	*Fusarium* sp.	3	6
2	*Phoma* sp.7	2	3
2	*Rhizopus oryzae*	6	11
2	*Rhizopus oryzae*	2	3
2	*Rhizopus oryzae*	1	2
2	sterile 1	2	3
2	sterile 9	2	4
3	*Aureobasidium pullulans*	5	9
3	Bacteria	6	12
3	Bacteria	6	11
3	sterile 1	2	4
3	sterile 6	3	5
3	sterile 7	1	2
3	sterile 8	3	6
4	*Diplodia* sp.	4	8
4	*Microsphaeropsis olivacea*	2	4
4	*Phoma* sp.4	2	3
4	*Phoma* sp.4	3	6
4	*Phoma* sp.4	5	10
4	*Phoma* sp.4	8	16
4	*Phoma* sp.6	4	8
4	sterile 5	4	8
5	*Aureobasidium pullulans*	8	15
5	*Aureobasidium pullulans*	8	15
5	*Aureobasidium pullulans*	8	16
5	sooty mould	3	6
5	sooty mould	4	7
5	sterile 4	1	1
6	*Aureobasidium pullulans*	10	19
6	*Cladosporium cladosporioides*	3	5
6	*Cladosporium cladosporioides*	9	17
6	*Diplodia* sp.	7	13
6	*Hormonema sp.*	11	22

6	*Microsphaeropsis olivacea*	4	8
6	sooty mould	3	6
6	sooty mould	8	15
6	sterile 5	8	15
7	*Aureobasidium pullulans*	3	5
7	*Aureobasidium pullulans*	8	15
7	*Aureobasidium pullulans*	8	16
7	Bacteria	5	10
7	Bacteria	7	13
7	Bacteria	6	11
7	Bacteria	7	14
7	Bacteria	1	2
7	Bacteria	5	9
7	Bacteria	3	6
7	*Cladosporium cladosporioides*	9	17
7	*Cladosporium cladosporioides*	8	16
7	dishes with nothing	4	7
7	*Phoma* sp.3	6	12
7	*Phoma* sp.3	9	18
7	*Phoma* sp.3	4	8
7	*Phoma* sp.3	2	3
7	*Phoma* sp.5	2	4
7	sterile 10	1	1
7	*Trichoderma* sp.	4	7
8	Bacteria	8	15
8	dishes with nothing	3	5
8	dishes with nothing	6	11
8	dishes with nothing	5	9
8	*Phoma* sp.1	10	19
8	*Phoma* sp.1	8	16
8	*Phoma* sp.2	6	12
8	*Phoma* sp.2	1	1
8	*Phoma* sp.2	5	10
8	*Phoma* sp.2	9	17
8	*Phoma* sp.2	11	21
8	*Phoma* sp.2	1	2
8	*Phoma* sp.2	7	14
8	*Phoma* sp.2	4	8
8	*Phoma* sp.2	3	6
8	*Phoma* sp.2	2	4
8	*Phoma* sp.2	2	3

8	*Phoma* sp.2	10	20
8	*Phoma* sp.2	9	18
8	sooty mould	11	22
8	*Trichoderma* sp.	7	13
9	*Chaetomium* sp.	3	6
9	*Lecythophora* sp.	8	15
9	sterile 3	9	18
9	sterile 3	7	13

*1a and 2a 2016 trees

APPENDIX II

GOOD'S HYPOTHESIS

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

= 1- (12/35) x 100

= 0.657 x 100

= 65.7%

APPENDIX III

Table 4. Frequency of Observed Fungi

Tree #	1a	2a	1	2	3	4	5	6	7	8	9
Fungi											
Alternaria alternata	0	0	2	0	0	0	0	0	0	0	0
Aureobasidium pullulans	1	0	4	1	1	0	3	0	3	0	0
Bacteria	0	4	0	1	2	0	0	0	7	1	0
Chaetomium sp.	0	0	0	0	0	0	0	0	0	0	1
Cladosporium cladosporioides	0	0	1	0	0	0	0	2	2	0	0
Coniothyrium fuckelii	0	0	2	0	0	0	0	0	0	0	0
Coniothyrium sp.	0	1	0	0	0	0	0	0	0	0	0
Coniothyrium sp. 1	1	0	0	0	0	0	0	0	0	0	0
Coniothyrium sporulosum	2	0	3	0	0	0	0	0	0	0	0
Diplodia sp.	0	0	0	0	0	1	0	1	0	0	0
dishes with nothing	0	0	0	0	0	0	0	0	1	3	0
Epicoccum purpurascens	1	0	3	1	0	0	0	0	0	0	0
Fusarium sp.	0	0	0	1	0	0	0	0	0	0	0
Hormonema sp.	0	0	0	0	0	0	0	1	0	0	0
Lecythophora sp.	0	0	0	0	0	0	0	0	0	0	1
Microsphaeropsis olivacea	0	0	0	0	0	1	0	1	0	0	0
Penicillium sp.	0	0	2	0	0	0	0	0	0	0	0
Phoma sp.1	0	0	0	0	0	0	0	0	0	2	0
Phoma sp.2	0	0	0	0	0	0	0	0	0	13	0
Phoma sp.3	0	0	0	0	0	0	0	0	4	0	0
Phoma sp.4	1	1	0	0	0	4	0	0	0	0	0
Phoma sp.5	0	0	0	0	0	0	0	0	1	0	0
Phoma sp.6	0	1	0	0	0	1	0	0	0	0	0
Phoma sp.7	0	0	0	1	0	0	0	0	0	0	0
Rhizopus oryzae	0	0	0	3	0	0	0	0	0	0	0
sooty mould	0	0	0	0	0	0	2	2	0	1	0
sterile 1	0	0	0	1	1	0	0	0	0	0	0
sterile 2	0	0	5	0	0	0	0	0	0	0	0
sterile 3	0	0	0	0	0	0	0	0	0	0	2
sterile 4	0	0	0	0	0	0	1	0	0	0	0
sterile 5	0	0	0	0	0	1	0	1	0	0	0
sterile 6	0	0	0	0	1	0	0	0	0	0	0
sterile 7	0	0	0	0	1	0	0	0	0	0	0
sterile 8	0	0	0	0	1	0	0	0	0	0	0
sterile 9	0	1	0	1	0	0	0	0	0	0	0
sterile 10	0	0	0	0	0	0	0	0	1	0	0
Stigmina sp.	0	0	5	0	0	0	0	0	0	0	0
Trichoderma sp.	0	0	0	0	0	0	0	0	1	1	0
Total	6	8	27	10	7	8	6	8	20	21	4