AN EXAMINATION OF BARK BEETLES AND THEIR ASSOCIATED BLUE STAIN FUNGI ON BOREAL JACK PINE (*Pinus banksiana*) AND WHITE SPRUCE (*Picea glauca*) IN THE THUNDER BAY REGION

by

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A CAUTION TO THE READER

This M.Sc.F thesis has been through a semi-formal process of review and comment by at least four readers. As well, it has been reviewed by an external examiner. It is made available for loan by the Faculty of Forestry and the Forest Environment for the purpose of advancing the practice of professional and scientific forestry.

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ABSTRACT

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Blue stain, caused by members of the Ophiostomatales, is of concern to the forest industry because it can quickly devalue timber by penetrating deep into the sapwood and creating dark blue to black staining. These fungi depend on insect vectors, such as bark beetles (Family Scolytidae), for the dispersal of their spores. Through a series of two field surveys, the objective of this thesis was to examine the diversity of ophiostomatoid fungi and their associated bark beetles in the Thunder Bay region, because of little research having been done on this topic in northwestern Ontario.

In 2006, a field study was undertaken to examine these associations on four conifer species: balsam fir (*Abies balsamea*), black spruce (*Picea mariana*), tamarack (*Larix laricina*) and jack pine (*Pinus banksiana*). One tree of each species was felled at Jack Haggerty Forest (36 kilometres north of Thunder Bay) which remained on the forest floor over the summer months. Discs were removed in October. Four species of bark beetles were observed on the wood samples. *Pityokteines sparsus* was abundant and the only species observed on balsam fir. *Polygraphus rufipennis* was commonly observed on black spruce and tamarack. *Dendroctonus simplex* and *Ips pini* were observed in low numbers. The former bark beetle was only observed on tamarack, while the latter was observed on black spruce and jack pine. Fungal isolations were taken from washings of adult bark beetles and their larvae as well as from bark beetle frass, stained wood and fruiting bodies of ophiostomatoid fungi growing within the galleries. Twenty-one species of ophiostomatoid fungi were isolated from the four conifer species. This included several new records in the Thunder Bay region, such as *Leptographium fruticetum*, *Ophiostoma abietinum*, *O. rectangulosporium*, *O. piceae* and *O. pulvinisporum*. Several new bark beetle-fungal associations were also observed. Most of these new associations occurred on tamarack and balsam fir.

A second field study was conducted on white spruce (*Picea glauca*) and jack pine in 2007. Four trees of each species at three forest properties (Jack Haggerty Forest, Silver Mountain and Quackenbush Woodlot) were felled. In early October, three discs representing the base, middle and tip, were removed from each tree. A total of eight species of bark beetles were observed. *Polygraphus rufipennis* made up the majority of the bark beetles found on white spruce while *Ips pini* and *Ips perturbatus* were

commonly observed on the jack pine samples. Twelve separate species of ophiostomatoid fungi were isolated from white spruce and 16 from jack pine. Several additional new records for the Thunder Bay area were observed, including *L. wingfieldii* and *O. tubicollis*. *Leptographium fruticetum* was the most dominant ophiostomatoid fungus isolated from white spruce, while *O. ips* was the most dominant ophiostomatoid fungus isolated from jack pine. Location had a significant effect on abundance of ophiostomatoid fungi, while there was no significant difference of abundance between the sections of the bait trees. In addition, host had a significant impact on the abundance of fungi isolated from both tree species. The results from this thesis were compared with the literature regarding specific bark beetle-fungal associations. A summary of the current knowledge of distribution and pathogenicity traits is discussed for each of the observed ophiostomatoid fungi and bark beetles.

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CHAPTER I
LITERATURE REVIEW

LITERATURE REVIEW

INTRODUCTION

The northern boreal forest is a conifer dominated region, which expands through Russia, Scandinavia and North America and encompasses about a third of the world's forests (CFA 2005). Thirty percent of the world's boreal forest is located in Canada (CFA 2005), constituting 77 per cent of Canada's forest cover (NRCan 2008a). In Ontario, this forest region covers 49.8 million hectares of Ontario's 88.3 million hectares of land area (OMNR 2008a), consisting of black spruce [*Picea mariana* (Mill.) B.S.P.], white spruce [*Picea glauca* (Moench) Voss], jack pine [*Pinus banksiana* Lamb.], balsam fir [*Abies balsamea* (L.) Mill.], tamarack [*Larix laricina* (Du Roi) K. Koch], trembling aspen (*Populus tremuloides* Michx.), balsam poplar (*Populus balsamifera* L.) and white birch (*Betula papyrifera* Marsh.).

The boreal forest is subjected to many natural disturbances throughout its life cycle, consisting of several abiotic and biotic factors. Three of the most serious abiotic factors contributing to tree stress or mortality in the boreal forest are fire, blowdown caused by windstorms and water stress caused by drought or flooding (Paine and Baker 1993). Trees that become stressed when subjected to these conditions are more vulnerable to the intrusion of insect and disease and may die if they are unable to defend against these attacks (Paine and Baker 1993). Most leaf-defoliating or wood-boring insects are a natural component of the boreal forest ecosystem and are not normally a nuisance in healthy forested areas. Insects can become problematic when their populations increase. Population bursts can be the result of many contributing factors,

such as ideal climatic conditions, severe weather and abundance of food; however, many of these contributing factors leading to severe outbreaks are not well known. Because native forest insects have coexisted with boreal trees for thousands of years, periodic insect outbreaks are typical and do not normally become unmanageable. However, as contributing factors continue to remain ideal, insect populations could increase further, leading to several years of outbreak and extensive tree mortality.

Disease epidemics are responsible for a considerable loss of volume of wood correlating with reduced growth and increased tree mortality. In Ontario, there are on average ten to twelve million cubic metres lost annually to disease (OMNR 2008a). Most of this loss is induced by fungi causing root rot, wood decay and die-back of trees. Diseases are also commonly encountered infecting trees and devaluing wood for timber production. One problem currently affecting the quality of timber production is sapstain, which is caused by three general groups of fungi: black yeast-like fungi (*e.g. Aureobasidium pullulans*, *Hormonema dematioides* and *Phialophora* spp.), dark moulds (*e.g. Alternaria alternata* and *Cladosporium* spp.) and blue stain fungi (*e.g. Ophiostoma* and *Ceratocystis* spp.) (Seifert 1993). Black yeast-like fungi and dark moulds are usually superficial and are not of much concern to the forest industry. In contrast, blue stain caused by ophiostomatoid fungi is of great concern because it can quickly devalue timber by penetrating deep into the sapwood and causing stained areas. Stained sapwood is a common problem encountered by forest managers when trees are in poor health or are left lying on the forest floor over a period of time before being processed. In case of a windstorm, fire or other natural disturbances, salvage logging is often performed immediately to prevent the staining of the sapwood by the intrusion of bark beetles carrying spores of blue stain fungi.

TAXONOMIC OVERVIEW OF THE OPHIOSTOMATOID FUNGI

Malloch and Blackwell (1993) recognize the Ophiostomatales as "Ascomycetes with long-necked perithecia, evanescent asci and hyaline ascospores lacking pores or slits"; however, some species of ophiostomatoid fungi have necks that are reduced or absent. The perithecia, or fruiting structures, are made of specialized pseudoparenchyma, often dark brown to black, with a globose or flask-shaped base within which asci are irregularly arranged (Upadhyay 1981). Eight unicellular ascospores are produced within each prototunicate ascus and are pushed up through the neck upon maturity and released through the ostiole (an opening) at the apex of the neck. Many species possess specialized hyphae around the ostiole, which aid in collecting the ascospores in a mucilaginous mass at the apex of the neck (Whitney and Blauel 1972; Upadhyay 1981). Ascospores can appear sheathed with a gelatinous layer around the spore or can appear unsheathed.

Taxonomically, members of the Ophostomatales are difficult to classify, making categorization of this group of fungi complicated. Olchowecki and Reid (1974) grouped all ophiostomatoid fungi within the genus *Ceratocystis* and divided them into four groups based on their ascospore shapes. Members of the Minuta group have elongated ascospores with a gelatinous sheath encompassing the spore and tapering at the ends. The Ips group contains members possessing ascospores with a rectangular or "pillow-shaped" sheath surrounding the spore. Members of the Fimbriata group have short, curved or "orange section-shaped" ascopores within a "hat-shaped" sheath. The final group is the Pilifera group, which contain members that do not possess an apparent sheath around the ascospores.

The proper application of names for ophiostomatoid fungi has been under constant revision for over a century as more is being understood about this complicated group. For example, the application of the genus *Ceratocystis sensu lato* has always been controversial among taxonomists (DeHoog and Scheffer 1984). Since the genus was established in 1890 by Halsted, who identified *Ceratocystis fimbriata* Ellis & Hals. as the causal agent for Black Rot of sweet potato, many of the ophiostomatoid fungi have been gradually moved to new genera as taxonomists investigated the group in more detail (Halsted 1890; Zipfel *et al.* 2006). One such genus, *Ophiostoma* Syd. & P. Syd, had been thought for years to be congeneric with *Ceratocystis* (Upadhyay 1993). However, Von Arx (1974a) separated the two genera on the basis of their anamorphs. Within the *Ceratocystis sensu lato* complex, he grouped all members with a *Chalara* (Corda) Rabenh. anamorph (now referred to as *Thielaviopsis* Went (Paulin-Mahady *et al.* 2002)) under the genus *Ceratocystis sensu stricto*, and all other members under the genus *Ophiostoma.* This includes all members possessing anamorphs within the genera *Leptographium* Legerb. & Melin, *Sporothrix* Hekt. & Perk., *Hyalorhinocladiella* Upadh. & Kendr., *Pesotum* Crane & Schokn or any other anamorph that produces blastic sympodial conidia (Von Arx 1974a; Upadhyay 1993).

The taxonomy of blue stain fungi has been an "ever-lasting" battle trying to find the most suitable name for any one fungus. Within the past decade, phylogenetic studies on these fungi have validated that the two major groups (*Ophiostoma* and *Ceratocystis*) are polyphyletic and has allowed taxonomists to distinguish among these fungi (Hausner *et al.* 1993b, Zipfel *et al.* 2006). Zipfel *et al.* (2006) constructed a table to denote confusion with the taxonomy, encompassing the changes made over time at the genus level, including important characteristics of the teleomorphs with their associated

anamorphs (Table 1). The teleomorphic and anamorphic states of these fungi are described in the following sections.

Teleomorphic States of Blue Stain Fungi

Ceratocystis

Ceratocystis is a genus that can be weakly or aggressively pathogenic to a large number of plants, chiefly angiosperms, found in agricultural landscapes as well as the natural environment (Kile 1993). An example of a disease caused by a member of *Ceratocystis* is a vascular wilt of oak (*Quercus* sp.) caused by *C. fagacearum* (Bretz) Hunt. This disease was first recognized in 1944 in Wisconsin and is responsible for the mortality of over half the oak in severely affected areas (Rexrode and Brown, 1983). White oak and closely related species appear to be most affected by the disease, where mycelial mats of the fungus expand and crack the host's bark, emitting an odour that attracts insects. Beetles, such as oak bark beetles (*Pseudopityophthorus* spp.), are known vectors of this fungus from diseased trees to healthy trees (Rexrode and Brown, 1983).

Since the establishment of the genus *Ceratocystis*, at one time or another, it encompassed all blue stain fungi and has since been under much revision (Table 1). The genus *Ophiostoma* was originally split from *Ceratocystis sensu lato* based on anamorph development, but since the split, there have been other attributes distinctively separating the two groups (De Hoog and Scheffer 1984). First, the anamorphs of *Ceratocystis sensu stricto* develop conidia through a ring wall-building process, whereas anamorphs of *Ophiostoma* develop conidia through an apical wall-building process (Mouton *et al.* 1993). Secondly, the cell walls of *Ceratocystis* contain chitin, but lack cellulose and rhamnose, while members of *Ophiostoma* contain chitin, cellulose and rhamnose in the

Table 1: Taxonomic changes made to the teleomorphic genera of the ophiostomatoid fungi since their discovery according to Zipfel *et al.* (2006)

Genera based on morphology		Ophiostoma	Ceratocystis	Grosmannia	Ceratocystiopsis	Europhium
Teleomorph:	ascomata	long to short-necked	long-necked	long to short-necked	short-necked	cleistothecia
	ascospores	various	various	various	falcate	hat-shaped
	sheath around ascospores	seldom	often	yes	yes	yes
Anamorph(s)		Sporothrix Hyalorhinocladiella Pesotum	Thielaviopsis	Leptographium Pesotum-like	Hyalorhinocladiella Sporothrix-like	Leptographium
Halsted 1890			**Ceratocystis gen. nov.**			
Hedgcock 1906						
Münch 1907			**Endoconidiophora gen. nov.**			
Sydow & Sydow 1919						
Nannfeldt 1932, Melin & Nannfeldt 1934						
Goidánich 1935, 1936						
Siemaszko 1939						
Davidson 1942						
Bakshi 1951, Moreau 1952		**Ceratocystis**	**Ceratocystis**	**Ceratocystis**		
Von Arx 1952, Von Arx & Müller 1954						
Hunt 1956		**Ceratocystis**	**Ceratocystis**	**Ceratocystis**	**Ceratocystis**	
Parker 1957						
Wright & Cain 1961, Griffin 1968, Olchowecki & Reid 1974		**Ceratocystis**	**Ceratocystis**	**Ceratocystis**	**Ceratocystis**	
De Hoog 1974, Harrington 1987			**Ceratocystis**			
Von Arx 1974b			**Ceratocystis**			
Weijman & De Hoog 1975			**Ceratocystis**			
Upadhyay & Kendrick 1975		**Ceratocystis**	**Ceratocystis**	**Ceratocystis**	**Ceratocystiopsis gen. nov.**	**Ceratocystis**
Upadhyay 1981		**Ceratocystis**	**Ceratocystis**	**Ceratocystis**		**Ceratocystis**
De Hoog & Scheffer 1984, Wingfield *et al.* 1993			**Ceratocystis**			
Von Arx & Van der Walt 1987			**Ceratocystis**			
Hausner *et al.* 1992, 1993a & b, 2000, 2003, Hausner & Reid 2003			**Ceratocystis**			
Jacobs & Wingfield 2001			**Ceratocystis**			
Zipfel *et al.* 2006						
Present Study			**Ceratocystis**			

cell walls (De Hoog and Scheffer 1984; Harrington 1987, Mouton *et al.* 1993). The use of the fungicide cycloheximide can also be used to distinguish the two genera, because most species of *Ophistoma* are resistant to this compound, while species of *Ceratocystis sensu stricto* are not (Harrington 1981). With the advancement of technology, Hausner *et al.* (1993b) proposed that *Ceratocystis sensu stricto* be removed from the Ophiostomatales and placed into the Microascales based on partial rDNA sequencing.

Ophiostoma

Ophiostoma is the genus containing the greatest number of blue stain fungi with over 140 known species (Zipfel *et al.* 2006), many of which act as weak parasites or saprophytes while others are economically important tree pathogens causing serious vascular wilts throughout the temperate regions of the world (Smalley *et al.* 1993; Seifert 1993). A well known pathogenic species is *Ophiostoma novo-ulmi* Brasier, which is the current causal agent of Dutch Elm Disease. This disease is responsible for wiping out most natural stands of native elms (*Ulmus* spp.) throughout North America (Et-Touil *et al.* 1999). The spores of the fungus are vectored by bark beetles from infected trees to healthy trees and is also capable of infecting healthy trees through grafts in the root system of adjacent elm trees.

Currently, there is much debate regarding the circumscription of the genus *Ophiostoma* due to the many morphological differences between species (Upadhyay 1981, Hausner et al. 1993a, Wingfield 1993a, Zipfel *et al.* 2006). Several genera (ie. *Ceratocystiopsis*, *Grosmannia* and *Europhium*) have already been established from species previously placed within *Ophiostoma* and more genera will likely be established as this genus becomes better defined (Zipfel *et al.* 2006). Some taxonomists feel that

Ophiostoma should not be divided into separate genera based on morphological characteristics because these characteristics are too variable and would complicate the taxonomy of these fungi even more (Wingfield 1993a, Hausner *et al.* 1993a). Zipfel *et al.* (2006) believed that due to the major morphological differences between species, there was need for *Ophiostoma* to be further divided, and separated the genus complex into three genera, including *Ophiostoma sensu stricto*, *Ceratocystiopsis* and *Grosmannia.* In addition, these authors insisted *Ophiostoma sensu stricto* could be divided into three groups based on morphological features. The first group contained species with pillow-shaped ascospores, which Olchowecki and Reid (1974) previously labelled the Ips group. The second group consisted of species with a *Sporothrix* anamorph, though a couple of species within this group have *Pesotum*-like anamorphs. The third group contained species with a *Pesotum* anamorph. However, Zipfel *et al.* (2006) did not suggest names for these groups and failed to provide sufficient data to support their theory.

Other Teleomorph Genera

In 1981, Upadhyay established the genus *Ceratocystiopsis* to include members of *Ophiostoma* that had short perithecial necks with convergent ostiolar hyphae and falcate ascospores (Upadhyay 1981; Mouton *et al.* 1993). This was earlier defined as the Minuta group by Olchowecki and Reid (1974). The species *Ceratocystiopsis falcata* (Wright & Cain) Upadhyay was an exception to the group as it was the only member with a *Chalara* anamorph and two-celled ascospores (Wingfield 1993b). Taxonomists began to feel the species was misclassified and it was suggested to belong to the genus *Ceratocystis*, even though it would be the only species in the group with falcate and

septate ascospores (Wingfield 1993b). In 2000, *Cp. falcata* was placed in the genus *Cornuvescia* using molecular data, and was no longer recognized as a member of the Ophiostomatales (Viljoen *et al.* 2000). Currently there are 11 species of *Ceratocystiopsis*, characterized by *Hyalorhinocladiella* and occasionally *Sporothrix*-like anamorphs (Zipfel *et al.* 2006). Hausner *et al.* (1993a) transferred members of the genus back into *Ophiostoma* after phylogenetic studies confirmed that the two genera were closely related. Despite Hausner *et al.*'s (1993a) efforts, Zipfel *et al.* (2006) reinstated the genus *Ceratocystiopsis* because they felt Hausner *et al.*'s (1993a) study was insufficient because only one *Ophiostoma* species was used to compare relatedness to *Ceratocystiopsis*.

The genus *Europhium* was established by Parker (1957) to include a single species of the Ophiostomatales that resembled *Ophiostoma*, but produced cleistothecia instead of perithecia and lacked ostioles. Several years later, Robinson-Jeffrey and Davidson (1968), placed three more species in this group. This genus was criticized because development of a neck was considered by some researchers to be easily affected by the environment and therefore an unreliable taxonomic character (Upadhyay 1981, De Hoog and Scheffer 1984, Harrington 1987). As a result, members within the genus *Europhium* were constantly regrouped into other genera, including *Ceratocystis* (Upadhyay 1981), *Ophistoma* (Harrington 1987; Hausner *et al.* 2000) and *Grosmannia* (Zipfel *et al.* 2006).

Zipfel *et al.* (2006) reinstated the genus *Grosmannia*, which was originally established in the 1930's by Goidánich (1936) to include all species with a *Leptographium* anamorph. The reinstatement of this genus was based on phylogenetic evidence and morphological characteristics. Through molecular work, Zipfel *et al.*

(2006) found strong evidence that supported monophyly of all members of the Ophiostomatales with a *Leptographium* anamorph. An earlier study by Hausner *et al.* (2000) failed to support this proposal and the authors concluded that the subdivision of *Ophiostoma* based on differences in their anamorphic states is artificial. Zipfel *et al.* (2006) stated their method of sequencing DNA was more accurate than the former study and incorporated 27 species into *Grosmannia*, including the four *Europhium* species.

Today, the teleomorphic genera found within the Ophiostomatales include *Ophiostoma*, *Ceratocystiopsis* and *Grosmannia*. This, however, is not accepted by all taxonomists. Wingfield (1993a) had previously argued that segregating out species based on ascospore morphology was illogical due to the variety of ascospore shapes found within the Ophiostomatales, while Hausner *et al.* (2000) felt segregating out species based on anamorph morphology, "although convenient, is artificial". Therefore many taxonomists may still feel *Grosmannia* and *Ceratocystiopsis* are synonyms of *Ophiostoma*. To avoid becoming overwhelmed by the constant changes and controversies surrounding the taxonomic nomenclature of these fungi, these genera will be represented as species of *Ophiostoma* in this thesis.

Anamorphic States of Blue Stain Fungi

Apart from the teleomorphic states of ophiostomatoid fungi being difficult to classify, the anamorphic states have received just as much, if not more, debate and controversy. Not too long ago, taxonomists recognized sixteen types of anamorphs amongst the Ophiostomatales (Upadhyay 1981), however because many have been proven to be synonymous to each other, taxonomists today recognize five distinct anamorphs. Members of the genera *Ceratocystis* have a *Chalara* anamorph, which is

now more commonly known as *Thielaviopsis*. Kim *et al.* (2005b) divided the anamorphs of *Ophiostoma* into three groups: 1) synnematous anamorphs (*e.g. Pesotum.*), 2) mononematous anamorphs (incorrectly referred to as macronematous by Kim) (*e.g. Leptographium*) and 3) mycelial anamorphs (*e.g. Sporothrix* and *Hyalorhinocladiella*). These anamorphs, along with their associated teleomorphs and taxonomic history, are summarized below.

Anamorphs of *Ceratocystis*

Chalara is a genus of anamorphic fungi that produces endoconidia singly or in basipetal chains from sessile or stalked phialides with basal venters and long collarettes (Kowalski 2006). Since Von Arx's (1974a) separation of *Ceratocystis* and *Ophiostoma*, all members of ophiostomatoid fungi with a *Chalara* anamorph were grouped into the genus *Ceratocystis*. Giving a proper name to this anamorphic genus has been complicated. One of the complications was how several species of *Chalara* produced aleuriocondia on top of the endoconidia, which was not a known characteristic of *Chalara*. It was proposed that these fungi were synanamorphs of *Chalara* and as a result two new genera were established: *Thielaviopsis* (producing aleurioconidia in chains) and *Chalararopsis* (producing aleurioconidia singly) (Paulin-Mahady *et al.* 2002).

In 1975, Nag Raj and Kendrick (1975) grouped *Thielaviopsis* and *Chalaropsis* as synonyms of *Chalara* due to the similarities in their phialidic states. Today, there are nearly 120 species of *Chalara* (Kowalski 2006), but recently it has been found that only a few are associated with the Ophiostomatales. Through rDNA sequencing, this group has been found to be polyphyletic, where most species of *Chalara* are shown to belong to the Leotiales (Paulin-Mahady *et al.* 2002). Paulin-Mahady *et al.* (2002) suggested the

genus *Chalara* should be split and adopted the genus *Thielaviopsis* to include all members of *Chalara* that had a known ophiostomatoid teleomorph and which the anamorph produced "endoconidia from phialides and larger, pigmented aleuroconidia in chains from specialized hyphae". However, a few species of *Chalara* that were transferred to the genus *Thielaviopsis* do not produce aleuroconidia. They were transferred over due to their *Ceratocystis* teleomorphs.

Synnematous Anamorphs

For many years, the synnematous anamorphs of the Ophiostomatales have been under much discussion and debate. In the early 1800's, the genus *Graphium* Corda was described as Hyphomycetes with "dark stalks, penicillated conidiophores, slimy heads and small aseptate conidia" (Seifert and Okada 1993). Over time, the description for this genus became more specific as more species were added and some removed (Okada *et al.* 1993).

Crane and Schoknecht (1973) proposed the genus *Pesotum* based on conidium ontogeny, including all *Graphium*-like anamorphs producing conida from conidiogenous cells with sympodial proliferations. Upadhyay (1981) revised the *Graphium* complex further into several genera based on conidium ontogeny and synnema pigmentation: 1) *Graphium sensu stricto*, 2) *Graphilbum* Upadh. & Kendr., 3) *Graphiocladiell*a Upadh., 4) *Hyalopesotum* Upadh. & Kendr., 5) *Pesotum*, 6) *Pachnodium* Upadh. & Kendr. and 7) *Phialographium* Upadh. & Kendr.

A little more than a decade later, Seifert and Okada (1993) disregarded the above-mentioned genera and suggested that they were all synonyms of *Graphium*. They proposed that the genus should be altered to include Hyphomycetes with "dark or lightly

pigmented synnemata, annellidic, phialidic, or apparently sympodially proliferating conidiogenous cells, and aseptate or septate conidia produced in slimy masses."

A problem that resulted with regrouping all synnematous anamorphs associated with blue stain into one genus was that the genus now included members of both the Ophiostomatales and Microascales. A few years later, Okada *et al.* (1998) grouped all *Graphium*-like anamorphs of the Ophiostomatales into the genus *Pesotum*, and *Graphium sensu stricto* was used to represent members of the Microascales. Presently, this is the most accepted solution.

Mononematous Anamorphs

The mononematous anamorphs of *Ophiostoma* consisted of three genera, known for about thirty years as "the *Leptographium* complex", which included *Leptographium*, *Verticicladiella* Hughes and *Phialocephala* Kendr. (Wingfield 1993a). The genus *Leptographium* was established in 1927 by Lagerberg *et al.* (1927) after examining fungi that caused the blueing of timber. Species within the genus *Leptographium* have distinguishable pigmented mononematous conidiophores with a cluster of conidiogenous cells producing a slimy mass of conidia at the apex of the conidiophore (Jacobs and Wingfield 2001). The long conidiophores and sticky spores are often found in bark beetle galleries, which is advantageous because spores stick to the bodies of passing beetles as the conidiophores brush against their bodies. When the adult bark beetles emerge from the tree, the spores are vectored to a new host tree and establish.

The confusion within the *Leptographium* complex was initiated when some members were transferred to *Verticicladiella* if their conidia were produced in a sympodial fashion (Hughes 1953). Since the establishment of *Verticicladiella*, there has

been debate about the synonymy of these two genera. Wingfield (1985) examined the conidial development of species in the two genera through electron microscopy and observed that conidia production was similar in all species examined. Based on these observations, the author concluded that the two genera were synonyms.

Phialocephala was established in the early 1960's and placed into the *Leptographium* complex based on the periclinal thickening of the phialides and evident collarettes (Kendrick 1961). Complications arose with this genus because many species did not have a known teleomorph and it appeared only a few species within this genus were ophiostomatoid (Jacobs and Wingfield 2001). Wingfield *et al.* (1987) examined many species of *Phialocephala* and transferred all anamorphs of *Ophiostoma* within this group to *Leptographium sensu stricto.* As a result, *Phialocephala* was disregarded as a member of the Ophiostomatales and *Leptographium sensu stricto* was considered the only member of the *Leptographium* complex.

Mycelial Anamorphs

Mycelial anamorphs of the Ophiostomatales have a simple morphology consisting of small conidiogenous cells arising from undifferentiated hyphae while producing their spores in a sympodial manner (De Hoog 1993, Zipfel *et al.* 2006). The two most recognized mycelial anamorphs of the Ophiostomatales are *Sporothrix* and *Hyalorhinocladiella* (Wingfield 1993a; Kim *et al.* 2005b; Zipfel *et al.* 2006). Differentiating these genera is problematic (De Hoog 1993). The main distinction between the two genera is that *Sporothrix* species typically produce peg-like denticles where conidia are developed, while species of *Hyalorhinocladiella* have inconspicuous scars on the conidiogenous cells (Benade *et al.* 1997).

Sporothrix is the most commonly produced anamorph of *Ophiostoma* (Zipfel *et al.* 2006), and is commonly found as a synanamorph of *Pesotum* (Zipfel *et al.* 2006; Benade *et al.* 1997). The genus *Sporothrix* is viewed as an artificial genus because it encompasses a large group of Hyphomycetes that are not found exclusively in the Ophiostomatales (De Hoog 1993). Segregating species into different groups is not favoured by many scientists because creating many morphologically similar genera would cause a lot of confusion (De Hoog 1993); however, other scientists feel this is necessary (Upadhyay 1981; Zipfel *et al.* 2006). The genus *Hyalodendron* has commonly been confused as an anamorph of *Ophiostoma* and differed from *Sporothrix* by producing conidia in short chains (Upadhyay 1981). However, it is now known that some species of *Sporothrix* can produce conidia in chains and the type species of *Hyalodendron* is a member of the Basidiomycota (De Hoog 1993). Because chains can be found in low abundance in many species of *Sporothrix*, De Hoog (1993) did not feel this was an important characteristic to define a separate genus and disregarded *Hyalodendron* as a member of the Ophiostomatales.

ECOLOGY OF THE OPHIOSTOMATALES

Members of the Ophiostomatales are among the first fungi to colonize freshly cut wood of both conifers and hardwoods (Seifert 1993; Jacobs and Wingfield 2001; Kim *et al.* 2005a) and most species appear to be vectored by arthropods, such as bark beetles (Malloch and Blackwell 1993). Many ophiostomatoid fungi are saprophytes and do little else than stain the sapwood of the colonized tree, while other species are highly pathogenic and can lead to mortality of the host tree in addition to heavy stains (Basham, 1970). The discolouration of sapwood, or sapstain, can cause serious economic losses to

the forest industry due to the reduction in value of the wood (Smalley 1993; Kim *et al.* 2005a). The discolouration of the wood is relatively cosmetic and does not significantly affect wood quality because the fungi do not break down the structural components of the wood (Seifert 1993; Kirisits 2004). Seifert (1993) reported that there was only a small loss in dried weight of infected wood. There was some loss in strength properties, but Seifert (1993) suggested this was insignificant unless the wood was used for applications requiring shock resistance.

The rapid growth rate along with the ability to tolerate low concentrations of oxygen is characteristic of ophiostomatoid fungi (Solheim and Krokene 1998). When the fungus is introduced to a tree by a bark beetle vector, the fungus quickly establishes in the bark beetle galleries and colonizes the cambium of the tree (Basham 1970). The high growth rate, especially in highly virulent species, allows the fungus to colonize the host's tissues before the host is able to build up its defences (Solheim and Krokene 1998). Upon colonization of the cambium, fungal hyphae begin to grow into the sapwood through the ray parenchyma cells, feeding on the food resources stored in the living cells (Ballard *et al.* 1984; Kirisits 2004). The fungal hyphae generally remain in the vessels, but can also enter tracheids through bordered pits or some species produce appressoria with penetration pegs to pierce through radial walls (Seifert 1993). When fungal hyphae grow through bordered pits, they begin to plug up the tree's vascular system, leading to reduced water transport up the stem and sometimes leading to wilting, dieback and eventual death of the tree (Ballard *et al.* 1984). This was observed by Basham (1970) who inoculated seedlings and saplings of loblolly pine with different species of ophiostomatoid fungi and found that the moisture content of the sapwood was

reduced at and above the point of infection, suggesting that the colonization of the sapwood blocked the sap flow to the crown.

Although ophiostomatoid fungi are termed blue stain fungi, the lesions caused by many species are not always blue, but can also be shades of brown, grey and black. The quality of the colour is largely affected by the water content of the wood, where saturated wood appears to have more pronounced lesions (Lagerberg *et al.* 1927). The staining of the wood is more an optical illusion because it is caused by pigmented hyphae growing in the tracheids and ray parenchyma cells (Seifert 1993). Along the cambium of an infected tree, stains can appear as lesions in long streaks down the length of the tree or in blotches where tissue is infected. In severe cases, sapstain can cover the entire perimeter of the stem. In cross-section, the stained wood appears wedge-shaped in the early stages of infection as fungal hyphae slowly work their way deep into the sapwood (Himelick 1982). The amount of stain is dependent on the species of ophiostomatoid fungus and duration of infection. Some species are highly pathogenic and cause deeply penetrating blue stain of the sapwood. Over time, the fungus can cause mortality of the tree, though most species are weakly pathogenic and cause little stain and no mortality (Mathre 1964; Basham 1970, Owen *et al.* 1987; Yamaoka *et al.* 1995).

Mathre (1964) discovered that mature ponderosa pine died in as little as 30 days when inoculated with pine sawdust and shavings mixed with mycelium of *Ceratocystis ips* (now *Ophiostoma ips* (Rumbold) Nannf.) over a 400cm^2 sapwood surface. However, when the fungus was used in smaller dosages in a separate study by Owen *et al.* (1987), it appeared to be much less pathogenic and resulted in almost no mortality of two-year old ponderosa pine seedlings over a 100-day period. In Owen *et al.*'s (1987) study, it was observed that *Ophiostoma clavigerum* (Rob.-Jeffr. and Davids.) Harr. was highly

pathogenic when artificially inoculated onto two-year ponderosa pine seedlings and was capable of causing up to 75% mortality of the seedlings within 100 days. The fungus was also capable of killing mature lodgepole pine trees within a year in a separate study by Yamaoka *et al.* (1995). A problem with the latter two studies was that they used a high amount of fungal inoculum that was not representative of natural inoculation by bark beetle vectors, even during large bark beetle outbreaks. When milder dosages, more representative of natural inoculum, were used of the pathogenic species *Ceratocystis montia* (now *Ophiostoma montium* (Rumbold) von Arx), it was determined that two growing seasons were required for noticeable symptoms to occur (Strobel and Sugawara 1986). The study still resulted in mortality of several trees by the end of the second growing season, which suggested that the fungus could cause mortality of the tree without the presence of its bark beetle vectors.

The penetration of ophiostomatoid fungi is dependent on a number of factors, such as temperature, moisture content and the availability of oxygen (Lindgren 1942). Mycelial growth of blue stain fungi is highest at temperatures between 15-30°C with optimal growth at about 25°C. Growth begins to cease when temperatures rise above 40°C or decrease below 4°C (Solheim and Krokene 1998; Hoffman 2003). Moisture contents between 60% and 80% are ideal for mycelial growth, while growth begins to cease when the moisture content is higher than 120% due to the lack of available oxygen and below 20% from the lack of moisture in the wood (Hoffman 2003). The moisture content values are based on an oven-dry weight method, where moisture content over 100% means there is more water in the wood than there is dry wood. Solheim and Krokene (1998) examined two species of *Ophiostoma* and suggested that highly virulent strains of ophiostomatoid fungi appeared to be more tolerant to oxygen deficiency and

grew at a quicker rate than less virulent strains. The authors examined growth patterns of *O. clavigerum* and *O. montium* under oxygen-deficient conditions by inoculating the two fungi into air-tight tubes of malt agar filled with nitrogen gas. *Ophiostoma montium* was able to grow slowly under oxygen-deficient conditions, but ceased after 11-18 days. *Ophiostoma clavigerum* did not appear to be affected much by the oxygen-deficient conditions and grew quicker than *O. montium*. *Ophiostoma clavigerum* was therefore considered to be more virulent. Under natural conditions, bark beetle galleries slightly increase the aeration under the bark, allowing these fungi to grow at a much quicker rate. Solheim and Krokene (1998) stated that high growth rate may be an adaptation to allow rapid colonization of the tree tissues before defences have been mobilized.

Bark Beetles and Their Mutualism With Ophiostomatoid Fungi

Bark beetles (Family Scolytidae) have long been recognized as vectors of blue stain fungi and have the ability to cause extensive damage to forest ecosystems through their mutualism (Malloch and Blackwell 1993, Schowalter and Filip 1993). There are two major groups of bark beetles: Xylomycetophagous bark beetles, or "ambrosia beetles", and phloeophagous bark beetles, or "true bark beetles" (Kirisits 2004). Ambrosia beetles are of little interest to this thesis because most of their associated fungi, known as ambrosia fungi, are not members of the Ophiostomatales. Also, ambrosia beetles do not feed on the host tree's phloem, but the adults bore into the xylem, lay eggs and cultivate their associated ambrosia fungi as a food source for developing larvae. True bark beetles include a larger group of bark beetles which live and breed under the bark, while feeding on the host tree's phloem. These beetles are of

particular interest because they carry along with them a variety of associated fungi, many of which are members of the Ophiostomatales.

Bark beetles are typically attracted to stressed, dying or dead trees (Schowalter and Filip 1993, Raffa *et al.* 1993, Paine *et al.* 1997). When trees become stressed by injury or by their surrounding environment, their defence against bark beetle attacks becomes impaired and the threshold number of beetles necessary to overcome the defences becomes smaller (Paine *et al.* 1997). In some cases spores can be introduced to healthy trees when there is an outbreak of beetles, such as the mountain pine beetle (*Dendroctonus ponderosae* Hopk.) in British Columbia (Kim *et al.* 2005a) or the spruce bark beetle (*Ips typographus* L.) in Central Europe (Wermelinger 2004). These conditions normally occur in pure stands of a single host tree or after environmental stressors (*e.g.* storm damage, drought, poor site conditions, other insect or disease outbreaks, etc.) which leave trees in a weakened state over a large area (Schowalter and Filip 1993; Paine *et al.* 1997). Forestry practices can also encourage bark beetle outbreaks (Dowding 1984). This occurs when there is dense planting and delayed thinning of monocultures of a susceptible tree species. At maturity, the thinning or final harvest of these stands creates a suitable habitat for bark beetles due to several factors, including the low availability of water on the site by the over crowding of trees and mechanical damage to the stems of living trees (Dowding 1984). The stumps that are left behind after harvest can also contribute to increasing beetle populations by attracting root and collar insects, including many bark beetle species (Dowding 1984). However, very few bark beetle species are aggressive and capable of colonizing and killing healthy living trees (Raffa *et al.* 1993).

Lieutier (1993) explained that conifers have evolved two defence responses to resist colonization by bark beetles. To prevent beetle invasion, most conifers have a preformed resin system located throughout the tree. Preformed resin ducts are not found in some conifers, such as *Abies*, *Tsuga* and *Cedrus* nor are they found in hardwoods (Paine *et al.* 1997). When a bark beetle bores through the bark, resin ducts are disrupted, resulting in a flow of resin that prevents entry of the beetle (Lieutier 1993). The resin is composed of anti-microbial and insecticidal compounds, which functions in cleaning the wound and sealing the entrance hole upon hardening (Dowding 1984; Paine *et al.* 1997). The second response occurs a few hours after the initial attack and is known as the "induced" or "hypersensitive response" (Lieutier 1993). In conifers, the vascular tissue is killed at the point of infection as resin enters and plugs up the sieve cells and tracheids. This response is induced by the host to prevent the establishment of bark beetles and their associated fungi (Lieutier 1993). In hardwoods, a similar response takes place involving tyloses (Dowding 1984). After the initial attack by a bark beetle, an outgrowth of parenchyma cells expands into vessel cells at the point of infection, stopping water flow. These outgrowths are impregnated with tannins, which in turn hide plant proteins from invaders. This is beneficial by preventing the establishment of associated fungi; however bark beetles are capable of eating the living tissue of the phloem at a quicker rate than it takes for the tannins to form (Dowding 1984). Therefore a heavy infestation of beetles can easily overcome the tree's defences.

Raffa *et al.* (1993) grouped bark beetles into three categories based on their ecological strategies: saprophytic, facultative parasitic and nearly obligate parasitic. Saprophytic, or opportunistic, bark beetles are the most common strategists. These bark beetles feed on dead plant material and rarely colonize living trees (Raffa *et al.* 1993,

Paine *et al.* 1997). Facultative parasitic, or secondary parasitic (Paine *et al.* 1997), bark beetles are typically saprophytic, but can parasitize stressed trees (Raffa *et al.* 1993). Under favourable environmental conditions, these strategists can attack healthy trees in high numbers, resulting in host mortality. Outbreaks are usually not severe or extensive (Paine *et al.* 1997). Nearly obligate parasitic, or primary parasitic (Paine *et al.* 1997) bark beetles can colonize healthy living trees and kill them with their large numbers. In cases where the host's defences have not been depleted, the beetles will continue to release aggregation pheromones until the host's defences are overcome. When beetle populations are low, however, healthy trees can normally withstand their attacks (Raffa *et al.* 1993, Paine *et al.* 1997). This is a necessary process for the bark beetle because the death of the tree determines the success of beetle reproduction (Sauvard 2004).

Basic Life Cycle of Bark Beetles

Most species of bark beetles share the same basic life cycle, which could be repeated several times a year depending on species, climatic and environmental conditions and geographic location (Sauvard 2004). Sauvard (2004) listed three phases to the basic life cycle of bark beetles: 1) reproduction, 2) development and 3) maturation and dispersal.

The reproduction phase begins when a mature bark beetle finds its mate on a host tree. Adults are often attracted to a host tree by pheromones produced by other bark beetles of the same species or by primary attractants, such as ethanol produced by the fermentation of tissue by bacteria and yeasts during an injury to a host tree (Bright 1993). Many species aggregate: large numbers of adults swarm to a single host over a short period of time, which is necessary for the mating process (Sauvard 2004).

Although some species mate on the surface of the host, most species mate under the bark; however beetles will not produce galleries and begin breeding until the host resistance stops and death of the tree or branch is certain (Paine *et al.* 1997). Depending on the species, the male or female bores a mating chamber in the phloem under the bark and waits for the arrival of the opposite sex. After mating, the female bores a breeding gallery in a new host or in the same mating chamber and lays her eggs singly or in groups on the sides of the gallery (Dowding 1984; Sauvard 2004). The pattern of the galleries is often species specific.

The entire development phase takes place under the bark, where three to five larval stages occur before pupation (Sauvard 2004). The eggs hatch within seven to ten days (Dowding 1984). Upon hatching, the larvae begin to move away from the breeding gallery, feeding on the phloem and moulting to larger instars. Before pupation occurs, the larvae build a small chamber in the phloem or outer bark (Sauvard 2004).

The final phase begins after pupation and includes the time it takes for the adult bark beetle to mature until mating is completed. After pupation, the adult requires some time to mature before it searches for a suitable host. During this time, it feeds on the phloem and stores energy reserves while its genitals develop and exoskeleton hardens (Sauvard 2004). Once the adult is ready to leave its gallery, it will either exit through a previously bored entrance hole, through a crack in the bark or, if necessary, will bore its own exit hole. At this point, the adult searches for a new host and mate and the cycle continues.

In climates such as Canada, bark beetles must undergo an overwintering period. When temperatures drop below 5-10°C, reproduction and development of bark beetles begin to stop (Sauvard 2004). Bark beetle larvae have a low tolerance to cold

temperatures and will die when temperatures drop below freezing, while adult beetles can tolerate below freezing temperatures (-15 to -30°C) before succumbing (Sauvard 2004). Therefore, bark beetles overwinter as adults. The overwintering process occurs for most species under the bark, where the temperature is higher than the air temperature and where snow sometimes acts as an insulator, especially for bark beetles breeding in logs on the forest floor. In some cases, such as *Ips typographus*, the adults overwinter in the forest litter (Sauvard 2004).

Adaptations of Blue Stain Fungi for Dispersal by Bark Beetles

Bark beetles are known to carry with them many microorganisms, including fungi, bacteria, mites and nematodes, making their associations quite complex (Dowding 1984; Stephen *et al.* 1993; Paine *et al.* 1997). Although many yeasts and members of the Basidiomycota are known to be associated with bark beetles, members of the Ascomycota, particularly ophiostomatoid fungi, are more commonly associated (Dowding 1984; Paine *et al.* 1997; Kim *et al.* 2005a). This is likely due to the ability of the fungi to grow on wood with high moisture content and survive under oxygen-deficient conditions (Solheim and Krokene 1998).

Bark beetles are largely responsible for the local and long distance dispersal of spores. Malloch and Blackwell (1993) listed several adaptations ophiostomatoid fungi have developed to make this possible. One of the most important adaptations for dispersal is the shape and adhesiveness of the spores. The sexual and asexual spores of ophiostomatoid fungi are typically produced in liquid droplets consisting of water and adhesive chemicals to aid the spores in adhering to passing bark beetles (Malloch and Blackwell 1993). Ascospores have a slightly greater advantage for dispersal than

conidia. This is because ascospores of most ophiostomatoid fungi have one or more concave surfaces, which allows the spore to adhere to the vector's body at more than one point (Malloch and Blackwell 1993). This allows ascospores to adhere to the vector for a longer period of time before being rubbed off, making them better adapted for long-distance dispersal. Conidia (produced by the anamorphic states), on the other hand, do not have concave surfaces. Therefore they only adhere to the vectors body by one point and are easily wiped off. This is good for local dispersion along the beetle galleries, especially since the anamorphic states of ophiostomatoid fungi germinate and establish quickly on bare substrates, allowing the fungus to spread quickly in the galleries (Malloch and Blackwell 1993).

Whitney and Blauel (1972) examined ascospore dispersion of eleven ophiostomatoid fungi in pine resin and distilled water. Masses of ascospores were stirred into a drop of resin and distilled water on separate Petri dishes containing water agar. The authors noticed that the masses of ascospores for most of the species examined broke up into single ascospores within the resin, while the masses did not break up as well in distilled water. The authors concluded from these results that ascospores are likely dislodged from the vector when it encounters resin as it bores into its host. The authors also examined the germination rate of the ascospores and observed that the ascospores mixed in the resin only germinated when they were in contact with the water agar. Whitney and Blauel (1972) suggested that because ascospores appear to be incapable of germinating when submerged in resin, a tree that exudes a large amount of resin upon invasion of a vector will reduce successful establishment of the fungus by flushing spores away from the wound and preventing the ascospores from coming in

contact with the wood. However, the ability of ascospores to disperse in the sap increases their chance of contacting the sapwood and establishing.

Another adaptation blue stain fungi have developed is the height of the conidiophores and ascomata (Malloch and Blackwell 1993). In the galleries, many ophiostomatoid species produce ascomata with long necks where ascospores collect at the apex in a sticky droplet. Some anamorphic states, such as *Leptographium* and *Pesotum* species, produce long conidiophores resembling a paint brush, where conidia are produced in a sticky droplet. As a beetle passes by, they brush against these droplets and carry spores for local dispersion or to a new host.

Adaptations of Bark Beetles to Vector Blue Stain Fungi

Ophiostomatoid fungi can positively or negatively affect bark beetles. Some of the benefits include plugging up the tree's vascular system and weakening the tree's defences against invading beetles, lowering the moisture content in the wood and providing a more favourable environment for the beetle brood (Ballard *et al.* 1984; Paine *et al.* 1997; Kim *et al.* 2005a; Romón *et al.* 2007). Ophiostomatoid fungi can also negatively affect bark beetles by inhibiting the development of essential fungi, such as yeasts, which are vital for larval feeding (Harrington 1993; Paine *et al.* 1997; Kirisits 2004; Romón *et al.* 2007).

There are several ways in which spores of blue stain fungi are transported by bark beetles. As previously mentioned, spores can adhere to the beetle's body surface with their mucilaginous coating. Other ways spores can be carried are in invaginations on the beetle's exoskeleton, in cuticular pits known as mycangia or internally through their digestive tract (Solheim 1993; Paine *et al.* 1997; Kirisits 2004; Kim *et al.* 2005a).

Mycangia are specialized structures often resembling a pouch or cavity in the cuticle where fungal spores and mycelia are carried and stored. Mycangia are not present in all bark beetle species, but when present, they can be located in the thorax, mandible, maxilla, head, posternum and/or elytra of the beetle (Paine *et al.* 1997). Within these cavities, glandular cells protect the fungal spores by producing secretions to keep the spores viable until they are released (Kirisits 2004).

Although ophiostomatoid fungi are often associated with mycangia, this is not likely the purpose of these structures because nutritionally beneficial yeasts are more commonly associated with mycangia than any ophiostomatoid species (Harrington 1993). This association is clearly present in the early stages of breeding development, since yeasts are among the most frequent fungi isolated from the wood and beetles (Kirisits 2004). Bark beetles can carry several yeast taxa in their mycangia often belonging to the genera *Candida* Berkhout, *Pichia* E.C. Hansen, *Hansenula* Syd. & P. Syd., *Saccharomyces* Meyen ex. E.C. Hansen and *Cryptococcus* Vuill. (Kirisits 2004). Yeasts are essential for early larval development because they are a nutritionally important part of the diet because wood is a nutrient deficient substrate (Harrington 1993; Paine *et al.* 1997; Kirisits 2004). When ovipositing, beetles will choose unstained wood so mycangial fungi can establish and inhibit the growth of blue stain fungi. If, for example, a blue stain fungus establishes first, it could inhibit the growth of essential mycangial fungi. This, in turn, would lower beetle reproduction by reducing the beetles' source of nutrients (Harrington 1993; Paine *et al.* 1997). Yeasts are an advantage to the beetle strictly for nutrition since they are not pathogenic to host trees (Kirisits 2004).

In a recent study, Cardoza *et al.* (2006) isolated *Ophiostoma abiocarpum* (Davidson) Harr. from nematode-harbouring structures, referred to as nematangia, found

on the hind wings of *Dendroctonus rufipennis* Kirby. The nematangia were present on 60% of the bark beetles and *O. abiocarpum* was extracted from 82% of the nematangia through a series of dilutions. More research is currently being done in order to see if more bark beetles possess these specialized structures.

ECONOMIC IMPACT OF BARK BEETLE OUTBREAKS

Bark beetles are among the most destructive forest insects in North America due to their ability to attack and kill healthy trees during outbreaks and devaluing the wood by introducing blue stain fungi. In recent years, much of North America has been experiencing a warmer than normal climate which is having a huge impact on beetle populations. Due to the dynamic nature of the boreal forest to subtle changes in the environment, it is believed that the boreal forest will be one of the planet's ecosystems most affected by climate change (Ogden 2008). The geographic distribution of plant communities or individual tree species will change with the changing climate, where some tree species will struggle to survive and others will become more successful (Paine and Baker 1993). Due to current trends, bark beetle activity has been increasing in areas that are receiving smaller amounts of annual precipitation and above normal temperatures. Drier summers will stress trees and make them more susceptible to bark beetle infestation. Warmer winters will result in fewer beetles succumbing to freezing temperatures and warmer summers will increase the growing season along with the length of exposure of host trees to bark beetle infestation. This would result in more infected trees as well as larger beetle populations caused by the increase of beetle broods per annum (Paine and Baker 1993; Ogden 2008).

Although most bark beetle species do not pose a threat to the forest industry, several examples exist of bark beetles and their associated fungi causing immense economic impact in North America. In the Yukon, the spruce bark beetle (*Dendroctonus rufipennis*) has been responsible for killing white spruce over a stretch of 380,000 hectares of land since 1994, which is the most severe infestation of this beetle ever recorded in Canada (Ogden 2008). This outbreak is thought to be the direct result of climate change, where climatic conditions have been warmer and drier than normal. As a result, white spruce in the Yukon have experienced more moisture stress and have become more susceptible to attacks of the spruce bark beetle (Ogden 2008). The Territory has integrated pest management strategies to salvage beetle-infested timber before it loses its value as well as to reduce beetle populations to an endemic level by controlling smaller attacks around the region most affected by the outbreak (Ogden 2008).

In current history, the most recognized example of a bark beetle outbreak in North America is the mountain pine beetle outbreak in British Columbia, which has had a devastating impact on lodgepole pine (*Pinus contorta* Douglas var. *latifolia* Engelmann). Three blue stain fungi are known to be associated with this beetle, namely *Ophiostoma clavigerum*, *Ophiostoma montium* and *Leptographium longiclavatum* Lee, Kim & Breuil (Rice *et al.* 2008). These fungi contribute to the mortality of infected trees and can stain the sapwood of infested trees within a few weeks (Rice *et al.* 2008; Kim *et al.* 2005a). Over the past few years, the mountain pine beetle has been responsible for the death of millions of lodgepole pine in British Columbia (Rice *et al.* 2008). This is a liability to the forest industry because Canada is the largest exporter of forest products, especially to the United States, and forest companies in British Columbia depend on

high quality timber for their wood products (NRCan 2007). Currently the mountain pine beetle outbreak has spread eastward into Alberta and into lodgepole pine x jack pine hybrid forests. This has resulted in an alarming concern across the country because the natural range of jack pine extends all the way to the east coast. However, the beetle's ability to infest natural jack pine populations is still unknown (Rice *et al.* 2008).

By the fall of 2006, it was estimated that 582 million cubic metres of trees in British Columbia were infested with the mountain pine beetle, which was an increase of 171 million cubic metres since 2005 (NRCan 2007). By early 2007, the epidemic had spread into Alberta, infesting over 3 million trees. If the infestation continues, it is estimated that 80% of the mature lodgepole pine in British Columbia could be gone by the end of 2013 (NRCan 2008b).

The Federal Mountain Pine Beetle Program was set up in 2006 by the Government of Canada, allocating $200 million to control the spread of the mountain pine beetle outbreak, to recover the economic value from beetle-killed timber and to help protect affected communities from the economic impact of the infestation (NRCan 2007). In Alberta, an action plan was immediately initiated by the government and forest industry to survey and control the beetle as soon as it crossed the border. Harvesting infected stands, felling and mulching single trees and prescribed burns were among the treatments utilized in Alberta's action plan. Crews were also sent out in helicopters to identify and destroy infested trees as well as setting pheromone traps in remote areas (NRCan 2007).

POTENTIAL SIGNIFICANCE OF THE PRESENT STUDY

Very little has been studied on the impact bark beetles and their associated fungi have on forest communities throughout many areas of North America. This is especially true in areas where there have been no economically important outbreaks. Concerns about the impact of these organisms on the economy are usually not taken seriously until severe outbreaks occur. Often by this point the outbreak cannot be controlled and strategies are instead put in place to salvage infested timber and control smaller attacks in the area (Ogden 2008). The two examples of bark beetle outbreaks mentioned previously illustrate this point, where extensive research was not implemented until the outbreaks were unmanageable. This trend is familiar throughout the world. In central Europe, an outbreak of the European spruce bark beetle (*Ips typographus* L.) occurred after severe storms in the 1990's, which prompted extensive research on the bark beetles and the complex interactions of their associated fungi (Wermelinger 2004). Research is required to survey forest communities across the country and observe which beetles and ophiostomatoid fungi are present in a given area. This way, when a particular beetle population begins to grow, researchers can predict the extent of damage and which control measures to use.

Even though associations between bark beetles and ophiostomatoid fungi have been studied since the mid twentieth century, many new associations are still being discovered. Due to the current outbreaks caused by the changing climate, people are becoming increasingly aware of these associations and demanding to know more information. Consequently, researchers are designing surveys in forest communities that have not previously been examined and are discovering new species of blue stain fungi as well as broader geographic ranges of bark beetles and associated fungi.

For example, Romón *et al.* (2007) examined the galleries of Monterey pine (*Pinus radiata* D. Don) in northern Spain, where thirteen bark beetle species were collected from three stands of Monterey pine. They observed that each bark beetle species had at least one primary ophiostomatoid fungus associated with it and up to ten additional associated species. Of the sixteen different *Ophiostoma* species isolated from the beetles and their galleries, many were new associations that had not previously been recorded. Alamouti *et al.* (2007) isolated fungi from bark beetles and their galleries on white spruce in northern British Columbia and the Yukon. In this study, three species of bark beetles were observed under the bark, most being the northern spruce engraver (*Ips perturbatus* Eichhoff). Thirteen species of ophiostomatoid fungi were isolated and identified, with the most commonly isolated fungus from *Ips perturbatus* being described as a new species. Four other ophiostomatoid fungi were commonly associated with *Ips perturbatus*, while others appeared to be more sporadic.

Currently, very little is known about bark beetles and their associated fungi in northwestern Ontario because no extensive surveys have been published. Bright (1976) published a compilation of bark beetles known, or suspected to occur, in Canada based on reference collections from the Canada Department of Agriculture and various forestry laboratories across Canada. Though this proves to be a strong representative of the bark beetle species known throughout Canada, extensive studies examining bark beetle galleries have not been published in northwestern Ontario.

Wright and Cain (1961) described four new species of *Ceratocystis* on conifers in Ontario. Collections were made by sampling conifer slash in forested areas around southern Ontario, but did not include northwestern Ontario. A few years later, a survey of ophiostomatoid fungi in Ontario was published by Griffin (1968), yet the samples

examined in this study had been collected on field trips throughout Ontario and did not include an extensive study in northwestern Ontario. Mussell (2004) completed a preliminary survey of bark beetles and their associated ophiostomatoid fungi on jack pine in the Thunder Bay region as an Honour's project. By examining bark beetle galleries on felled jack pine, she observed two bark beetle species and isolated several species of ophiostomatoid fungi. The bark beetles examined in her study were *Ips pini* (Say) and *Polygraphus rufipennis* (Kirby), which yielded several species of *Ophiostoma* (including *O. ips* and *O. piceae* (Münch) Syd. & P. Syd.) and several unidentified ophiostomatoid fungi in the genera *Leptographium*, *Pesotum*, *Sporothrix* and *Hyalorhinocladiella*. Mussell's (2004) survey of fungal associates of bark beetles on jack pine in the Thunder Bay region had promising results; however, the study was relatively small and it was suggested by the author that more research about these interactions on jack pine is necessary.

Many studies have been established to examine fungal associates of destructive bark beetles, such as the mountain pine beetle, however very few studies have been published about the less aggressive species (Alamouti *et al.* 2007). Less aggressive bark beetles are often not studied because they are generally saprophytic and rarely colonize living trees (Raffa *et al.* 1993). However, some species could have associated fungi that are highly pathogenic and an outbreak of the beetle could lead to disastrous results.

More research is required to expand the taxonomic range of bark beetles and ophiostomatoid fungi and to understand their associations with each other. The Thunder Bay region has not received any economically important outbreaks of bark beetles and, as a result, no studies have been published on bark beetles and their associated fungi in the area. Currently, there is a small outbreak of the eastern larch beetle (*Dendroctonus*

simplex LeConte) in Rosyln village, which is located just west of Thunder Bay (OMNR 2008b). This small outbreak is believed to be the result of a larger epidemic of the eastern larch beetle in Minnesota, which has resulted in high mortality of tamarack in over 65,000 acres of Minnesota's forests (MDNR 2008). The outbreak appears to be linked to extended periods of drought in the past decade (MDNR 2008). With a changing climate that could favour bark beetle epidemics, it is vital that we understand the effects of potential outbreaks by first identifying the endemic bark beetles and their associated fungi in the area. This study will provide a preliminary look at the biodiversity of bark beetles and their associated ophiostomatoid fungi in the Thunder Bay region to reach a better understanding about the associations in the area as well as identifying their potential pathogenic nature through the use of literature.

CHAPTER II
YEAR ONE FIELD AND LAB STUDY

PURPOSE

An early scientific study was established to investigate the presence of conifer-inhabiting bark beetles and their associated ophiostomatoid fungi in the Thunder Bay region. To achieve this, a single balsam fir, black spruce, jack pine and tamarack each were felled as bait trees during the summer of 2006 and destructively sampled for bark beetle galleries. Galleries were examined for the presence of bark beetles, and ophiostomatoid fungi were isolated from both the beetles and their galleries. The information collected from this survey will attempt to fill in some of the gaps in the literature by defining any new associations that might exist between bark beetles and ophiostomatoid fungi identified in this study. Finally, a summary was conducted from the literature of the pathogenicity for each of the bark beetles and ophiostomatoid fungi identified in the present study.

MATERIALS AND METHODS

STUDY AREA

The study area was established at Jack Haggerty Forest, located approximately 36 kilometres north of Thunder Bay in Fowler and Jacques Townships and covering a total area of 1039.1 hectares (Anderson *et al.* 2007). The property is owned by Lakehead University and managed for educational purposes by the Faculty of Forestry and the Forest Environment since 1953 (Luckai 2009). Entrance to the study area is located in Block 14 on Beaverkit Lake Road, which branches off Island Lake Road. The study area has a latitude of 48°38' N and a longitude of 89°23' W.

SAMPLING DESIGN

A total of four healthy trees of different species (balsam fir, black spruce, jack pine and tamarack) showing no signs of disease or decay was selected for this study. On May 26, 2006, each tree was felled and left on the forest floor in an opened area for five months. The felled trees were inspected on October 16, 2006 for the presence of bark beetle entry and exit holes. Sections of areas on the trees that were visibly infested with bark beetles were cut into longitudinal slabs with a chain saw and placed in plastic bags. The bags were labelled with the tree species and collection date. The longitudinal slabs were further cut into10cm x 10cm sections with a band saw by Dr. M. Leitch (Faculty of Forestry and the Forest Environment, Lakehead University). The sections were rebagged and stored in the forestry freezer until examined.

ISOLATION AND IDENTIFICATION OF BARK BEETLES AND FUNGI

Sampling for ophiostomatoid fungi began on October 21, 2006. A plastic bag containing the sections of jack pine was removed from the forestry freezer and two or three of the sections were allowed to thaw out overnight in the laboratory refrigerator. The remaining sections were placed back into the freezer to reduce the amount of mould growing on the wood sections and would be sampled at a later date. Wood sections were not kept in the refrigerator for longer than a single night to reduce contamination by moulds, such as *Penicillium* spp., due to moisture accumulating in the bag. This procedure was used for all tree species throughout the study.

Modified 2% malt extract agar [20g Bacto™ malt extract, 1g Bacto™ yeast extract (DIFCO certified), 15g Bacto™ agar and 1000mL distilled water], was employed as the standard medium for isolating ophiostomatoid fungi. Each batch of medium was placed in 2L flasks and placed in an autoclave for 30 minutes at 121°C and at 1.7kg/cm² chamber pressure. Once removed from the autoclave, the flasks were placed in a water bath at 45°C until the medium was cool enough to handle. Antibiotics (30mg SIGMA streptomycin sulphate and 300mg SIGMA penicillin-G) were then added and mixed into the medium to reduce the amount of bacteria growing out of the isolations. Once mixed, the agar was poured into sterile 9cm plastic Petri dishes under a transfer hood and left overnight to harden and to reduce the amount of condensation on the Petri dish lids. The plates were then wrapped in parafilm™ to prevent drying of the agar.

Before any sampling was taken from the wood sections, the work area was surface sterilized with 70% ethanol and all tools sterilized with a flame to kill any fungal or bacterial spores present. Since bark beetles feed on the cambium and phloem tissues of the tree, their galleries were visible directly under the bark. The bark of each sample

was carefully peeled off using a sterilized knife and examined under a dissecting microscope for the presence of bark beetles and evidence of ophiostomatoid fungi. Five sampling methods for the extraction of ophiostomatoid fungi were implemented on each tree: 1) adult washings, 2) larval washings, 3) sampling from ophiostomatoid fruiting bodies and conidiophores, 4) sampling from stained wood tissue and 5) sampling from bark beetle frass.

Adult bark beetles and their larvae were removed from their galleries using a pair of sterilized forceps and placed into separate sterile 2mL plastic vials filled with distilled water. The vials were shaken for about 30 seconds and the contents poured onto a fresh Petri dish of agar. The adult beetle or larva was then removed and placed into a 2mL plastic vial containing 70% ethanol and labelled for later identification.

Isolations from the fruiting bodies or conidiophores of ophiostomatoid fungi were taken by either plucking entire perithecia with a sterilized needle or by collecting spore masses at the apices of mature perithecia or conidiophores. Spore masses were collected by placing a small piece of agar on the tip of a sterilized needle, touching the spore mass with the agar, and inoculating a fresh Petri dish of agar. Four fruiting bodies or spore masses from the same fungal species were inoculated per Petri dish.

Isolations from the stained wood were sampled from within or along the edges of the bark beetle galleries using a sharp sterilized scalpel blade. Four chips of stained wood were removed from the same gallery and inserted in a fresh Petri dish of agar so that half of each wood chip was inserted into the agar. Finally, the same process was used for isolations of the bark beetle frass, where four pieces from a single gallery were removed with a sterilized needle and placed on a fresh Petri dish of agar.

A unique code was given to each of the cultures to indicate which tree each isolate came from and which sampling method was used (Table 2). For example, the code 'BFb_1' would represent an isolate which was taken from balsam fir and was the first isolation taken from a fruiting body or conidiophore on that tree. If the isolation was taken from stained wood around or within a gallery, a 'g' would replace the 'b' and a 'w' would replace the 'b' if an isolate taken from an adult bark beetle washing, and so on. If more than one fungus was isolated from a single sampling method, as was often the case, the letter 'a', 'b', 'c', etc. was used. For example, the code 'BFb_{1c}' would represent the third of at least three fungi isolated from the first fruiting body isolated from balsam fir.

Table 2: Explanation of the coding system used in the year one field and lab study

Code	Description
BFb_{1a}	The **first fungus** isolated from the **first fruiting structure** from **balsam fir**
BFb_{1b}	The **second fungus** isolated from the first fruiting structure from balsam fir
BFb_{1c}	The **third fungus** isolated from the first fruiting structure from balsam fir
BFb_{2a}	The first fungus isolated from the **second fruiting structure** from balsam fir
BFf_{1a}	The first fungus isolated from the first **frass sample** from balsam fir
Sbl_{1a}	The first fungus isolated from the first **larval washing** from **black spruce**
Tw_{1a}	The first fungus isolated from the first **adult washing** from **tamarack**
Pjg_{1a}	The first fungus isolated from the first **stained wood sample** from **jack pine**

After inoculations were placed onto a Petri dish of agar using any of the above mentioned sampling methods, the Petri dish was rewrapped in parafilm™ and labelled with tree species, the sampling method used and the date of isolation. This information was also recorded in a laboratory book. The Petri dishes were then placed upside down (to reduce condensation build-up) in an incubator set at 20°C. The developing cultures were left in the incubator for three or four days to allow for mycelial growth. Anything that grew out from the initial isolation was transferred onto a fresh Petri dish of agar. To

ensure purification, a small section of hyphae was removed from the edge of a colony. The Petri dishes were placed back into the incubator to be identified at a later date.

Identification began when the cultures got older. Each isolate was prepared on a glass slide using a 1% aqueous solution of Phloxine and examined through a compound microscope for morphological features. Many of the cultures were sterile for the first couple of months and had to remain incubating until they showed signs of spore production. Fungi were named down to genus, and where possible to species by referring to taxonomic keys and monographs of ophiostomatoid fungi (Griffin 1968; Olchowecki and Reid 1974; Upadhyay 1981; Hutchison and Reid 1988a; Grylls and Seifert 1993; Jacobs and Wingfield 2001). If a fungus could not be identified, the culture was placed back in the incubator until the development of spores or any other morphological characteristics that would make it possible to identify the fungus.

Cultures that remained sterile over a period of time and anamorphs of ophiostomatoid fungi not producing their teleomorphic state were transferred to a fresh Petri dish of agar containing a piece of sterilized wood. The wood pieces were of the same tree species from which the fungus was isolated and was used to help induce the fungus to sporulate or form perithecia (Hutchison and Reid 1988a). Wood chips were cut from each tree species using a sharp razor blade and were roughly 4cm x 2cm in size and about 1mm thick. The wood chips were then wrapped in aluminum foil and sterilized in the lab's autoclave prior to being added to Petri dishes containing 2% malt extract agar. Another way to induce sporulation and the formation of perithecia was to place cultures in a light chamber (Hutchison and Reid 1988a), where they were exposed to a sixteen hour light/eight hour dark regime.

Representatives of ophiostomatoid fungi were transferred into agar slants and placed in the refrigerator to inhibit growth after the fungus had become established. These representatives were sent to Dr. G. Hausner and J. Reid at the Department of Microbiology, University of Manitoba, to confirm identity or provide a potential name that could be given to the representatives. At the University of Manitoba, rDNA data was obtained and the results compared in the internal transcribed spacer (ITS) sequence database. The exact methods and primers used for the ITS analysis can be found in Hausner *et al.* (2005) and Hausner and Wang (2005). Bark beetles were tentatively identified by L. Sevean and S. Andersen through the use of taxonomic keys (Bright 1976; Wood 1982) and sent out to Dr. D. Bright at Colorado State University for verification.

STATISTICAL ANALYSES

The Simpson's diversity index (D) (Simpson 1949) was used to measure the sample diversity of all ophiostomatoid fungi isolated from their host tree and each bark beetle species. This index is the most suitable and recommended diversity index to date when working with small sample sizes (less than 1000 individuals) (Mouillot and Leprêtre 1999). The Simpson's diversity index was designed to take into account the number of each ophiostomatoid species isolated from its host as well as the relative abundance of each species isolated. The index measures the probability that two ophiostomatoid fungi randomly isolated from the host tree belong to the same species. The index is defined as:

$$D = 1 - \sum_{i=1}^{i=S} p_i^2$$

where p_i is the relative abundance of the ith species in a sample containing S species. The value of D ranges between 0 and 1, where 0 means no diversity and 1 means infinite diversity. Therefore the greater the value, the greater the probability is that two randomly isolated individuals will belong to different species. Fungal dominance was calculated using Camargo's index ($1/S$) (Camargo 1993), where S represents species richness, which is defined as the number of competing species observed in a community. A species was considered dominant if $p_i > 1/S$.

RESULTS

FUNGAL DIVERSITY OF OPHIOSTOMATOID FUNGI

The sampling methods performed on the four tree species in this study yielded a large diversity of ophiostomatoid fungi (Table 3). A total of 93 isolates of ophiostomatoid fungi were isolated from balsam fir, representing six different species. These species included *Ophiostoma bicolor* Davidson & Wells, *O. ips*, *O. rectangulosporium* Ohtaka, Masuya & Yamaoka, a *Hyalorhinocladiella* or *Sporothrix*-like fungus, a *Hyalorhinocladiella*-like fungus resembling the anamorph of *O. bicolor*, and a *Pesotum*-like fungus resembling the anamorph of *O. ips*. Three ophiostomatoid fungi were considered dominant based on Camargo's Index, including the *Hyalorhinocladiella* or *Sporothrix*-like fungus, *O. bicolor* and *O. rectangulosporium*. The latter fungus was the most frequently encountered and represented 30.1% of all the ophiostomatoid fungi isolated from balsam fir. The former two fungi each represented 22.6% of the ophiostomatoid cultures. The Simpson's Index of diversity for the ophiostomatoid fungi isolated from balsam fir was 0.79, which suggests that there is a 79% probability that two randomly isolated individuals will belong to different species.

Forty-one isolates of ophiostomatoid fungi were acquired from the sampling methods implemented on black spruce, which comprised of eight separate species, including *Leptographium fruticetum*, *O. ips*, *O. piceaperdum* (Rumbold) Arx, two unidentified *Ophiostoma* species, two *Pesotum*-like fungi (one of which resembled the anamorph of *O. ips*), and a *Sporothrix*-like fungus. *Ophiostoma ips* was the most commonly encountered ophiostomatoid fungus on black spruce, making up 35% of the

Table 3: Species of ophiostomatoid fungi isolated from balsam fir, black spruce, jack pine and tamarack at Jack Haggerty Forest (Isolation frequencies in parentheses)

Genus	Balsam Fir	Black Spruce	Jack Pine	Tamarack
Hyalorhinocladiella/Sporothrix-like	21[a] (22.6%)	-	-	-
[1]*Hyalorhinocladiella*-like #1	8 (8.6%)	-	-	-
[2]*Hyalorhinocladiella*-like #2	-	-	-	5 (8.1%)
Leptographium fruticetum	-	7[a] (17.1%)	-	4 (6.5%)
Leptographium abietinum	-	-	-	16[a] (25.8%)
[3]*Ophiostoma* #1	-	1 (2.4%)	-	-
[4]*Ophiostoma* #2	-	2 (4.9%)	-	-
[5]*Ophiostoma* #3	-	-	-	1 (1.6%)
Ophiostoma abietinum	-	-	-	2 (3.2%)
Ophiostoma bicolor	21[a] (22.6%)	-	-	-
Ophiostoma ips	6 (6.5%)	14[a] (34.1%)	2[a] (66.7%)	-
Ophiostoma minus	-	-	1 (33.3%)	23[a] (37.1%)
Ophiostoma piceae	-	-	-	1 (1.6%)
Ophiostoma piceaperdum	-	1 (2.4%)	-	
[6]*Ophiostoma pulvinisporum*	-	-	-	3 (4.8%)
Ophiostoma rectangulosporium	28[a] (30.1%)	-	-	-
[7]*Pesotum*-like #1	9 (9.7%)	5 (12.2%)	-	1 (1.6%)
Pesotum-like #2	-	1 (2.4%)	-	-
[8]*Pesotum*-like #3	-	-	-	2 (3.2%)
Sporothrix	-	-	-	4 (6.5%)
Sporothrix-like	-	10[a] (24.4%)	-	-
Total	93	41	3	62
Species Richness	6	8	2	11
Camargo's Index	0.17	0.13	0.50	0.09
Simpson's Diversity Index	0.79	0.84	0.44	0.78

[a]Dominant species. Species were considered dominant if the relative abundance (P_i) was greater than Camargo's Index ($1/S$)

[1]Resembling anamorph of *Ophiostoma bicolor*

[2]Resembling anamorph of *Ophiostoma* #3

[3]Containing a *Leptographium* anamorph

[4]Containing a *Sporothrix*-like anamorph

[5]Containing a *Hyalorhinocladiella*-like anamorph

[6]Containing a *Pesotum*-like anamorph

[7]Resembling anamorph of *Ophiostoma ips*

[8]Resembling anamorph of *Ophiostoma pulvinisporum*

ophiostomatoid cultures. *Leptographium fruticetum* and the *Sporothrix*-like fungus were also dominant species isolated from black spruce with isolation frequencies of 17.1% and 24.4% respectively. The Simpson's Index of diversity for the ophiostomatoid fungi isolated from black spruce was 0.84.

Eleven different species of ophiostomatoid fungi were identified from tamarack, consisting of a total of 62 isolates. The isolates consisted of five different *Ophiostoma* species, including an unidentified species with a *Hyalorhinocladiella*-like anamorph, *O. abietinum* Marm. & Butin, *O. minus* (Hedgc.) Syd. & P. Syd., *O. piceae* and *O. pulvinisporum* Zhou & Wingf. Six other ophiostomatoid fungi were also observed, including an unidentified *Hyalorhinocladiella*-like fungus, *L. fruticetum* Alamouti, Kim & Breuil, *L. abietinum* (Peck) M.J. Wingf., two unknown *Pesotum*-like fungi and an unidentified species of *Sporothrix*. Two species were considered dominant according to Camargo's Index. The most frequently isolated ophiostomatoid fungus isolated was *O. minus*, which represented 37.1% of the ophiostomatoid isolates on tamarack. *Leptographium abietinum* was also a dominant species, with an isolation frequency of 25.8%. The Simpson's Index of diversity for the ophiostomatoid fungi isolated from the tamarack samples was 0.78.

Only two species of ophiostomatoid fungi were isolated from jack pine, including *O. ips* and *O. minus*, and comprised of a total of three isolations. *Ophiostoma ips* was the dominant species, occurring twice out of the three ophiostomatoid fungi isolated successfully from jack pine. The Simpson's Index of diversity for the ophiostomatoid fungi isolated from jack pine was 0.44.

FUNGAL DIVERSITY ON BARK BEETLES ISOLATED FROM EACH TREE

A total of four bark beetles were isolated during the present study, including *Pityokteines sparsus* (LeConte) on balsam fir, *Polygraphus rufipennis* on black spruce and tamarack, *Ips pini* on black spruce and *Dendroctonus simplex* on tamarack. Each bark beetle yielded at least two species of ophiostomatoid fungi via adult bark beetle washings (Table 4). Isolation frequencies are based on number of times a particular fungus is isolated from each bark beetle species. Some adult bark beetles yielded more than one ophiostomatoid fungus while others did not yield any ophiostomatoid fungi. Therefore, the total isolation frequencies in Table 4 do not add up to 100%.

All of the bark beetles observed under the bark of balsam fir were of the species *Pityokteines sparsus* (LeConte). The balsam fir samples observed in this study were overwhelmed with an abundance of interwoven galleries of *P. sparsus*. The bark was easy to peel off and in some cases crumbled due to the high number of galleries. No larvae were observed in the galleries; however there were a few pupae present in the galleries and were used as a substitution for the larval washings. The adult beetle washings of *P. sparsus* yielded a total of 15 isolates of ophiostomatoid fungi, comprising five different species. The two dominant species isolated were a *Hyalorhinocladiella* or *Sporothrix*-like fungus and *Ophiostoma retangulosporium*. These fungi were isolated from 16.1% (5) of the adult beetle washings. *Ophiostoma bicolor* and *Hyalorhinocladiella*-like #1 were isolated from 6.5% (2) of the adult washings and *Pesotum*-like #1 was isolated once (3.2%) from an adult washing. The Simpson's Diversity Index for ophiostomatoid fungi found on adult bark beetles was 0.74.

The most abundant bark beetle species observed in galleries under the bark of the black spruce samples was *P. rufipennis*. *Ips pini* was less common and was only

Table 4: The number of bark beetles extracted from balsam fir, black spruce and tamarack and their associated ophiostomatoid fungi (Isolation frequencies in parentheses)

	Balsam Fir	Black Spruce		Tamarack	
Fungal Species	*Pityokteines sparsus*	*Polygraphus rufipennis*	*Ips pini*	*Polygraphus rufipennis*	*Dendroctonus simplex*
Hyalorhinocladiella/Sporothrix-like	5[a] (16.1%)	-	-	-	-
Hyalorhinocladiella-like #1	2 (6.5%)	-	-	-	-
Hyalorhinocladiella-like #2	-	-	-	1 (3.8%)	-
Leptographium abietinum	-	-	-	3[a] (11.5%)	1 (12.5%)
Leptographium fruticetum	-	5[a] (22.7%)	-	2 (7.7%)	-
Ophiostoma #2	-	1 (4.5%)	-	-	-
Ophiostoma bicolor	2 (6.5%)	-	-	-	-
Ophiostoma minus	-	-	-	5[a] (19.2%)	1 (12.5%)
Ophiostoma piceaperdum	-	1 (4.5%)	-	-	-
Ophiostoma rectangulosporium	5[a] (16.1%)	-	-	-	-
Pesotum-like #1	1 (3.2%)	-	1 (33%)	1 (3.8%)	-
Sporothrix	-	-	-	2 (7.7%)	-
Sporothrix-like	-	4[a] (18.2%)	3[a] (100%)	-	-
Total	15	11	4	14	2
Total number of beetles (n)	31	22	3	26	8
Species Richness	5	4	2	6	2
Camargo's Index	0.20	0.25	0.50	0.17	0.50
Simpson's Diversity Index	0.74	0.64	0.38	0.80	0.50

[a]Dominant species. Species were considered dominant if the relative abundance (P_i) was greater than Camargo's Index ($1/S$)

sampled from sparingly, as most of the adults were dead and partially consumed by predators (mostly members of the Hymenoptera and Diptera) in the galleries. Only fresh specimens were used for the adult washings to reduce the isolation of non-ophiostomatoid fungi. There was an abundant amount of galleries under the bark with a high number of *P. rufipennis* adults situated within them, but very few larvae were observed. There were many dark stains apparent under the bark, but they were primarily restricted to areas of the cambium colonized by larvae of round-headed wood borers (Family Cerambycidae) and flat-headed wood borers (Family Buprestidae).

A total of 22 adult bark beetles of *P. rufipennis* was selected for washings, yielding a total of 11 ophiostomatoid fungi. Only three representatives of *I. pini* were in ample shape to use for adult washings and yielded four ophiostomatoid fungi. Two dominant species of ophiostomatoid fungi were observed on *P. rufipennis*, including *Leptographium fruticetum* and the *Sporothrix*-like species. The first fungus was isolated from 22.7% (5) of the *P. rufipennis* adults and the *Sporothrix*-like fungus was isolated from 18.2% (4) of the adult washings. The *Sporothrix*-like fungus was also the dominant fungus isolated from the *I. pini* adults, occurring on all three specimens. The unidentified *Ophiostoma* #2 and *O. piceaperdum* were each isolated once (4.5%) from *P. rufipennis* and the *Pesotum*-like #1 specimen was isolated once (33%) from *I. pini*. The Simpson's Diversity Index for ophiostomatoid fungi found on *P. rufipennis* was 0.70 and 0.38 on the *I. pini* adults.

Polygraphus rufipennis was the most abundant species observed under the bark of tamarack and was therefore more frequently sampled than *D. simplex*. There was a moderate number of bark beetle galleries under the bark and a high number of *P. rufipennis* adults within the galleries. Wood borer larvae and their galleries were also

commonly encountered throughout the cambium, which often intersected bark beetle galleries. Very few bark beetle larvae were present in the galleries and in some cases pupae of *P. rufipennis* were used as a substitution for larvae. The only ophiostomatoid fungus isolated from bark beetle larvae/pupae was a single species of *O. minus*. The most frequently isolated fungi from larvae/pupae were common contaminants, such as *Penicillium* and *Candida* (see Appendix I).

A total of 26 *P. rufipennis* adults was selected for sampling, which resulted in 14 ophiostomatoid fungi of six different species. Two ophiostomatoid fungi were dominant, including *O. minus* and *L. abietinum*. The former fungus was isolated from five (19.2%) of the *P. rufipennis* adults and the latter was isolated three (11.5%) times. *Leptographium fruticetum* and the unidentified species of *Sporothrix* were both isolated twice (7.7%), while *Pesotum*-like #1 and *Hyalorhinocladiella*-like #2 were each isolated once (3.8%) from adult washings of *P. rufipennis*. Eight representatives of *D. simplex* were collected and sampled, yielding only a single isolate of *O. minus* and a single isolate of *L. abietinum*. Neither fungus isolated from *D. simplex* was considered dominant since the value for relative abundance was the same value as Camargo's Index. The Simpson's Diversity Index for the ophiostomatoid fungi found on *P. rufipennis* was 0.72 and was 0.5 for *D. simplex.*

Only two representatives of *I. pini* were sampled from jack pine and neither yielded ophiostomatoid fungi. The only other evidence of bark beetles under the bark were remnants (mostly belonging to *I. pini*) scattered throughout the bark beetle and wood borer galleries. The galleries were overwhelmed with bacteria, wood borers, predators and pockets of common moulds, such as *Trichoderma*. Fruiting bodies of ophiostomatoid fungi were observed throughout the galleries, but did not grow out when

transferred onto agar medium. They were instead overgrown by *Trichoderma* and bacteria. No larvae of bark beetles were encountered in any of the jack pine samples.

FUNGAL DIVERSITY OF NON-OPHIOSTOMATOID FUNGI

Many non-ophiostomatoid fungi were isolated in this study (see Appendix I). These fungi were identified to genus and, when possible, species. Less emphasis was taken to identify any non-ophiostomatoid fungi to species and in some cases a tentative name of genus was not assigned. Any fungus that was either sterile or possessed features that were not distinct enough to distinguish identity down to genus was labelled as an unidentified fungus.

A total of 244 isolates were obtained from the sampling methods utilized on balsam fir. The most common fungus isolated was an unknown Hyphomycete, which made up 17.2% of the total isolates. This fungus grew slowly on the agar medium and had a *Gliocladium*-like appearance with penicillus-like conidiophores. A proper name was not given to this fungus because it was non-ophiostomatoid and not of particular importance to the study. *Candida* and *Penicillium* were commonly isolated from balsam fir and together comprised of 20.1% of all the isolates. Other non-ophiostomatoid wood-inhabiting fungi isolated from balsam fir included *Cytospora*, several *Lecythophora*-like fungi, *Cladosporium sphaerospermum*, *Exophiala jeanselmei* and several other fungi known to cause wood-stain or cankers (Hutchison and Reid 1988b).

There were a total of 214 isolates acquired from all of the sampling methods implemented on black spruce. As was found with the balsam fir, *Candida* and *Penicillium* were commonly isolated, making up 28% of all isolates. *Calcarisporium arbuscula* was also commonly encountered throughout the sampling process, making up

15.9% of the total isolates. The fungus was originally mistaken for a *Sporothrix*-like fungus; however, its cultures were very quick-growing and produced black sclerotia. This fungus was considered a contaminant due to its quick growth rate and rapid spore production. Several fungi isolated from black spruce known to cause wood-stain or cankers included an *Exophiala*-like species, *Cytospora*, *Phoma*, *Coniothyrium* and *Lecythophora*. Again, these fungi were not of particular importance to the study.

The sampling methods performed on the tamarack samples yielded a total of 187 isolates. *Penicillium* and *Candida* were again two of the most commonly isolated fungi. *Penicillium* made up 32.8% and *Candida* 12.9% of the total isolates. Many other common moulds encountered included members within the genera *Trichoderma*, *Aspergillus*, *Lecythophora*, *Phoma*, *Acremonium*, *Cladosporium* and *Sporotrichum*.

Only 88 isolates were acquired from the sampling methods performed on the jack pine samples. Cellular slime moulds made up 9.1% of the total fungi isolated and were not identified to genus. These cultures only appeared on agar in Petri dishes that were highly contaminated with bacteria. The most common fungus isolated was *Trichoderma*, which made up 43.2% of the total isolates. *Verticillium* and *Penicillium* were also commonly isolated, making up 18.2% and 13.6% of the total fungi isolated respectively. Other non-ophiostomatoid fungi isolated from jack pine were *Exophiala jeanselmei*, *Aspergillus*, *Lecythophora*, a *Phialophora*-like fungus and *Rhizomucor*, many of which can be associated with causing wood-stain.

DISCUSSION

The examination of ophiostomatoid fungi and their associated bark beetles on balsam fir, black spruce, jack pine and tamarack revealed a diverse number of fungi and several species of bark beetle associates. It was intended that this study would provide a general representation of the bark beetle species and ophiostomatoid fungi present in the Thunder Bay region. However, since the present study was restricted to one sample of each tree species at one location in the Thunder Bay region, it is expected that there is a far greater diversity of bark beetles and ophiostomatoid fungi in the region. In addition, the jack pine examined in this study gave poor representation of the diversity of ophiostomatoid fungi on the host because it was overwhelmed with bacteria, moulds and parasites. These results were much unlike the results found in Mussell's (2004) study on bark beetles and their associated fungi on jack pine. Bark beetles and ophiostomatoid fungi identified in the present study are described in the following sections.

BARK BEETLES AND THEIR AGRESSIVENESS

Four bark beetle species were observed upon inspection of their galleries within the four conifer species at Jack Haggerty Forest. These species were *Pityokteines sparsus*, *Polygraphus rufipennis*, *Ips pini* and *Dendroctonus simplex*.

Pityokteines sparsus, also known as the balsam fir bark beetle, are on average 2.1-2.5mm in length and range from Alberta to Newfoundland (Bright 1976). This bark beetle is considered the "primary enemy of balsam fir" (Wood 1982). *Pityokteines sparsus* is an opportunistic, or saprophytic, bark beetle that commonly infests dead or

dying trees. The bark beetle commonly occurs in high numbers under the bark of balsam fir due to their high rate of reproduction (Hosking 1975). The beetles observed in this study were found in high densities under the bark of the balsam fir samples and no other bark beetle species were present. This was not surprising since the balsam fir examined in this study were left in the open. According to Hosking (1975), larval development of *P. sparsus* occurs twice as quickly in opened areas than under closed canopies, which most likely accounted for the large number of beetles observed in the present study. The probability that these bark beetles will ever pose a threat to the economy is unlikely, since they do not appear to be able to successfully attack healthy trees. However, *P. sparsus* could be a problem when trees are victim to windthrow or have been felled and left in the sun over a period of time before being processed. This is because the beetles can attack in high numbers with early brood development and a second brood in late summer or early fall (Hosking 1975). This would be especially true if the blue stain fungi associated with *P. sparsus* were capable of establishing quickly in the galleries and causing severe staining of the wood.

Polygraphus rufipennis, known also as the four-eyed spruce bark beetle, was commonly isolated from black spruce and tamarack. The primary host for the beetle is spruce (Hosking 1975), yet it can infest all conifers in its range throughout most of North America (Bright 1976). The average length of these saprophytic bark beetles is 2.1-3.1mm and they are typically found on dead or dying trees (Bright 1976). There was a high number of *P. rufipennis* adults under the bark of both the black spruce and tamarack samples, though their densities were not as high as the *Pityokteines sparsus* adults in the balsam fir samples. *Polygraphus rufipennis* can have as many as three broods per season in the southern parts of its range, resulting in high beetle populations

(Bright 1976). Large areas of stressed trees can lead to epidemics of *P. rufipennis* (Bowers *et al.* 1996). Bowers *et al.* (1996) concluded that *P. rufipennis* was aggressive enough to kill healthy trees when populations were high; however, their attacks were rarely successful without the presence of other stress-related factors, such as defoliation caused by spruce budworm.

Ips pini, commonly referred to as the pine engraver, occurs primarily on pine and occasionally on spruce (Bright 1976), which was representative of the present study. Though few specimens were observed, remnants of these beetles were apparent throughout the galleries in the black spruce and jack pine specimens. Only three representatives were used in this study and were all isolated from black spruce. Ranging from 3.5-4.2mm in length, *I. pini* is widely distributed in North America and is found transcontinental in Canada on slash and dead or dying trees (Bright 1976; Furniss *et al.* 1995). *Ips pini* is not normally aggressive towards healthy trees and is rarely capable of attacking trees without the assistance of primary invaders (Wood 1982). However, they can often build up high populations and kill trees up to 20cm diameter (Bright 1976).

Dendroctonus simplex, also referred to as the eastern larch beetle, was isolated from tamarack in this study. This beetle measures 3.4-5.0mm in length and is found across Canada throughout the range of tamarack (Bright 1976). Only eight *D. simplex* adults were observed under the bark of tamarack in the present study. *Dendroctonus simplex* are facultative parasites and typically prefer dead or dying trees; however, they are capable of causing significant mortality in tamarack stands when trees have been stressed by harsh environmental conditions. Currently in Minnesota, an outbreak of *D. simplex* over the past seven years has lead to high levels of mortality to tamarack on over 65,000 acres of forests (MDNR 2008). Severely hit areas have seen 30-50%

mortality of tamarack by *D. simplex* and as high as 80% in some areas. It is believed the droughts that hit Minnesota in 2002-2003 and 2006-2007 are to blame for the outbreak (MDNR 2008). The drought of 2006-2007 is believed to be the cause of the current *D. simplex* outbreak in Roslyn Village, just west of Thunder Bay (OMNR 2008b).

OPHIOSTOMATOID FUNGI: THEIR DISTRIBUTIONS, HOSTS AND VECTORS

Ten ophiostomatoid fungi identified to species were isolated from conifers, including seven species of *Ophiostoma* and two species of *Leptographium* (Table 3). These species include *O. abietinum*, *O. bicolor*, *O. ips*, *O. minus*, *O. piceae*, *O. piceaperdum*, *O. pulvinisporum*, *O. retangulosporium*, *L. abietinum* and *L. fruticetum*. Several other species of ophiostomatoid fungi were isolated from the conifer samples, but were not identified to species. These include representatives in the genera *Ophiostoma*, *Pesotum*, *Sporothrix* and *Hyalorhinocladiella*.

Ophiostoma ips was one of the most commonly isolated fungi in the present study and was found growing in bark beetle galleries on balsam fir, black spruce and jack pine. Interestingly, this fungus was not isolated from any of the adult beetle washings, but had been commonly isolated from perithecia growing in the galleries, from stained wood and occasionally from bark beetle frass (Appendix I). The lack of cultures from adult bark beetles may have been due to the lack of *Ips pini* used in the study. *Ophiostoma ips* is commonly associated with *Ips pini* and other *Ips* species (Griffin 1968; Raffa and Smalley 1988; Furniss *et al.* 1995, Haberkern *et al.* 2002; Mussell 2004). There were many remnants of these beetles within the galleries, which could explain the presence of the fungus. The fungus is quick growing and produces dark brown to black stains on sapwood of infected trees (Upadhyay 1981; Hutchison and

Reid 1988a; Seifert 1993). *Ophiostoma ips* is considered highly pathogenic when healthy trees are given high artificial dosages of the fungus (Mathre 1964; Fernández *et al.* 2004); however, it is only moderately pathogenic in low dosages and not as pathogenic as some blue stain species (Owen *et al.* 1987). *Ophiostoma ips* is frequently isolated from conifers, such as pine and spruce (Griffin, 1968, Olchowecki and Reid 1974; Upadhyay 1981; Hutchison and Reid 1988a; Seifert 1993), but has not been recorded on balsam fir.

No studies have extensively examined ophiostomatoid fungi on balsam fir, which may be because balsam fir is not considered an economically important tree in Ontario. *Ophiostoma bicolor* and *O. rectangulosporium* were commonly encountered on balsam fir and were both associated with *Pityokteines sparsus*. *Ophiostoma bicolor* appeared on 6.5% of the *P. sparsus* adult washings, but has never before been associated with the beetle. *Ophiostoma bicolor* grows very quickly, but does not produce pronounced staining of the wood (Upadhyay 1981; Sallé *et al.* 2005). Sallé *et al.* (2005) studied the virulence of several fungi including *O. bicolor* through mass inoculations on living spruce trees. The inoculations tests failed to show high level of virulence by the fungus.

Ophiostoma rectangulosporium was the more common of the two identified ophiostomatoid fungi on balsam fir and was isolated from 16.1% of the adult beetles. This species was first described in 2006 from bark beetle infested *Abies* in Japan (Ohtaka *et al.* 2006). The only other record of this fungus was by Romón *et al.* (2007), who reported isolating an *O. rectangulosporium*-like fungus from *Pinus radiata* in northern Spain. If these are the same species, it suggests that the fungus has a potentially large geographic range and is therefore not surprising to be found in North America. Currently, nothing is known about the pathogenicity of this fungus. *Ophiostoma*

rectangulosporium is morphologically similar to the fungus *Ceratocystis brunneocrinita* Wright & Cain (Wright and Cain 1961; Upadhyay 1981; Ohtaka *et al.* 2006), yet even though Ohtaka *et al.* (2006) mentioned this similarity, molecular comparisons of *O. rectangulosporium* to *C. brunneocrinita* were not performed. Ohtaka *et al.* (2006) claimed the fungus was a new species based on very slight morphological differences between the two species, such as length of ascospores and colour of ostiolar hyphae. The size of *O. rectangulosporium*'s ascospores in Ohtaka *et al.*'s (2006) study were 2.0-3.5μm x 1.0-1.7μm, while Wright and Cain (1961) documented that the ascospores of *C. brunneocrinita* were 3.5-5.0μm x 1.4-2.4μm. In the present study, ascospore measurements of *O. rectangulosporium* were 3.0-4.0μm x 1.5-2.0μm, which is more comparable to the ascospores of *C. brunneocrinita*. This suggests that the two species may indeed be conspecific and have a larger geographic range than expected.

Ceratocystis brunneocrinita was the tentative name assigned to the *O. rectangulosporium* cultures in the present study before representatives were sent away for molecular analysis. *Ceratocystis brunneocrinita* is specific to balsam fir and appears to be commonly associated with *Pityokteines sparsus* (Griffin 1968). The name *O. rectangulosporium* was the suggested name based on the ITS database used for rDNA sequencing (Hausner pers. comm.). Unfortunately, *C. brunneocrinita* was not included in the database. As mentioned in Ohtaka *et al.*'s (2006) paper, *C. brunneocrinita* should belong to the genus *Ophiostoma* because of its *Hyalorhinocladiella* anamorph, but has not been transferred over to date.

Although eight species of ophiostomatoid fungi were isolated from the black spruce samples, only three were successfully identified to species. These fungi consisted of *L. fruticetum*, *O. ips* and *O. piceaperdum*.

Leptographium fruticetum was isolated several times from fruiting structures, bark beetle frass and from *Polygraphus rufipennis* adults collected from the tamarack and black spruce samples. There is currently very little known about this fungus since it was not described until 2006 (Alamouti *et al.* 2006). To date, it has only been recorded on *Picea glauca* and *Picea engelmannii* x *glauca* and associated with *Ips perturbatus* in western Canada. The present study found the fungus on tamarack and black spruce and associated with *Polygraphus rufipennis*. This is also the only recorded case of *L. fruticetum* in Ontario. However, this fungus may have been recorded in many other studies throughout North America as an unknown *Leptographium* species and its range of host, insect vectors and distribution are likely much broader. Currently, no studies have been published about *L. fruticetum*'s pathogenicity.

Ophiostoma piceaperdum occurred once on the black spruce samples from an adult washing of *Polygraphus rufipennis*. This fungus has a large range of hosts and bark beetle vectors and is cosmopolitan in distribution (Wright and Cain 1961; Upadhyay 1981; Hutchison and Reid 1988a; Jacobs and Wingfield 2001). *Ophiostoma piceaperdum* has been reported along the Great Lakes region in the United States and Ontario (Wright and Cain 1961; Griffin 1968; Haberkern *et al.* 2002), but has not yet been recorded in northwestern Ontario. It is not surprising to find this fungus in northwestern Ontario because of its large geographic range. There has been some debate about the pathogenicity of *O. piceaperdum*. Several studies have suggested the fungus to be highly pathogenic to its host tree, while other studies have determined the fungus to be moderately or non-pathogenic (Jacobs and Wingfield 2001; Sallé *et al.* 2005). Sallé *et al.* (2005) suggested the fungus may be an important associate of *Ips typographus* due to its ability to produce significant dark reaction zones through the use of mass and low-

intensity inoculations. It was concluded that the fungus could be considered a pathogen of Norway spruce (*Picea abies* L.), but some strains of the fungus appear to be more pathogenic than others.

There were a total of 11 species of ophiostomatoid fungi isolated from tamarack and six were identified to species, including *L. abietinum*, *L. fruticetum*, *O. abietinum*, *O. minus*, *O. piceae* and *O. pulvinisporum*.

Leptographium abietinum represented over a quarter of the ophiostomatoid fungi isolated from tamarack and was associated with *Polygraphus rufipennis* and *Dendroctonus simplex* in the present study. *Leptographium abietinum* is a commonly occurring blue stain fungus that is primarily found throughout North America, including Thunder Bay district (Kendrick 1962; Harrington and Cobb 1988; Jacobs and Wingfield 2001; Haberkern *et al.* 2002; McBeath *et al.* 2004); however, isolates have also been reported from England (Kendrick 1962) and Scotland (Harrington and Cobb 1988). *Leptographium abietinum* is not host specific because it has been reported on a wide range of conifers throughout its distribution (Jacobs and Wingfield 2001). The fungus has also been found associated with a wide variety of bark beetles, including both bark beetles in the present study (Haberkern *et al.* 2002; McBeath *et al.* 2004). Although *L. abietinum* was isolated from western Siberian larch (*Larix sukaczewii* N. Dyl.) in a study by McBeath *et al.* (2004), there have been no records of this fungus on any other *Larix* species to date. *Leptographium abietinum* is not regarded as being pathogenic, though some studies suggest it is mildly pathogenic and may assist bark beetles by hastening the decline of its host (Jacobs and Wingfield 2001). This fungus was tentatively named *O. americanum* Jacobs & Wingf. in the present study based on the descriptions of the *Leptographium* anamorph in Jacobs and Wingfield (2001), since the conidia were

curved and much larger than the description of *L. abietinum*. However, molecular analyses by Dr. G. Hausner found it to be genetically similar to *L. abietinum*. Interestingly, *O. americanum* has been associated with *Dendroctonus simplex* and was isolated from *Larix decidua* Mill. in northern Vermont (Jacobs *et al.* 1997b). Jacobs *et al.* (1997b) considers *O. americanum* to be pathogenic, but its role with *D. simplex* is unknown. It is uncertain whether these two fungi are synonyms, but it is recommended that further analyses be undertaken.

Ophiostoma abietinum was described in Mexico in 1990 from the galleries of *Pseudohylesinus* sp. on *Abies vejari* (Marmolejo and Butin 1990). In 1998, a pest risk assessment on importing unprocessed pine and fir wood from Mexico was published by the United States Department of Agriculture, which listed *O. abietinum* as a serious vascular wilt and wood-staining fungus with high risk potential of establishing in the United States from Mexico (Tkacz *et al.* 1998). Currently it has not been recorded in the United States, but the report suggested that the fungus was likely present in the United States along the Mexican border. The present study suggests that the fungus occurs in Canada and more than likely has a wider distribution. Recently, Zhou *et al.* (2006) reported *O. abietinum* in South Africa. The fungus was isolated from the bark beetle *Orthotomicus erosus* in an earlier study, but the authors did not mention the host tree from which the beetles were extracted. However, there was no indication of an unknown species of *Ophiostoma* in a paper published from their earlier study (Zhou *et al.* 2001). An unknown species of *Sporothrix* was mentioned, but the fungus was also isolated from two other bark beetle species that were not credited as being vectors of the fungus. The bark beetles collected in the study were taken from *Pinus patula* and *P. elliottii*; however, it was not clear from which tree the *O. abietinum*-bearing bark beetles were

isolated. Since the bark beetles were collected from *Pinus* spp., it is possible that *O. abietinum* also occurs on *Pinus* spp. *Ophiostoma abietinum* has never been recorded in North America on tamarack. The fungus was not isolated from bark beetles in this study, but may be associated with *Polygraphus rufipennis*. Very little research has been done on this fungus, so its pathogenicity is still unknown.

Ophiostoma pulvinisporum is another Mexican species of ophiostomatoid fungi that was described in 2004 from *Dendroctonus mexicanus* on *Pinus pseudostrobus* and from *Ips calligraphus* on *P. maximinoi* (Zhou *et al.* 2004). In the present study, the fungus was not isolated from a bark beetle, but is possibly associated with *Polygraphus rufipennis*. In Zhou *et al.*'s (2004) paper, a Canadian isolate of *Ophiostoma ips* isolated from *Pinus contorta* was used for comparative purposes but had been switched over to *O. pulvinisporum* by the authors without any discussion on the matter. Cultures of *O. pulvinisporum* resemble *O. ips* because the teleomorphs produce long-necked perithecia with no ostiolar hyphae, and possess pillow-shaped ascospores. Both fungi also possess a *Pesotum* anamorph and a *Hyalorhinocladiella* synanamorph. The Canadian culture used for comparison in their study was an American Type Culture Collection (ATCC) strain that was once called *Ceratocystis montia* and then moved to *C. ips* before being moved to *O. pulvinisporum* by Zhou *et al.* (2004) (Hausner pers. comm.). Furthermore, spore sizes from the *Pesotum* anamorph in the present study did not fit the description of the spore sizes in Zhou *et al.*'s (2004) paper. The authors distinguished the two fungi from each other through ITS rDNA sequences and explained the two fungi differed in growth rates and mating systems. In order to determine whether these fungi are two separate species, it is felt more comparisons of *O. pulvinisporum* to *O. ips* and related species is necessary.

Ophiostoma piceae is one of twelve species found within the *O. piceae* complex: a group of ophiostomatoid fungi that possess *Pesotum* and *Sporothrix* synanamorphs (Harrington *et al.* 2001; Chung *et al.* 2006). *Ophiostoma piceae* has been associated with a wide range of hosts, including both conifers and hardwoods, and is cosmopolitan in distribution (Griffin 1968; Upadhyay 1981; Hutchison and Reid 1988a; Brasier and Kirk 1993; Harrington *et al.* 2001). *Ophiostoma piceae* is the most common sapstain fungus found on conifers in Canada, yet it does not appear to be pathogenic, causing little to no stain and minimal damage to infected conifer wood (Griffin 1968; Seifert 1993). In addition, even though the fungus can quickly colonize freshly cut material, its growth is superficial and is usually restricted close to the point of infection (Griffin 1968; Seifert 1993). Through sexual compatibility tests, Brasier and Kirk (1993) separated *O. piceae* into two intersterile mating groups, confirming a previously discussed theory that the fungus is heterothallic. In addition, one group was commonly isolated from hardwoods (OPH), while the other group originated predominantly from conifers (OPC). The authors suggested the two groups were biologically separate species, where the former group represented *Ophiostoma quercus* (Georgev.) Nannf. and the latter group *O. piceae*. This was later confirmed through molecular analysis (Harrington *et al.* 2001).

No published records of *O. piceae* have been reported from the Thunder Bay district, but discovering it at this location does not come as a surprise since it has already been found in other areas along the perimeter of Lake Superior in Ontario (Griffin 1968) and the United States (Haberkern *et al.* 2002). In addition, Mussell (2004) had previously isolated *O. piceae* from jack pine in the Thunder Bay region, which she reported in her unpublished thesis. Since tamarack has rarely been studied for bark

beetle-fungal associations, *O. piceae* has not yet been reported from tamarack, though the fungus has been reported on *Larix* sp. (Hutchison and Reid 1988a).

Ophiostoma minus was the most frequently isolated ophiostomatoid fungus from tamarack and was isolated once from the jack pine samples. It is assumed the fungus would have been encountered more often from the jack pine samples if they were not severely contaminated with bacteria and *Trichoderma* because of the high number of fruiting bodies in the galleries. *Ophiostoma minus* is commonly associated with a wide variety of hosts, especially pine, and has been commonly associated with a number of bark beetles over North America, Europe, North Africa and Japan (Upadhyay 1981; Seifert 1993; Solheim *et al.* 1993; Gorton and Webber 2000; Jamaa *et al.* 2007). This fungus is an early colonizer of recently felled or severely weakened trees and has the ability to stain the entire sapwood of a tree within a few months (Griffin 1968). As a result, *O. minus* is considered an economically important blue stain fungus throughout its range (Griffin 1968; Upadhyay 1981; Gorton and Webber 2000). *Ophiostoma minus* can be pathogenic in high dosages with the ability to create large lesions and kill young trees (Solheim *et al.* 1993; Gorton and Webber 2000), yet was the least pathogenic fungus when compared to *Leptographium wingfieldii* and *O. ips* (Jamaa *et al.* 2007). The latter two fungi are noted for causing serious staining of sapwood, but are only pathogenic when trees are given excessively large dosages of the fungus through artificial means (Owen *et al.* 1987; Jacobs and Wingfield 2001). To date, no isolates of *O. minus* has been isolated from tamarack or in association with *Polygraphus rufipennis* and *Dendroctonus simplex*.

NON-OPHIOSTOMATOID ASSOCIATES

Many of the fungi isolated in this study were non-ophiostomatoid; some of which were frequently encountered. Members of the genera *Penicillium*, *Candida* and *Trichoderma* were commonly isolated in all of the sampling methods and were often isolated more than any of the ophiostomatoid fungi. These genera are frequently isolated from wood, and members of *Penicillium* and *Trichoderma* can cause green discolorations to sapwood by producing copious amounts of conidia; however, they are not considered sapstain organisms since they cannot penetrate the wood (Seifert 1993). Dark moulds, such as *Alternaria alternata* and *Cladosporium* spp., are also commonly isolated from wood and produce large amounts of conidia. Their growth is not strictly superficial and therefore these fungi are capable of staining sapwood (Seifert 1993). *Alternaria* was not isolated in the present study, but there were several isolates of *Cladosporium*. Throughout this investigation, several other genera of non-ophiostomatoid fungi known to cause wood-stain had been isolated, including *Coniochaeta*, *Phialophora*, *Lecythophora*, *Cytospora*, *Phoma* and *Exophiala* (Hutchison and Reid 1988b).

An interesting find was that of *Myxotrichum deflexum*, which had been isolated twice from bark beetle washings in the present study. One isolate was taken from *Pityokteines sparsus* on balsam fir and the other from *Polygraphus rufipennis* on tamarack. *Myxotrichum deflexum* forms ascomata (gymnothecia) with a cage-like peridium (reticuloperidium) made up of an aggregation of rigid, thick-walled and branched hyphae (Greif and Currah 2003). Greif and Currah (2003) felt the reticuloperidium was an adaptation by the fungus for arthropod distribution. The reticuloperidium benefits the fungus by clinging to the hairs of passing insects.

Myxotrichum deflexum can be dispersed through the air. Therefore, when an insect carrying these gymnothecia leaves its host tree, it can disperse some of the spores into the air and carry the rest with it to a new host tree.

The isolation of ophiostomatoid fungi from jack pine proved to be disappointing in this study because the samples were of poor quality. This may have been a result of leaving the bait logs on the forest floor for too long before harvesting the galleries. The samples were highly infested with parasites and wood borer larvae which invaded many of the bark beetle galleries. Also, the galleries and bark beetle frass had a slimy appearance likely caused by the existing bacteria. The presence of common moulds was also observed growing throughout the galleries. A large number of isolations were taken from the jack pine samples; however, after a few days the Petri dishes were completely contaminated with bacteria and no fungi other than cellular slime moulds were observed. It appeared the galleries were so overwhelmed with bacteria that the streptomycin sulphate and penicillin-G that was added to the agar medium were not working. Acidified Malt Extract Agar was therefore used to stop the bacteria from outgrowing the fungi. This media was prepared by adding 10mL of 25% lactic acid to 1L of modified 2% malt extract agar media after autoclaving. Acidified Malt Extract Agar was only used for the jack pine samples. After more isolations were made from the jack pine samples, the bacteria was no longer a problem, but the mycoparasite *Trichoderma* was very abundant and inhibited most fungi from growing. No more isolations were taken from jack pine in the present study since it was assumed the samples collected would not provide an accurate representation of ophiostomatoid fungi on jack pine.

CONCLUSION

Several new bark beetle-fungus associations were observed in this study along with new records of hosts and geographic distributions for some of the identified ophiostomatoid fungi.

Tamarack yielded new records of six distinct fungi: *Leptographium abietinum*, *L. fruticetum*, *Ophiostoma abietinum*, *O. piceae*, *O. pulvinisporum* and *O. minus*. *Leptographium fruticetum* was isolated for the first time from black spruce and was never reported in Ontario or with *Polygraphus rufipennis*. The fungus may have been recorded in other studies as an unknown *Leptographium* species since it was only described in 2006. The geographic distribution, hosts and insect vectors of this fungus should increase over time as more research is undertaken. *Ophiostoma abietinum* has only been reported in Mexico and South Africa. This study provides the first reported case of the fungus in North America. This was also the first report of *O. pulvinisporum* in Ontario, which had only been reported in Mexico and western Canada. *Ophiostoma piceae* is common throughout the world, but has never been reported in the Thunder Bay District, even though it has been commonly isolated throughout the Great Lakes region. *Ophiostoma minus* also occurs in many parts of the world, yet no studies have reported it on *Polygraphus rufipennis* or *Dendroctonus simplex*.

Balsam fir also yielded new records of ophiostomatoid fungi. This study is the first to report *O. ips* on balsam fir, but this was not surprising because of its large host range. *Ophiostoma rectangulosporium* has never been reported in North America and this is the first record of it on balsam fir or as an associate of *Pityokteines sparsus*.

Ceratocystis brunneocrinita is a common associate of balsam fir and may be a synonym of *O. rectangulosporium* because of morphological similarities and the lack of molecular comparisons between the two fungi. If this is the case, this would be the first record of *C. brunneocrinita* in the Thunder Bay District, but not in Ontario (Griffin 1968). Finally, *O. bicolor* has never been associated with *Pityokteines sparsus* in any other studies.

As expected, a large diversity of ophiostomatoid fungi was isolated in this study including several new records for the Thunder Bay region. Tamarack and balsam fir yielded more unique fungi than black spruce and jack pine. This may be due to the limited information available on bark beetles and their associated fungi for tamarack and balsam fir. Tamarack yielded the highest diversity of ophiostomatoid fungi, many of which had never been reported on tamarack or in Ontario. Jacobs *et al.* (1997a) examined bark beetle-fungus associations on tamarack in eastern North America and discovered a new species of *Ophiostoma*. The authors concluded that further studies on beetle-fungus associations on tamarack are necessary because of the limited available information. Wright and Cain (1961) and Griffin (1968) sampled from conifer slash (including tamarack) on field trips throughout Ontario, yet these were not extensive studies and many species of ophiostomatoid fungi would have been missed. Therefore, it is not surprising to find many new fungal associations on tamarack, especially with fungi that have large host ranges, such as *L. abietinum*, *O. piceae* and *O. minus*.

Further research is necessary to examine the bark beetle-fungal associations in the Thunder Bay region for each tree species examined in this study, since the diversity of ophiostomatoid fungi is expected to be much greater than this study has revealed.

CHAPTER III
YEAR TWO FIELD AND LAB STUDY

PURPOSE

A second study was established to investigate the presence of conifer-inhabiting bark beetles and their associated ophiostomatoid fungi in the Thunder Bay region and to answer the following question: Does the abundance of ophiostomatoid fungi differ significantly between locations and along the length of the tree? The year two field and lab study was initiated in the early summer of 2007. A design was set up to identify a representative population of ophiostomatoid fungi and their bark beetle vectors on two boreal conifers in the Thunder Bay region. In order to achieve this, three locations were selected within the Thunder Bay region and the isolation sample size for each tree species was increased. At each location, four jack pine and four white spruce trees were felled and remained on the forest floor as bait trees over the summer of 2007. In the autumn, the trees were destructively sampled for bark beetle galleries by removing three discs from each tree. The galleries were examined in the laboratory for the presence of bark beetles and ophiostomatoid fungi.

The study was set up with an experimental design to test the following hypotheses: 1) there is no difference in abundance of ophiostomatoid fungi between locations, and 2) there is no difference in abundance of ophiostomatoid fungi along the length of each tree. In addition, it is believed that the diversity of fungi and bark beetles will increase with a larger study area. The information collected from this study will be used to fill in some of the gaps in the literature by defining any new associations that exist between the specific bark beetles and ophiostomatoid fungi identified.

MATERIALS AND METHODS

STUDY AREA

Three sites, or locations, were selected for the present study, including Jack Haggerty Forest (JHF), Silver Mountain (SM) and Quackenbush woodlot (Q) (Figure 1). The jack pine felled at JHF were located within a small jack pine plantation in Block 14 (48°38' N 89°23' W). This young plantation was situated directly on the opposite side of Beaverkit Lake Road where the jack pine in the year one study was located. The white spruce were located in Block 7 (48°38' N 89°24' W) by one of the area's main gravel pits, which branches off Wartman Road. A brief description of JHF is summarized in Chapter 2. Silver Mountain (48°15' N 89°54' W) is a university-owned property located about 74 kilometres southwest of Thunder Bay in Lybster township and contains approximately 33 hectares of productive forest land (Luckai 2009). The Faculty of Forestry and the Forest Environment uses the property for educational purposes, but the property is not managed by the faculty due to the rugged terrain and the high number of abandoned mine shafts in the area. The Quackenbush Woodlot (48°23' N 89°21' W) is a property recently donated to the University. The property contains about 14 hectares of managed and unmanaged forested land and is located at the end of Laval Street about four kilometres west of Thunder Bay (Klassen 2007).

SAMPLING DESIGN

Four healthy white spruce and four healthy jack pine trees showing no signs of disease or decay were selected as bait trees at each of the three locations. The trees

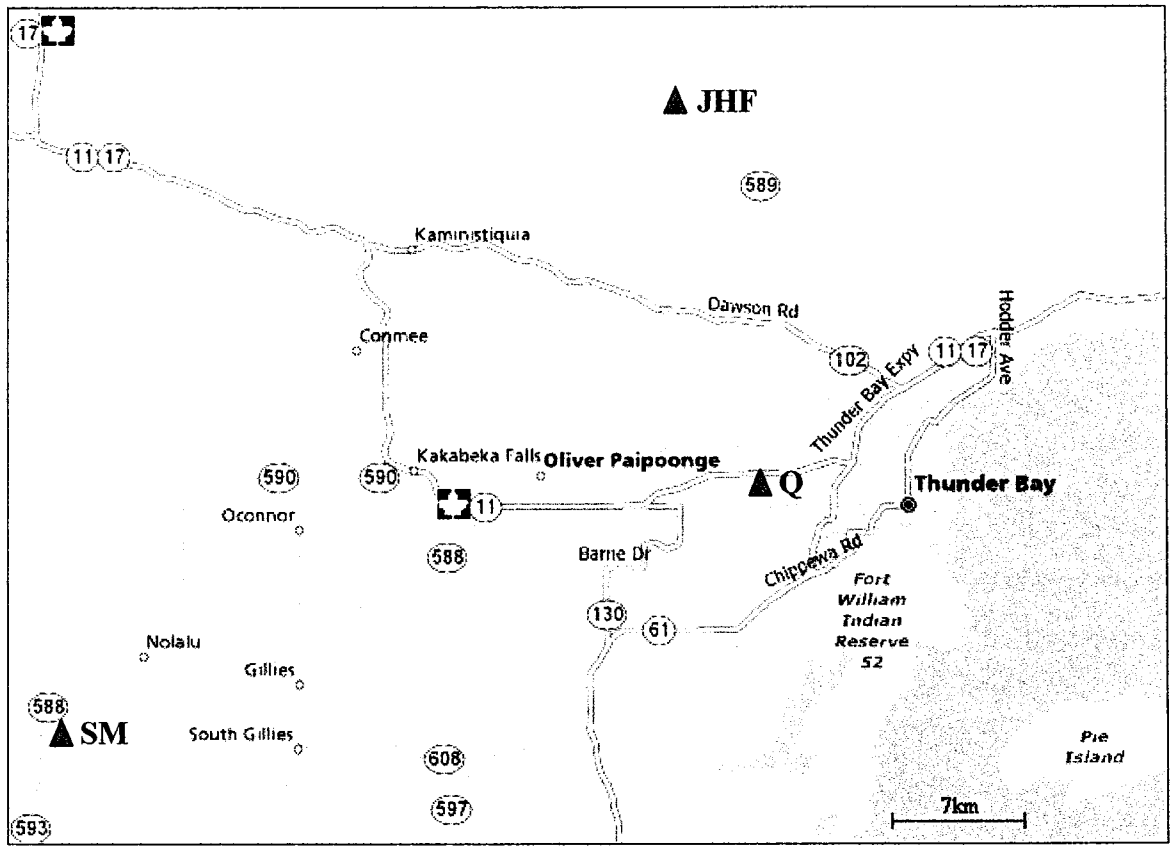

Figure 1: Location of forested properties (represented by red triangles) used in the year two field and lab study

Source: MapQuest®

selected for this study ranged from 14.9cm dbh (diameter at breast height) to 23.4cm dbh (see Appendix III). Although all of the trees were supposed to be of equal calliper (20cm dbh) to reduce experimental error, it was not possible to meet this criterion at all three locations for each tree species. On June 21 and 22, 2007, each of the trees were felled and remained in an open area on the forest floor over the summer. Each tree was uniquely identified using water-resistant labels. The felled trees were harvested for bark beetle galleries on October 3 to October 5, 2007 by removing three 10cm-thick discs from each tree with a chain saw. The first disc, or section, was removed at the tree's dbh to represent the base of the tree. The second section was removed three metres up from the first section to represent the middle of the tree, and the third section was removed

another three metres up the tree to represent the top of the tree. Each of the sections was individually placed in a plastic bag along with a label identifying the location (JHF, SM or Q), tree species (jack pine or white spruce), tree replicate number (1, 2, 3 or 4) and the level of the tree to which the sections belonged (1, 2 or 3). The sections were placed into cardboard boxes according to tree species and location and were then placed into the forestry freezer until examined.

ISOLATION PROCEDURE

The isolation procedures used in the present study were the same as those employed in the year one study unless otherwise mentioned (see Chapter 2). A systematic approach was taken while isolating from the wood samples to ensure that an equal number of isolations were taken from each tree. Therefore, each sampling method (ie. adult washing, larval washing, fruiting structures, stained wood and beetle frass) was undertaken five times on each section of each tree. Ideally, this would mean that 25 isolation attempts were taken from each section and 75 isolation attempts from each tree; however, many sections lacked bark beetles among other things and, as a result, five isolations could not be taken from each sampling method for those sections. Instead, the assumption was that each section had an equal opportunity of being sampled from 25 times. In addition, the number of cultures isolated from each section had the potential to be much higher than 25, since each sampling method used could yield multiple fungi.

A new coding system was implemented for the present study. A unique code was given to each of the fungal cultures to indicate which sampling method was used as well as the location, host, tree number and the section of the tree from which the fungus was isolated (Table 5). For example, the code '$JHFPj_1s_1b_1$' would represent an isolate

Table 5: Explanation of the coding system used in the year two field and lab study

Code	Description
$JHFPj_1s_1b_{1a}$	The **first fungus** isolated from the **first fruiting structure** on the **first section** of wood within the **first jack pine tree** at **Jack Haggerty Forest**
$JHFPj_1s_1b_{1b}$	The **second fungus** isolated from the first fruiting structure on the first section of wood within the first jack pine tree at Jack Haggerty Forest
$JHFPj_1s_1b_{2a}$	The first fungus isolated from the **second fruiting structure** on the first section of wood within the first jack pine tree at Jack Haggerty Forest
$JHFPj_1s_2b_{1a}$	The first fungus isolated from the first fruiting structure on the **second section** of wood within the first jack pine tree at Jack Haggerty Forest
$JHFPj_2s_1b_{1a}$	The first fungus isolated from the first fruiting structure on the first section of wood within the **second jack pine tree** at Jack Haggerty Forest
$JHFSw_1s_1b_{1a}$	The first fungus isolated from the first fruiting structure on the first section of wood within the first **white spruce** tree at Jack Haggerty Forest
$JHFPj_1s_1f_{1a}$	The first fungus isolated from the first **frass sample** on the first section of wood within the first jack pine tree at Jack Haggerty Forest
$JHFPj_1s_1l_{1a}$	The first fungus isolated from the first **larval washing** on the first section of wood within the first jack pine tree at Jack Haggerty Forest
$JHFPj_1s_1w_{1a}$	The first fungus isolated from the first **adult washing** on the first section of wood within the first jack pine tree at Jack Haggerty Forest
$JHFPj_1s_1g_{1a}$	The first fungus isolated from the first **stained wood sample** on the first section of wood within the first jack pine tree at Jack Haggerty Forest
$SMPj_1s_1b_{1a}$	The first fungus isolated from the first fruiting structure on the first section of wood within the first jack pine tree at **Silver Mountain**
$QPj_1s_1b_{1a}$	The first fungus isolated from the first fruiting structure on the first section of wood within the first jack pine tree at **Quackenbush Woodlot**

which was taken from the first fruiting structure found on the first section of the first jack pine tree located at the Jack Haggerty Forest property. Similar to the year one study, if the isolate was instead taken from stained wood, a 'g' would replace the 'b', and a 'w' would replace the 'b' if the isolate was taken from an adult bark beetle washing, and so on. If more than one fungus was isolated from a single sampling method, as was the case most of the time, the letter 'a', 'b', 'c', etc. was added to the end of the code. For example, the code '$JHFPj_1s_1b_{1c}$' would represent the third of at least three fungi isolated from the first fruiting structure found on the first section of wood.

STATISTICAL ANALYSIS

The overall sources of variation in the abundance of ophiostomatoid fungi isolated from the jack pine and white spruce were compared and analyzed using univariate and multivariate analyses of variance (ANOVA and MANOVA, respectively). ANOVAs were performed on each species of ophiostomatoid fungus occurring on jack pine and on those occurring on white spruce. MANOVAs examined the impact of the main effects (e.g. locations) on all of the dependent variables (i.e. ophiostomatoid fungi) for each tree species. The analyses were executed using SPSS Statistics® 17.0 (SPSS Inc. Chicago, Illinois) with the linear model illustrated below:

$$Y_{ijkl} = \mu + L_i + T_{(i)j} + S_k + LS_{ik} + TS_{(i)jk} + \varepsilon_{(ijk)l}$$

$$i = 1,2,3 \quad j = 1,2,3,4 \quad k = 1,2,3 \quad l = 1,2,3,\ldots,25$$

Where:

- Y_{ijkl} = the average occurrence of the fungus from the l^{th} replicate isolated from the k^{th} section of the j^{th} tree within the i^{th} location;
- μ = the overall mean;
- L_i = the fixed effect of the i^{th} location;
- $T_{(i)j}$ = the random effect of the j^{th} tree within the i^{th} location;
- S_k = the fixed effect of the k^{th} section;
- LS_{ik} = the interaction effect of the i^{th} location with the k^{th} section;
- $TS_{(i)jk}$ = the interaction effect of the j^{th} tree within the i^{th} location with the k^{th} section;
- $\varepsilon_{(ijk)l}$ = the random residual error due to the l^{th} replicate isolated from the k^{th} section of the j^{th} tree within the i^{th} location.

A preliminary run of the linear model was tested for each tree species in order to check which treatment combinations in the model could be pooled with the experimental error. All of the treatment combinations for each tree species proved to be insignificant after the trial run. The mean squares of each treatment combination were therefore pooled with the experimental mean squares to increase the degrees of freedom (df) of the linear model. This is acceptable because by failing to reject the Null hypothesis, their

mean squares are assumed to be an estimate of the experimental error. Another run of the linear model was executed with the pooled data in order to determine which of the main effects were significant. Finally, multiple comparisons of the significant main effects (*e.g.* location) were performed using Duncan's multiple-range test to estimate statistically significantly different means among the treatments (*e.g.* JHF, SM or Q).

A second linear model was developed to analyze the overall sources of variation in the abundance of ophiostomatoid fungi that occurred on both tree species. This model is illustrated below:

$$Y_{ijklm} = \mu + H_i + L_j + HL_{ij} + T_{(j)k} + HT_{(j)ik} + S_l + HS_{il} + LS_{jl} + HLS_{ijl} + TS_{(j)kl} + HTS_{(j)ikl} + \varepsilon_{(ijkl)m}$$

$$i = 1,2 \quad j = 1,2,3 \quad k = 1,2,3,4 \quad l = 1,2,3 \quad m = 1,2,3,\ldots,25$$

Where:

- Y_{ijklm} = the average occurrence of the fungus from the m^{th} replicate isolated from the l^{th} section of the k^{th} tree within the j^{th} location from the i^{th} host tree species;
- μ = the overall mean;
- H_i = the fixed effect of the i^{th} host tree species
- L_j = the fixed effect of the j^{th} location;
- HL_{ij} = the interaction effect of the i^{th} host tree species with the j^{th} location;
- $T_{(j)k}$ = the random effect of the k^{th} tree within the j^{th} location;
- $HT_{(j)ik}$ = the interaction effect of the i^{th} host tree species with the k^{th} tree within the j^{th} location;
- S_l = the fixed effect of the l^{th} section;
- HS_{il} = the interaction effect of the i^{th} host tree species with the l^{th} section;
- LS_{jl} = the interaction effect of the j^{th} location with the l^{th} section;
- HLS_{ijl} = the interaction effect of the i^{th} host tree species with the j^{th} location and the l^{th} section;
- $TS_{(j)kl}$ = the interaction effect of the k^{th} tree, within the j^{th} location, with the l^{th} section;
- $HTS_{(j)ikl}$ = the interaction effect of the i^{th} host tree species with the k^{th} tree, within the j^{th} location, and with the l^{th} section;
- $\varepsilon_{(ijkl)m}$ = the random residual error due to the m^{th} replicate isolated from the l^{th} section of the k^{th} tree within the j^{th} location and from the i^{th} host tree species.

Individual ANOVAs and a MANOVA for the ophiostomatoid fungi isolated from both jack pine and white spruce were executed using the above model. After performing a preliminary run of the linear model, it was determined that all of the treatment combinations, apart from the HL_{ij} (tree species x location) treatment combination, could be pooled. A final run of the linear model with the pooled data was executed following by Duncan's multiple-range tests to estimate significantly different means among the treatment groups.

The Simpson's diversity index (D) was used to measure the sample diversity of all ophiostomatoid fungi isolated from their host tree and each bark beetle species. Fungal dominance was calculated using Camargo's index ($1/S$). An explanation of these two indices is illustrated in Chapter 2.

ALTERATIONS FROM THE YEAR ONE FIELD AND LAB STUDY

In the year one field and lab study (Chapter 2), jack pine did not yield a large number of ophiostomatoid fungi because most of the cultures were contaminated with bacteria and mycophagous fungi, such as *Trichoderma*. However, there were a large number of ophiostomatoid fruiting bodies within the bark beetle galleries. As a result, jack pine was reselected for the present study because it had potential to yield a greater diversity of ophiostomatoid fungi than observed in the year one study.

Trichoderma is a soil-inhabiting mycoparasite and may have entered the bark beetle galleries of the jack pine in the year one study from the soil. In addition, slabs were only removed from the sides of the jack pine log, which may have had a higher concentration of *Trichoderma* than the top of the log. To overcome the same problem in the present study, three thick discs were removed from each tree so areas contaminated

with common moulds (often appearing green in the galleries) could be avoided since sampling could occur anywhere along the perimeter of the disc. Additionally, areas within the galleries that had a wet or slimy appearance were also avoided since this appearance is typically caused by a high concentration of bacteria. It was assumed that removing discs from different lengths of the tree and isolating from several trees per location would further reduce the occurrence of non-ophiostomatoid fungi and increase the abundance and diversity of ophiostomatoid fungi.

RESULTS

BARK BEETLES, OPHIOSTOMATOID FUNGI AND THEIR DIVERSITY ON JACK PINE

Condition of Trees

Details about the condition, the abundance of bark beetle galleries and a list of bark beetle species isolated from each of the jack pine sections are presented in Appendix III. All four of the felled jack pine trees at the JHF location were exposed to mild to severe damage by woodpeckers stripping the bark. Trees 1 and 2 received a moderate amount of damage, where patches of the lower and mid sections of the main stem were stripped of bark; however there was no need to adjust the heights of the sections removed. This was not the case for tree 4, which was almost completely stripped of bark. The three sections removed from this tree had to be adjusted to areas on the stem that had the least amount of damage. The three 10cm-thick discs were removed in a way that still represented the base, middle and top of the tree. Tree 3 received the least damage, which also required no adjusting of the sections.

Fruiting bodies of an unidentified species of *Stereum* were found growing along the entire length of trees 1 and 2. When the bark was removed for sampling, the mycelium of this fungus was observed growing throughout the galleries. These areas were avoided upon sampling to reduce contamination.

On average, there was a moderate to high number of bark beetle galleries found under the bark of the jack pine sections at JHF. Most sections had a moderate number of adult bark beetles present in the galleries, while their larvae were not present on most

sections. Tree 4 had a higher number of larvae than the other three trees. There was a moderate to high level of wood-borers and other "invasive insects" present in most sections. "Invasive insects" refer to all arthropods, apart from bark beetles and wood-borers, found within the galleries, many of which are members of the Hymenoptera and Diptera. When the jack pine discs of each of the four trees were examined in cross-section, there was a low to moderate amount of stain extending into the sapwood.

The jack pine trees at the other two locations were of much better quality. Tree 1 at the SM location had a high amount of woodpecker damage to the main stem. Slight adjustments had to be made when removing the third section in order to attain a disc with a low amount of damage. None of the other trees at this location had any woodpecker damage. When examining under the bark of the four jack pine trees at SM, there was on average a moderate to high number of bark beetle galleries and wood-borers present as well as a low abundance of other "invasive insects". Tree 4 had a high number of bark beetle adults, while they were less abundant and often not present in the other three trees. No bark beetle larvae were present in any of the jack pine at SM. In cross section, the sections had on average a moderate to high amount of sapstain extending deep into the sapwood.

Each of the jack pine trees at Q had no apparent defect upon harvesting of the bark beetle galleries. However, when isolating from these trees in the lab, many of the sections had mould problems, which was evident by the dry green spore masses growing within the bark beetle galleries and along the exposed cross section of sapwood. These were expected to be species of *Trichoderma* and *Penicillium*. The mould problem was possibly the result of the forestry freezer breaking down during the winter months of 2008. While the freezer was inoperable, the sections of all of the trees examined in this

study were placed outside in the shade where temperatures remained below freezing. Snow was placed over the boxes to prevent sun from overheating the bags. Unfortunately, there were a couple of days where the outdoor temperature reached above freezing before the bags were moved to Dr. M. Leitch's (Faculty of Forestry and the Forest Environment, Lakehead University) personal freezer. The sections of wood remained in this freezer until the forestry freezer was repaired. All sections of trees 1 and 4 had mould problems and a single section of tree 2; these spots were avoided when isolating from galleries and may not have made much of an impact to the end results. When examining under the bark, there were few to no bark beetle galleries on most sections of wood. Adult bark beetles and their larvae were abundant on a few sections and were few to none on the other sections. Each tree had a high amount of wood borers present and other "invasive insects" were low to moderate in numbers. The sapstain in cross-section was on average moderate on most sections, but very high on tree 3.

Diversity of Ophiostomatoid Fungi

A total of 16 species of ophiostomatoid fungi was isolated from jack pine from the three forest properties, as illustrated in Table 6. The jack pine from JHF yielded ten species of ophiostomatoid fungi with a combined total of 218 isolates. These species included *Leptographium wingfieldii* M. Morelet, *Ophiostoma abietinum*, *O. bicolor*, *O. ips*, *O. minus*, *O. piceae*, an unknown *Hyalorhinocladiella*-like fungus, two unknown *Pesotum*-like fungi and an unknown species of *Sporothrix*. Four species were considered dominant based on Camargo's Index, including *O. ips*, *O. minus*, *Hyalorhinocladiella*-like #1 and *Pesotum*-like #1. These four species were also the most frequently occurring fungi when combining the total number of ophiostomatoid fungi isolated from the three

locations. The Simpson's Diversity Index for the ophiostomatoid fungi isolated from JHF was 0.82.

Table 6: Species of ophiostomatoid fungi isolated from jack pine

Species	Location				
	JHF	SM	Q	**Total**	Frequency
Hyalorhinocladiella-like #1	52[a]	8	50[a]	**110[a]**	20.9%
[1]*Hyalorhinocladiella*-like #2	-	-	8	**8**	1.5%
[2]*Hyalorhinocladiella*-like #3	-	-	5	**5**	0.9%
Leptographium wingfieldii	5	9	10	**24**	4.6%
[3]*Ophiostoma* #1	-	1	8	**9**	1.7%
[4]*Ophiostoma* #2	-	1	-	**1**	0.2%
Ophiostoma abietinum	13	15[a]	2	**30**	5.7%
Ophiostoma bicolor	1	2	-	**3**	0.6%
Ophiostoma ips	40[a]	64[a]	1	**105[a]**	19.9%
Ophiostoma minimum-like	-	-	5	**5**	0.9%
Ophiostoma minus	23[a]	14	13	**50[a]**	9.5%
Ophiostoma piceae	3	1	30[a]	**34[a]**	6.5%
Ophiostoma tubicollis-like	-	-	1	**1**	0.2%
[5]*Pesotum*-like #1	55[a]	32[a]	-	**87[a]**	16.5%
Pesotum-like #2	18	12	-	**30**	5.7%
[6]*Sporothrix* #1	9	16[a]	-	**25**	4.7%
Total	219	175	133	**527**	
Species Richness	10	12	10	**16**	
Camargo's Index	0.10	0.08	0.10	**0.06**	
Simpson's Diversity Index	0.82	0.80	0.78	**0.86**	

[a]Dominant species. Species were considered dominant if the relative abundance (P_i) was greater than Camargo's Index ($1/S$)
[1]Resembling anamorph of *Ophiostoma minimum*
[2]Resembling anamorph of *Ophiostoma tubicollis*
[3]Containing a *Leptographium* anamorph (may be *O. piceaperdum*)
[4]Containing a *Pesotum*-like anamorph
[5]Resembling anamorph of *Ophiostoma ips*
[6]Resembling anamorph of *Ophiostoma abietinum*

The sampling methods employed on the jack pine sections at SM yielded a total of 175 isolates of ophiostomatoid fungi, comprising of 12 separate species. These species included *L. wingfieldii*, two unidentified species of *Ophiostoma*, *O. abietinum*,

O. bicolor, *O. ips*, *O. minus*, *O. piceae*, an unidentified *Hyalorhinocladiella*-like fungus, two unidentified *Pesotum*-like fungi and an unidentified species of *Sporothrix*. The latter four fungi were assumed to be the same species found on jack pine at JHF. *Ophiostoma ips* was the most dominant species isolated from SM and made up 64 of the 175 isolates. *Ophiostoma abietinum*, *Hyalorhinocladiella*-like #1 and *Pesotum*-like #1 were also considered dominant species based on Camargo's Index. The Simpson's Index of Diversity at SM was 0.80.

The jack pine sampled from Q provided the fewest number of ophiostomatoid isolates (133). From these isolates, ten species were differentiated, including an unidentified species of *Ophiostoma* with a *Leptographium* anamorph (assumed to be the same species isolated at SM), *O. abietinum*, *O. ips*, *O. minimum*-like, *O. minus*, *O. piceae*, *O. tubicollis*-like and three unidentified *Hyalorhinocladiella*-like fungi. Two of the identified species of *Ophiostoma* (*O. minimum*-like and *O. tubicollis*-like) had only been given tentative names. The *O. minimum*-like cultures were identified as either *O. minimum* or *O. minutum* Siemaszko based on molecular analyses. *Ophiostoma minimum* was chosen as the tentative name since its morphological features fit the descriptions in the literature better than *O. minutum* (Olchowecki and Reid 1974; Jacobs and Wingfield 2001). *Ophiostoma tubicollis* (referred to as *Ceratocystis tubicollis* Olchow. & J. Reid in the literature) was the tentative name given to this culture based on the descriptions by Olchowecki and Reid (1974). This was the only culture that produced fruiting bodies, but its anamorph resembled the *Hyalorhinocladiella*-like #3 cultures, which were isolated five times. This culture has not been verified through molecular analysis.

Two dominant ophiostomatoid fungi were isolated from the jack pine trees at Q. *Hyalorhinocladiella*-like #1 consisted of 50 of the 133 isolates and *O. piceae* made up

30 of the total isolates. The former fungus was assumed to be the same species isolated from the previous two locations and was also a dominant species at the JHF location, while the latter fungus was isolated from the previous two locations, though not as frequent. The Simpson's Index of Diversity at Q was 0.78.

When combining the total number of each ophiostomatoid fungus from all locations, five species were considered to be dominant, including *Hyalorhinocladiella*-like #1, *O. ips*, *O. minus*, *O. piceae* and *Pesotum*-like #1. Each of these fungi were isolated from each location apart from *Pesotum*-like #1. This fungus was the most frequently occurring ophiostomatoid fungus from the JHF location and resembled the anamorph of *O. ips*. These two fungi made up a total of 36.4% of the total ophiostomatoid fungi isolated from jack pine in the present study. The most dominant ophiostomatoid fungus isolated from jack pine was *Hyalorhinocladiella* #1, which comprised of 20.9% of the isolates. *Ophiostoma minus* and *O. piceae* were also dominant species according to Camargo's Index, making up 9.5% and 6.5% of the total ophiostomatoid fungi isolated respectively. The Simpson's Index of Diversity for the combined total of ophiostomatoid fungi isolated from jack pine was 0.86.

Fungal Diversity on Bark Beetles Isolated from Jack Pine

Seven species of bark beetles were observed on the jack pine samples, which comprised of *Ips perturbatus* (Eichhoff), *I. pini*, *I. grandicollis* (Eichhoff), *Pityogenes plagiatus plagiatus* (LeConte), *Polygraphus rufipennis*, *Dryocoetes autographus* (Ratzeburg) and *Hylurgops rugipennis pinifex* (Fitch). Each of these bark beetle species had at least one species of ophiostomatoid fungus associated with it, apart from the *Hylurgops rugipennis pinifex* specimens (Table 7).

The bark beetle species varied at each location. *Ips pini* and *I. perturbatus* were the two most common bark beetles observed under the bark. The former beetle was observed at the SM and JHF location and was sampled from 33 times, while the latter beetle was only found at the SM site and sampled from 24 times. Thirty-three isolates of ophiostomatoid fungi were isolated from the *I. pini* adult washings and comprised of eight separate species. Two species were considered dominant according to Camargo's Index, including *Hyalorhinocladiella*-like #1 (22.2%) and *Pesotum*-like #1 (48.1%). *Ophiostoma ips* was isolated from 14.8% of the *I. pini* adults, but was not considered to be a dominant species. There were 24 isolates of ophiostomatoid fungi, consisting of six species, associated with *I. perturbatus*. *Pesotum*-like #1 and *O. ips* were the two dominant ophiostomatoid fungi from the adult washings, occurring on 37.5% and 20.8% of the bark beetles respectively.

Ips grandicollis was the only other bark beetle found on jack pine at SM and only one specimen was collected, which yielded a single isolate of *O. ips* and no other ophiostomatoid fungi. *Pityogenes plagiatus plagiatus* was only observed at JHF and yielded four species of ophiostomatoid fungi. *Pesotum*-like #1 was the only dominant fungus isolated from this bark beetle, representing 62.5% of the ophiostomatoid isolates.

Polygraphus rufipennis was third bark beetle species observed at JHF and was also observed at Q. Twelve representatives were isolated from beetle washings, which yielded a total of eleven ophiostomatoid fungi consisting of six separate species. Three species were considered dominant, including *Hyalorhinocladiella*-like #1 (33.3%), *Hyalorhinocladiella*-like #2 (16.7%) and *Ophiostoma piceae* (16.7%). The final two bark beetle species observed on jack pine in the present study were only observed at Q. *Dryocoetes autographus* was the most commonly occurring bark beetle at this location

Table 7: The number of bark beetles extracted from jack pine and their associated ophiostomatoid fungi (Isolation frequencies in parentheses)

Fungal Species	*Ips perturbatus*	*Ips pini*	*Ips grandicollis*	*Pityogenes plagiatus plagiatus*	*Polygraphus rufipennis*	*Dryocoetes autographus*	*Hylurgops rugipennis pinifex*
Hyalorhinocladiella-like #1	-	6[a] (22.2%)	-	-	4[a] (33.3%)	4[a] (28.6%)	-
Hyalorhinocladiella-like #2	-	-	-	-	2[a] (16.7%)	5[a] (35.7%)	-
Leptographium wingfieldii	1 (4.2%)	1 (3.7%)	-	-	1 (8.3%)	-	-
Ophiostoma abietinum	4 (16.7%)	2 (7.4%)	-	1 (12.5%)	-	-	-
Ophiostoma bicolor	-	-	-	1 (12.5%)	-	-	-
Ophiostoma ips	5[a] (20.8%)	4 (14.8%)	1 (100%)	-	1 (8.3%)	-	-
Ophiostoma minimum-like	-	-	-	-	1 (8.3%)	1 (7.1%)	-
Ophiostoma minus	2 (8.3%)	3 (11.1%)	-	-	-	1 (7.1%)	-
Ophiostoma piceae	-	-	-	-	2[a] (16.7%)	2 (14.3%)	-
Pesotum-like #1	9[a] (37.5%)	13[a] (48.1%)	-	5[a] (62.5%)	-	-	-
Pesotum-like #2	-	1 (3.7%)	-	-	-	-	-
Sporothrix #1	3 (12.5%)	3 (11.1%)	-	1 (12.5%)	-	-	-
Total	24	33	1	8	11	13	0
Total number of beetles (n)	24	27	1	8	12	14	3
Species Richness	6	8	1	4	6	5	0
Camargo's Index	0.17	0.13	1.00	0.33	0.17	0.20	N/A
Simpson's Diversity Index	0.76	0.78	0.00	0.45	0.78	0.72	N/A
Location[*]	SM	JHF, SM	SM	JHF	JHF, Q	Q	Q

[a]Dominant species

[*]JHF= Jack Haggerty Forest, SM= Silver Mountain, Q= Quackenbush Woodlot

and was sampled from 14 times. Five species of ophiostomatoid fungi were associated with this bark beetle, making up a total of 13 isolates. The two dominant species were *Hyalorhinocladiella*-like #1 and *Hyalorhinocladiella*-like #2, which were isolated from 28.6% and 35.7% of the bark beetles respectively. *Hylurgops rugipennis pinifex* was observed and collected three times from jack pine, yet yielded no ophiostomatoid fungi.

BARK BEETLES, OPHIOSTOMATOID FUNGI AND THEIR DIVERSITY ON WHITE SPRUCE

Condition of Trees

All of the white spruce trees examined at each of the forest properties had no evident defects when harvested for bark beetle galleries. There was a slight mould problem on four of the sections at the JHF location, but this was again assumed to be due to the forestry freezer breaking down. Under the bark of most sections, there was a high number of bark beetle galleries, most belonging to *Polygraphus rufipennis*. These galleries contained numerous bark beetle adults and larvae, especially on trees 2 and 3. There was on average a moderate to high amount of wood borers present on each tree and a low amount of "invasive insects" with the exception of tree 4, which had a moderate to high amount of "invasive insects". The amount of sapstain visible in cross section was considerably low on trees 2, 3 and 4, but slightly more on tree 1. It was also observed that the sapstain appeared much darker in areas colonized by wood borers.

There was a high number of bark beetle galleries on most of the sections at SM, but fewer on tree 1. All of the bark beetle galleries appeared to belong to *P. rufipennis*. There was a high number of adult bark beetles and their larvae within the galleries of most sections. The number of wood borers was moderate to low and there were almost

no other "invasive insects" under the bark. The sapstain in cross section of most of the samples was also very low.

The trees inspected for this study at Q proved to show the most promising results because each tree section had a high number of galleries filled with adult bark beetles and their larvae. Each tree had a low to moderate number of wood borers apart from tree 2, where there was a moderate to high level of wood borers. There was a low occurrence of other "invasive insects" on all trees and the sapstain on each section was also very low. The white spruce examined at Q yielded the highest number of fungal isolates than any other tree species and location combination.

Diversity of Ophiostomatoid Fungi

Fewer ophiostomatoid fungi, consisting of 12 species, were isolated from white spruce (Table 8) than jack pine. Nine species were isolated from white spruce at JHF, making up a total of 106 isolates. The species included an unidentified species of *Leptographium* that is related to *Ophiostoma abiocarpum* based on its ITS sequence, *L. fruticetum*, an unidentified species of *Ophiostoma* with a *Leptographium* anamorph, *O. abietinum*, *O. ips*, *O. minimum*-like, *O. minus*, *O. piceae* and an unidentified species of a *Pesotum*-like fungus. The two species of *Leptographium* were the dominant species isolated from white spruce at JHF according to Camargo's Index, where *L. fruticetum* was the most commonly encountered ophiostomatoid fungus, making up 62 of the 106 isolates. The Simpson's Diversity Index for the ophiostomatoid fungi isolated from the white spruce at JHF was 0.61.

The SM location provided the least number of ophiostomatoid fungi on white spruce. A total of ten isolates consisting of four species was encountered, including an

unidentified *Leptographium* species assumed to be the same species encountered at JHF, *L. fruticetum*, *O. abietinum* and *O. minus*. Each of these fungi, apart from *O. abietinum*, were considered dominant. The Simpson's Index of Diversity at SM was 0.72.

Table 8: Species of ophiostomatoid fungi isolated from white spruce

Species	Location				
	JHF	SM	Q	**Total**	Frequency
Hyalorhinocladiella-like #1	-	-	13	**13**	4.2%
[1]*Hyalorhinocladiella*-like #2	-	-	24[a]	**24**	7.7%
[2]*Leptographium* #1	18[a]	3[a]	27[a]	**48[a]**	15.5%
Leptographium fruticetum	62[a]	3[a]	81[a]	**146[a]**	47.1%
[3]*Ophiostoma* #1	2	-	4	**6**	1.9%
Ophiostoma abietinum	10	1	19[a]	**30[a]**	9.7%
Ophiostoma bicolor	-	-	9	**9**	2.9%
Ophiostoma ips	1	-	1	**2**	0.6%
Ophiostoma minimum-like	10	-	1	**11**	3.5%
Ophiostoma minus	1	3[a]	1	**5**	1.6%
Ophiostoma piceae	1	-	14	**15**	4.8%
[4]*Pesotum*-like #1	1	-	-	**1**	0.3%
Total	106	10	194	**310**	
Species Richness	9	4	11	**12**	
Camargo's Index	0.11	0.25	0.09	**0.08**	
Simpson's Diversity Index	0.61	0.72	0.77	**0.73**	

[a]Dominant species. Species were considered dominant if the relative abundance (P_i) was greater than Camargo's Index ($1/S$)
[1]Resembling anamorph of *Ophiostoma minimum*
[2]Related to *Ophiostoma abiocarpum*
[3]Containing a *Leptographium* anamorph (may be *O. piceaperdum*)
[4]Resembling anamorph of *Ophiostoma ips*

Contrary to jack pine, the white spruce from Q provided the highest number of ophiostomatoid isolates (194) and highest species richness (11). The species isolated from white spruce at Q included two unidentified *Hyalorhinocladiella*-like fungi, an unidentified *Leptographium* species that was assumed to be the same species as the other two locations, *L. fruticetum*, an unidentified *Ophiostom*a species that was also observed

on white spruce at JHF, *O. abietinum*, *O. bicolor*, *O. ips*, *O. minimum*-like, *O. minus* and *O. piceae*. *Leptographium fruticetum* was the most dominant ophiostomatoid fungus isolated at this location, representing 81 of the total isolates. Other dominant ophiostomatoid fungi included *Hyalorhinocladiella*-like #2, *Leptographium* #1 and *O. abietinum*. The Simpson's Index of Diversity at Quackenbush woodlot was 0.77.

Three species were considered dominant when combining the totals of all the ophiostomatoid fungi isolated from white spruce, including *Leptographium* #1, *L. fruticetum* and *O. abietinum*. All three of these fungi were commonly encountered at all three forest properties, where the former two were considered dominant species at all three locations. *Ophiostoma minus* was the only other species encountered at all three locations and was considered dominant at SM; however, it only made up 1.6% of the total isolates. *Leptographium fruticetum* was by far the most dominant ophiostomatoid fungus, making up nearly half (47.1%) of all of the ophiostomatoid isolates. *Leptographium* #1 and *O. abietinum* made up 15.5% and 9.7% of the total ophiostomatoid isolates respectively. The Simpson's Index of Diversity for the combined total of ophiostomatoid fungi isolated from white spruce was 0.73.

Fungal Diversity on Bark Beetles Isolated from White Spruce

Only three species of bark beetles were observed on the white spruce samples, including *Polygraphus rufipennis*, *Dryocoetes autographus* and *Crypturgus borealis* Swaine (Table 9). *Crypturgus borealis* was only observed once at Q and yielded no fungi when an adult washing was performed. *Polygraphus rufipennis* was the most abundant bark beetle observed and occurred in high numbers at all three locations. A total of 123 representatives of *P. rufipennis* adults was used in the present study,

yielding 75 cultures of ophiostomatoid fungi, consisting of nine species. Two species were considered dominant based on Camargo's Index. *Leptographium fruticetum* was the most dominant and found on nearly a third (30.1%) of the bark beetles. *Ophiostoma abietinum* was not isolated as frequently, but was considered dominant and was isolated from 7.3% of the adult bark beetles. *Dryocoetes autographus* was observed at JHF and more commonly at Q. From the 30 specimens sampled, only four fungi were isolated, comprising of three separate species. The only dominant species was *O. abietinum*, which occurred on 6.7% of the adult bark beetles.

Table 9: The number of bark beetles extracted from white spruce and their associated ophiostomatoid fungi (Isolation frequencies in parentheses)

Fungal Species	*Polygraphus rufipennis*	*Dryocoetes autographus*	*Crypturgus borealis*
Hyalorhinocladiella-like #1	4 (3.3%)	-	-
Hyalorhinocladiella-like #2	7 (5.7%)	-	-
Leptographium #1	7 (5.7%)	-	-
Leptographium fruticetum	37[a] (30.1%)	1 (3.3%)	-
Ophiostoma #1	1 (0.8%)	-	-
Ophiostoma abietinum	9[a] (7.3%)	2[a] (6.7%)	-
Ophiostoma minimum-like	8 (6.5%)	-	-
Ophiostoma minus	1 (0.8%)	-	-
Ophiostoma piceae	1 (0.8%)	1 (3.3%)	-
Total	75	4	0
Total number of beetles (n)	123	30	1
Species Richness	9	3	0
Camargo's Index	0.11	0.33	N/A
Simpson's Diversity Index	0.71	0.63	N/A
Location*	JHF, SM, Q	JHF, Q	Q

[a]Dominant species

*JHF= Jack Haggerty Forest, SM= Silver Mountain, Q= Quackenbush Woodlot

UNIVARIATE AND MULTIVARIATE ANALYSIS OF VARIANCE

Jack Pine

The ANOVAs performed for each of the ophiostomatoid fungi isolated from jack pine revealed inconsistent significance between each of the treatment groups at the $p<0.05$ level (Table 10). Five of the sixteen fungi showed significant differences between the location treatment means, four between the tree replication treatment means and two fungi showed significant difference between the section treatment means.

Table 10: ANOVA and MANOVA of the ophiostomatoid fungi isolated from jack pine (bold values are significant at the $p<0.05$ level)

Fungal Species	Source of Variation	Location	Tree	Section
(A) Univariate Analysis				
Hyalorhinocladiella-like #1	F	2.251	1.191	0.401
	P	0.161	0.348	0.674
Hyalorhinocladiella-like #2	F	24.000	0.140	0.368
	P	**<0.001**	0.998	0.696
Hyalorhinocladiella-like #3	F	1.000	1.000	1.000
	P	0.405	0.469	0.384
Leptographium wingfieldii	F	0.389	0.947	3.316
	P	0.689	0.506	0.055
Ophiostoma #1	F	2.898	3.434	3.667
	P	0.107	**0.009**	**0.042**
Ophiostoma #2	F	1.000	1.000	1.000
	P	0.405	0.469	0.384
Ophiostoma abietinum	F	2.183	2.224	2.081
	P	0.169	0.061	0.149
Ophiostoma bicolor	F	1.000	4.000	1.000
	P	0.405	**0.004**	0.384
Ophiostoma ips	F	3.762	3.791	0.150
	P	0.065	**0.005**	0.862
Ophiostoma minimum-like	F	3.947	1.181	0.186
	P	0.059	0.354	0.831

Ophiostoma minus	F	0.198	8.189	4.618
	P	0.824	**<0.001**	**0.021**
Ophiostoma piceae	F	5.247	0.556	0.248
	P	**0.031**	0.818	0.782
Ophiostoma tubicollis-like	F	1.000	1.000	1.000
	P	0.405	0.469	0.384
Pesotum-like #1	F	8.467	1.149	0.778
	P	**0.009**	0.373	0.472
Pesotum-like #2	F	4.846	1.042	0.541
	P	**0.037**	0.440	0.590
Sporothrix #1	F	5.035	1.215	2.314
	P	**0.034**	0.335	0.122

(B)Multivariate Analysis				
Pillai's Trace	P	**0.020**	**0.012**	0.760
Wilks' Lambda	P	**0.014**	**0.016**	0.783
Hotelling's Trace	P	**0.011**	**0.024**	0.808
Roy's Largest Root	P	**0.003**	**0.005**	0.379

*F= F-value, P= P-value

Duncan's multiple-range tests were performed on each of the significant treatment groups to determine which of the treatment means differed significantly from each other (Table 11). Duncan's multiple-range tests were not performed on the tree replication treatments since the variables of this treatment are considered dependent and cannot be compared from location to location. When examining the results in Table 11, four of the five fungi that differed significantly in abundance from the location treatment were not significantly different between SM and JHF, but their abundance in Q differed significantly from the other two locations. The abundance of *Sporothrix* #1 was significantly different between SM and Q, but was not significantly different between JHF and the other two locations.

The two fungi that showed significant difference in abundance from the section treatment were not significantly different between sections 2 and 3. *Ophiostoma* #1 was

also not significantly different between sections 1 and 3, but was significantly different between sections 1 and 2. *Ophiostoma minus* was found to be significantly different in abundance between section 1 and the other two sections.

All of the tests of the MANOVA showed that the location treatment and tree replication treatment had significant impact on the abundance of ophiostomatoid fungi, while none of the tests found significant difference between the section treatment means.

Table 11: Results of Duncan's multiple range test on significant treatment groups for ophiostomatoid fungi isolated from jack pine

Location			
Dependent Variable	JHF	SM	Q
Hyalorhinocladiella-like #2	0.00^{a}	0.00^{a}	0.67^{b}
Ophiostoma piceae	0.25^{a}	0.08^{a}	2.50^{b}
Pesotum-like #1	4.58^{a}	2.67^{a}	0.00^{b}
Pesotum-like #2	1.50^{a}	1.00^{a}	0.00^{b}
Sporothrix #1	$0.75^{a, b}$	1.33^{a}	0.00^{b}
Section			
Dependent Variable	1	2	3
Ophiostoma #1	0.50^{a}	0.08^{b}	$0.17^{a, b}$
Ophiostoma minus	2.25^{a}	1.17^{b}	0.75^{b}

^{a}=subset 1, ^{b}=subset 2

White Spruce

The ANOVA tests applied to each ophiostomatoid fungus isolated from white spruce showed that seven of the twelve fungi were significantly different in abundance between the location treatment means and one fungus showed significant difference between the section treatment means at the $p<0.05$ level (Table 12).

The results of the Duncan's multiple-range tests revealed that four of the seven fungi showing significant differences in abundance from the location treatment were

Table 12: ANOVA and MANOVA of the ophiostomatoid fungi isolated from white spruce (bold values are significant at the $p<0.05$ level)

Fungal Species	Source of Variation*	Location	Tree	Section
(A) Univariate Analysis				
Hyalorhinocladiella-like #1	F	11.791	0.549	2.338
	P	**0.003**	0.823	0.120
Hyalorhinocladiella-like #2	F	22.590	1.499	0.704
	P	**<0.001**	0.209	0.505
Leptographium #1	F	3.020	1.560	0.381
	P	0.099	0.189	0.687
Leptographium fruticetum	F	8.384	2.195	0.332
	P	**0.009**	0.064	0.721
Ophiostoma #1	F	3.000	0.772	0.579
	P	0.100	0.643	0.569
Ophiostoma abietinum	F	9.851	0.411	0.200
	P	**0.005**	0.915	0.820
Ophiostoma bicolor	F	22.091	0.791	1.941
	P	**<0.001**	0.628	0.167
Ophiostoma ips	F	0.500	0.957	0.478
	P	0.622	0.500	0.626
Ophiostoma minimum-like	F	6.167	0.971	1.941
	P	**0.021**	0.489	0.167
Ophiostoma minus	F	0.706	1.056	4.661
	P	0.519	0.430	**0.021**
Ophiostoma piceae	F	11.681	0.676	0.388
	P	**0.003**	0.722	0.683
Pesotum-like #1	F	1.000	1.000	1.000
	P	0.405	0.469	0.384
(B)Multivariate Analysis				
Pillai's Trace	P	**<0.001**	0.285	0.376
Wilks' Lambda	P	**<0.001**	0.350	0.333
Hotelling's Trace	P	**<0.001**	0.424	0.302
Roy's Largest Root	P	**<0.001**	0.071	0.063

*F= F-value, P= P-value

significantly different between Q and the other two locations, while their abundance was not significantly different between JHF and SM (Table 13). This was the same trend

observed for four of the five fungi that showed significant differences in abundance from the location treatment on jack pine. *Ophiostoma minimum*-like was significantly different in abundance between JHF and the other two locations, but was not significantly different between SM and Q. The abundance of *Leptographium fruticetum* was not significantly different between JHF and Q, but was significantly different between SM and the other two locations. *Ophiostoma abietinum* was found not to be significantly different in abundance between JHF and SM as well as between JHF and Q, but was significantly different between SM and Q. *Ophiostoma minus* was the only fungus that was determined to be significantly different in abundance from the section treatment, where it was significantly different between section 1 and the other two sections, but not significant between sections 2 and 3.

Table 13: Results of Duncan's multiple range test on significant treatment groups for ophiostomatoid fungi isolated from white spruce

Location			
Dependent Variable	JHF	SM	Q
Hyalorhinocladiella-like #1	0.00^{a}	0.00^{a}	1.08^{b}
Hyalorhinocladiella-like #2	0.00^{a}	0.00^{a}	2.08^{b}
Leptographium fruticetum	5.33^{a}	0.25^{b}	6.75^{a}
Ophiostoma abietinum	$0.83^{a, b}$	0.08^{a}	1.58^{b}
Ophiostoma bicolor	0.00^{a}	0.00^{a}	0.75^{b}
Ophiostoma minimum-like	0.92^{a}	0.00^{b}	0.08^{b}
Ophiostoma piceae	0.08^{a}	0.00^{a}	1.17^{b}
Section			
Dependent Variable	1	2	3
Ophiostoma minus	0.22^{a}	0.00^{b}	0.00^{b}

^{a}=subset 1, ^{b}=subset 2

All of the tests of the MANOVA revealed that the location treatment had a significant impact on the abundance of ophiostomatoid fungi isolated from white spruce,

but did not find the tree replication or section treatments to have a significant impact on abundance.

Table 14: ANOVA and MANOVA of the ophiostomatoid fungi isolated from both jack pine and white spruce (bold values are significant at the $p<0.05$ level)

	Source of Variation*	Tree Species	Location	Tree	Section	Species x Location
(A) Univariate Analysis						
Hyalorhinocladiella-like #2	F	7.873	55.373	0.536	1.062	7.873
	P	**0.007**	**<0.001**	0.842	0.353	**0.001**
Ophiostoma #1	F	0.632	7.085	1.100	2.739	1.897
	P	0.430	**0.014**	0.378	0.073	0.160
Ophiostoma abietinum	F	0.000	0.527	0.813	1.418	8.144
	P	1.000	0.608	0.606	0.251	**0.001**
Ophiostoma bicolor	F	8.292	8.739	1.297	2.708	17.431
	P	**0.006**	**0.008**	0.260	0.076	**<0.001**
Ophiostoma ips	F	35.617	3.517	2.787	0.096	10.383
	P	**<0.001**	0.074	**0.009**	0.909	**<0.001**
Ophiostoma minimum-like	F	1.977	3.845	0.955	1.251	7.304
	P	0.165	0.062	0.487	0.294	**0.002**
Ophiostoma minus	F	15.662	0.168	3.627	3.395	0.859
	P	**<0.001**	0.848	**0.001**	**0.041**	0.429
Ophiostoma piceae	F	1.321	14.611	0.433	0.157	0.772
	P	0.255	**0.001**	0.911	0.855	0.467
Pesotum-like #1	F	30.765	8.880	1.109	0.828	9.201
	P	**<0.001**	**0.007**	0.372	0.442	**<0.001**
(B)Multivariate Analysis						
Pillai's Trace	P	**<0.001**	**<0.001**	0.097	0.139	**<0.001**
Wilks' Lambda	P	**<0.001**	**<0.001**	0.099	0.137	**<0.001**
Hotelling's Trace	P	**<0.001**	**<0.001**	0.101	0.136	**<0.001**
Roy's Largest Root	P	**<0.001**	**<0.001**	**0.012**	**0.038**	**<0.001**

*F= F-value, P= P-value

Jack Pine and White Spruce

Univariate ANOVAs were also performed on the ophiostomatoid fungi isolated from both hosts, which showed inconsistent significance between each of the treatment

groups at the $p<0.05$ level (Table 14). *Ophiostoma abietinum* had an F-value of 0 and a P-value of 1 for the tree species treatment group, which was the result of the same number of *O. abietinum* cultures being isolated from both tree species. Five of the nine fungi were found to have significant differences in abundance between the tree species treatment means, five between the location treatment means, two between the tree replication treatment means, one between the section treatment means and six species showed significant difference between the means of the tree species x location treatment combination.

Duncan's multiple-range tests were performed on each of the significant location and section treatment groups to estimate significantly different means among the treatment groups (Table 15). Duncan's multiple-range tests were not required for the tree species treatment group since there were only two tree species to compare. All five of the fungi that differed significantly in abundance from the location treatment were not significantly different between SM and JHF, but their abundance in Q differed significantly from the other two locations. *Ophiostoma minus* was the only fungus found to be significantly different in abundance from the section treatment. The abundance of this fungus was significantly different between JHF and Q, but was not significantly different between SM and the two other locations.

Interaction plots for the significant interactions between the tree species and location treatment groups are illustrated in Figure 2. The estimated marginal means of these interactions plots are noticeably much different between location for jack pine and white spruce. The interaction plots in Figure 2 therefore show how the ophiostomatoid fungi have been isolated at significantly different frequencies between the three locations and two tree species. For example, Figure 2c shows a moderate abundance of

Table 15: Results of Duncan's multiple range test on significant treatment groups for ophiostomatoid fungi isolated from both jack pine and white spruce

Location			
Dependent Variable	JHF	SM	Q
Hyalorhinocladiella-like #2	0.00[a]	0.00[a]	1.38[b]
Ophiostoma #1	0.08[a]	0.04[a]	0.50[b]
Ophiostoma bicolor	0.00[a]	0.08[a]	0.38[b]
Ophiostoma piceae	0.17[a]	0.04[a]	1.83[b]
Pesotum-like #1	2.33[a]	1.33[a]	0.00[b]
Section			
Dependent Variable	1	2	3
Ophiostoma minus	1.33[a]	0.58[a, b]	0.37[b]

[a]=subset 1, [b]=subset 2

O. abietinum isolated from jack pine at JHF and SM and a low abundance at Q, whereas it was isolated at a high abundance on white spruce at Q, a moderate abundance at JHF and a low abundance at SM. If the abundance of *O. abietinum* was not significantly different within the tree species and location interaction, then this fungus would have been isolated at a similar abundance from both tree species at all three locations.

Three of the four tests of the MANOVA performed on the ophiostomatoid fungi isolated from both tree species found that the tree species treatment, location treatment and tree species x location treatment combination were statistically significant. Roy's largest root was the only test that found all treatment groups and the tree species x location treatment combination to have a significant impact on abundance.

FUNGAL DIVERSITY OF NON-OPHIOSTOMATOID FUNGI

Similar to the year one study, many non-ophiostomatoid fungi were isolated in the present study (see Appendix II). A total of 1100 isolates (~50 species) were obtained from jack pine and 1605 (~43 species) from white spruce.

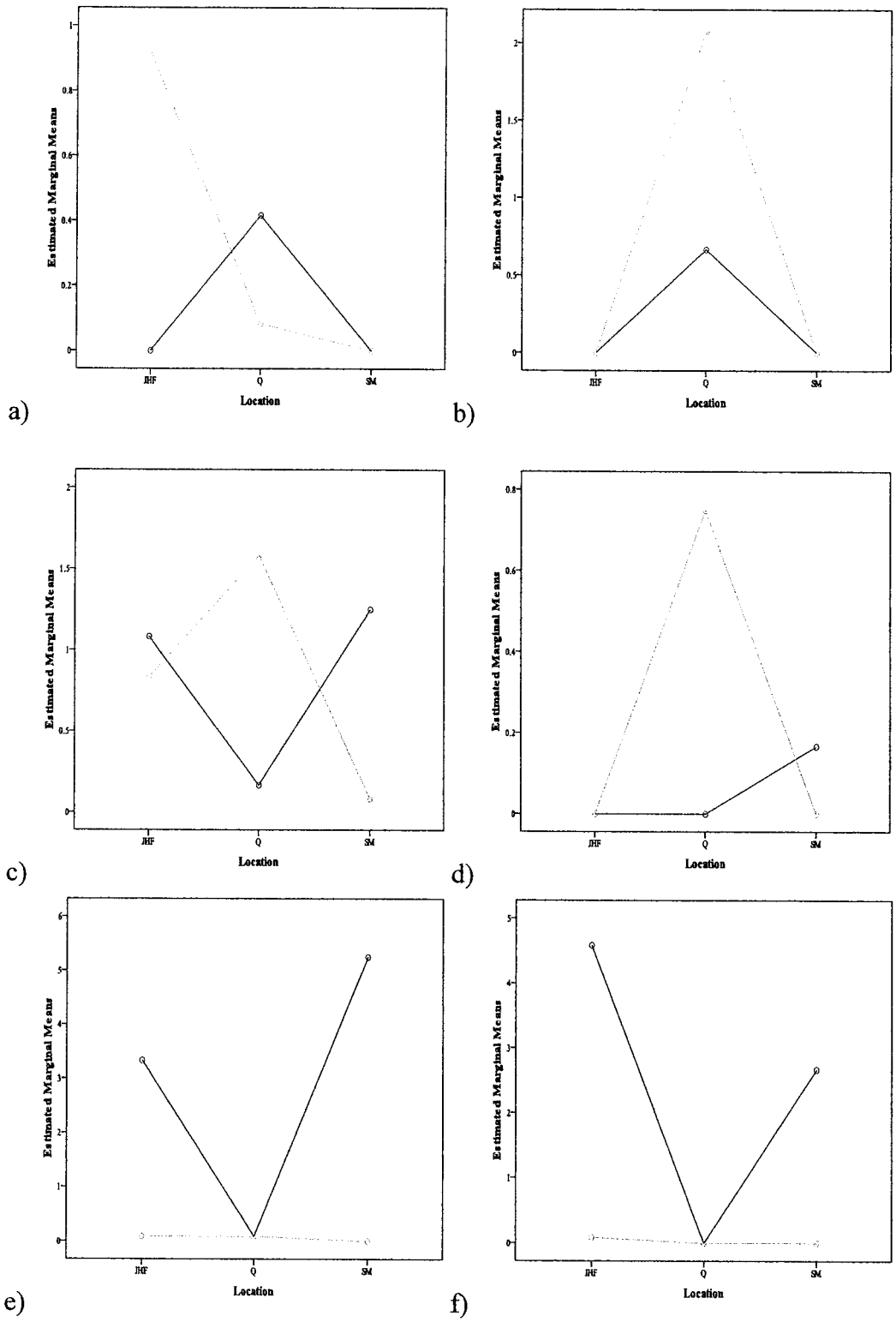

Figure 2: Estimated marginal means of a) *Ophiostoma minimum*-like, b) *Hyalorhinocladiella*-like #2, c) *Ophiostoma abietinum*, d) *Ophiostoma bicolor*, e) *Ophiostoma ips*, and f) *Pesotum*-like #1 (The blue line represents jack pine and the green line represents white spruce)

Ophiostomatoid fungi were the most dominant fungi isolated from jack pine. *Hyalorhinocladiella*-like #1, *Ophiostoma ips* and *Pesotum*-like #1 were the top three fungi isolated from jack pine and made up 10.0%, 9.5% and 7.9% of the total isolates respectively. The most common non-ophiostomatoid fungi included *Candida* (7.7%), *Rhizomucor* (5.2%), *Penicillium* (4.9%), *Dipodascus* (4.7%), *Trichoderma* (4.4%) and an unknown Coelomycete (4.1%).

The fungi isolated from white spruce consisted primarily of non-ophiostomatoid fungi, particularly *Candida* (20.4%), *Calcarisporium arbuscula* (17.6%), an unknown Hyphomycete (17.3%), *Penicillium* (5.0%) and *Cladosporium sphaerospermum* (1.5%). *Cladosporium sphaerospermum* was especially bad in the white spruce removed from the Q location and *Calcarisporium arbuscula* was frequently isolated from the trees felled at SM and JHF. *Leptographium fruticetum* was the most commonly isolated ophiostomatoid fungus, making up 9.1% of the total isolates.

DISCUSSION

OCCURRENCE OF BARK BEETLES

Eight bark beetle species were observed on jack pine and white spruce, including *Ips perturbatus*, *I. pini*, *I. grandicollis*, *Pityogenes plagiatus plagiatus*, *Hylurgops rugipennis pinifex*, *Polygraphus rufipennis*, *Dryocoetes autographus* and *Crypturgus borealis*. All but two of these bark beetles (*H. rugipennis pinifex* and *C. borealis*) yielded at least one and as many as nine ophiostomatoid fungi from the adult washings. Each of the bark beetle species collected in the present study, apart from *C. borealis*, were observed on jack pine, while only three were observed on white spruce: *C. borealis*, *D. autographus* and *P. rufipennis*.

The three species of bark beetles observed on white spruce were also observed in Haberkern *et al.*'s (2002) extensive survey of bark beetles and their fungal associates on white spruce throughout the lower Great Lakes region. The authors also reported several other bark beetle species that were not observed on white spruce in the present study. For example, *I. pini* and *I. grandicollis* were observed in Haberkern *et al.*'s (2002) study, but were only observed on jack pine in the present study. *Ips pini* was, however, observed on black spruce in the year one study, which confirms that this bark beetle colonizes spruce in the area. As mentioned in Chapter 2, *I. pini*'s primary host is pine, but it will also occasionally colonize spruce.

In Mussell's (2004) survey on fungal associates of bark beetles colonizing jack pine, only two bark beetle species were observed: *Ips pini* and *P. rufipennis*. Mussell's (2004) study was not as extensive as the present study because Mussell (2004) only

examined jack pine at one location (Jack Haggerty Forest) with fewer samples of bark beetle galleries. The present study observed a high number of *I. pini* on jack pine at JHF as well as a few specimens of *P. rufipennis* and *Pt. plagiatus plagiatus*. *Ips pini* was only observed once at SM and not at Q, while *P. rufipennis* was observed several times at Q and not at SM. This illustrates how the diversity of bark beetles can vary from location to location with only about 100 km difference between the two most distant forest properties. *Pityogenes plagiatus plagiatus* was not observed in Mussell's (2004) study and were uncommon (three specimens) in the present study. Mussell (2004) examined 19 samples from four jack pine logs where there was evidence of bark beetle activity. It is believed if more samples were examined along the length of the logs that more bark beetle species would have been observed in her study. Even in the present study, if more trees were examined and more samples removed at this location, there would likely be a higher diversity of bark beetles present. In North America, there are at least 27 bark beetle species known to colonize jack pine (Bright 1976).

Two of the bark beetles observed in the present study were also present in the year one study: *P. rufipennis* and *I. pini*. A short description of these two bark beetles can be found in Chapter 2. *Polygraphus rufipennis* is a common invader of spruce, but has also been observed colonizing all conifers within its range. It was observed in high numbers on white spruce at all three forest properties and was only present in low numbers on jack pine at two of the forest properties. Very few studies have examined the fungal associates of *P. rufipennis*. This thesis is the first to report *L. wingfieldii*, *O. minimum*, *L. fruticetum*, *O. abietinum* and *O. minus* as associates of *P. rufipennis*. *Leptographium fruticetum* was the most dominant fungus isolated from *P. rufipennis* and occurred on 30.1% of the adults. In the year one study, this fungus was also

dominant on the *P. rufipennis* adults isolated from black spruce and occurred on 22.7% of the adults. Therefore it can be suggested that this fungus may depend on the presence of *P. rufipennis* for the dispersal of its spores.

Ips pini was commonly observed on jack pine at JHF, but was only observed once at SM. Although this bark beetle can colonize white spruce, it was not present on any of the white spruce bait logs examined in the present study. Even though *I. pini* commonly colonizes jack pine, very few studies have examined their fungal associates. Of the few studies that have examined fungal associates of *I. pini*, it is agreed that *O. ips* is commonly vectored by this bark beetle (Raffa and Smalley 1988; Furniss *et al.* 1995; Mussell 2004). Although, *O. ips* was not one of the dominant ophiostomatoid fungi isolated from *I. pini*, the *Pesotum*-like #1 cultures, which resembled the anamorph of *O. ips*, were dominant and occurred on 48.1% of the *I. pini* adults. With further molecular analyses, it is proposed that these two fungi may prove to be conspecific.

Ophiostoma abietinum has never been reported on *I. pini* based on the extent of this literature search. *Ophiostoma abietinum* was originally described as an extralimital species (see Chapter 2) but was commonly isolated in both studies in the present thesis. It was interesting to find it associated with bark beetles this far north because records of the fungus have only been reported in Mexico and South Africa.

Six of the eight bark beetles observed in the present study were not recorded in the year one study. The northern spruce engraver (*Ips perturbatus*) was only observed on jack pine at SM and made up all but two of the bark beetle specimens observed on jack pine at this location. *Ips perturbatus* measures about 4.2-4.8mm in length and is found transcontinental in Canada (Bright 1976). Its hosts are primarily spruce, but it also colonizes jack pine and lodgepole pine (Bright 1976). *Ips perturbatus* does not appear to

be overly aggressive since these bark beetles normally colonize weakened, dead or fallen trees (Wood 1982; Alamouti *et al.* 2006); however, they have been reported to attack and kill healthy trees during outbreaks (Ross *et al.* 2001; Alamouti *et al.* 2006). The fungal associates of *I. perturbatus* have not been thoroughly examined, which may be because the beetles do not normally pose a threat to the forest industry. To date, *L. wingfieldii*, *O. abietinum*, *O. ips* and *O. minus* are new records on *I. perturbatus*.

Ips grandicollis, known in the literature as the eastern five-spined ips, was isolated once from jack pine at the SM property. Ranging from 2.8-4.7mm in length, *I. grandicollis* is commonly found on logging slash or trees in stress throughout eastern Canada and the United States (Bright 1976). This bark beetle is rarely reported as a nuisance, but is capable of killing healthy trees, especially in times of drought or other factors leading to widespread stress of trees (Bright 1976; Morgan 1989). Very few studies have examined the fungal associates of *I. grandicollis*, as is the case for most species of *Ips* (Alamouti *et al.* 2006). In the present study, *O. ips* was the only ophiostomatoid fungus isolated from *I. grandicollis*, which is similar to Haberkern *et al.*'s (2002) study, where *O. ips* was the dominant fungus of *I. grandicollis*.

Pityogenes plagiatus plagiatus averages between 1.7-2.4mm in length and is located in eastern Canada, but has been recorded as far west as Alberta (Bright 1976). Its hosts are primarily jack pine, red pine (*Pinus resinosa* Aiton) and spruce and it commonly colonizes slash and fallen branches, and sometimes stems of standing dying trees (Wood 1982). Eight specimens were observed and examined and they all occurred on jack pine at JHF. Very little is known about this bark beetle and it does not appear to be a threat to living trees. Currently, there appears to be no published literature on the

fungal associates of *Pt. plagiatus plagiatus*, and as a result *O. abietinum* and *O. bicolor* are two new associates.

Hylurgops rugipennis pinifex commonly colonizes a wide variety of conifer hosts, including *Pinus*, *Picea* and sometimes *Larix*. This bark beetle measures about 4.0-5.0mm in length and occurs throughout eastern Canada and through to Alberta (Bright 1976). *Hylurgops rugipennis pinifex* prefers the thicker portion of its host tree, colonizing primarily stumps, logs and the lower part of standing trees, including the roots (Bright 1976). Interestingly enough, all three of the bark beetles isolated in the present study came from section one (base) of a jack pine tree, which had a diameter of 22.2cm. There appears to be no research about the fungal associates of *H. rugipennis pinifex* and there were no ophiostomatoid associates identified from the adults examined.

Dryocoetes autographus was observed on jack pine and white spruce in the present study, which is normal of this species, since it has been observed on all species of conifers within its geographic range (Bright 1976). However, even though the bark beetle appears to colonize a wide range of conifers, there have been no recorded cases of *D. autographus* on jack pine. *Dryocoetes autographus* averages between 3.4 and 5.0mm in length and occurs transcontinental in Canada as well as eastern and western United States and Eurasia (Bright 1976). Very little is known about this bark beetle, but it appears that it does not attack healthy trees and is often observed colonizing the base of dying or stressed trees as well as stumps and large roots (Wood 1982). Adult bark beetles may produce more than one brood, but it takes one to two years to complete their life cycle (Wood 1982). Several studies have examined fungal associates of *D. autographus*, though none have viewed the bark beetle to be problematic (Haberkern *et*

al. 2002; Kirisits 2004; Romón *et al.* 2007). This study reports *L. fruticetum* and *O. abietinum* as new associates of *D. autographus*.

Crypturgus borealis are small bark beetles (1.1-1.3mm in length) that inhabit the galleries of other wood-boring insects in order to gain access to the phloem tissues (Wood 1982). One specimen of *C. borealis* was collected in the present study on white spruce at the Q property. *Crypturgus borealis* has a large host range, colonizing all species of coniferous trees within its range and is found transcontinental in Canada and in the western United States (Bright 1976). However, this bark beetle has never been reported in the Thunder Bay region and was reported for the first time in Wisconsin, Minnesota and Michigan in 2002 (Haberkern 2002). *Crypturgus borealis* is more than likely native to the region, but has gone unnoticed either due to its small size, its economic unimportance or the lack of research. This bark beetle does not pose a threat to living trees as it requires existing entrance holes from other wood boring insects in order to feed on the phloem. No information is available on any ophiostomatoid associates of *C. borealis*. Although Haberkern *et al.* (2002) examined a large number of *C. borealis* specimens, they did not report any fungal associates in their study, which may be due to their small size. The present study did not identify any ophiostomatoid fungi associated with these bark beetles.

OCCURRENCE OF OPHIOSTOMATOID FUNGI

Throughout the present study, nine of the ophiostomatoid fungi isolated were tentatively identified to species, including two species of *Leptographium* and seven species of *Ophiostoma*. These species include *L. wingfieldii*, *L. fruticetum*, *O. abietinum*, *O. bicolor*, *O. ips*, *O. minimum*-like, *O. minus*, *O. piceae* and *O. tubicollis*-like. As in the

year one study, several other ophiostomatoid fungi of the genera *Ophiostoma*, *Leptographium*, *Pesotum*, *Sporothrix* and *Hyalorhinocladiella* were isolated, but have not yet been identified to species. Occurrence and pathogenicity of the ophiostomatoid fungi identified in the present study that were also observed in the year one study are found in Chapter 2.

In Mussell's (2004) survey, representatives of *O. ips*, *O. piceae*, three unidentified species of *Ophiostoma* and several unidentified species of *Leptographium*, *Pesotum*, *Sporothrix* and *Hyalorhinocladiella* were isolated from bark beetles colonizing jack pine at JHF. Similar to the present study, Mussell (2004) observed that *O. ips* made up a large number of the ophiostomatoid cultures and was closely associated with *I. pini*. Molecular analyses of some of these fungal cultures afterwards confirmed *Leptographium wingfieldii* and *Graphium pseudormiticum* were among the isolates in Mussell's (2004) survey (Dr. L.J. Hutchison *pers. comm.*).

Occurrence and pathogenicity of *L. fruticetum*, *O. abietinum*, *O. bicolor*, *O. ips*, *O. minus* and *O. piceae* are summarized in Chapter 2. Representatives of these fungi were found on both tree species except for *L. fruticetum*, which only occurred on white spruce. In addition, the latter was the most dominant ophiostomatoid fungus isolated from white spruce at all three locations. This was also a dominant species on black spruce in the year one study. Since *L. fruticetum* has just recently been described (Alamouti *et al.* 2006), very little is known about the biology of this fungus. It appears the fungus may be economically important, since it has commonly been encountered on spruce, and even tamarack in the year one study (see Chapter 2). It is felt that the pathology of this fungus should be examined further.

Ophiostoma abietinum was frequently isolated from both tree species and was a dominant fungus (9.7%) with the combined total of ophiostomatoid cultures on white spruce. It was surprising that this fungus occurred as often as it did throughout both studies since it has never been recorded in North America. This study is the first to report *O. abietinum* in Canada on white spruce, jack pine as well as on tamarack.

Ophiostoma ips was frequently encountered in the present study and made up about 20% of the total ophiostomatoid cultures isolated from jack pine. In addition, the *Pesotum*-like #1 cultures resembled the anamorph of *O. ips*, but perithecia did not appear on these cultures even after they were transferred onto wood samples to stimulate the development of fruiting bodies. As already stated, it is believed that the *Pesotum*-like #1 cultures are the anamorphic state of *O. ips*, which would make the total number of *O. ips* cultures to nearly double in size. *Ophiostoma ips* was commonly isolated from *Ips perturbatus* and *I. pini* in the present study. Since this fungus has the ability to be highly pathogenic in high concentrations (Mathre 1964; Fernández *et al.* 2004), it is suggested that this fungus should be taken seriously in case of an outbreak by either of the two bark beetles mentioned above.

Two species of ophiostomatoid fungi that had not been isolated in the year one study were *Leptographium wingfieldii* and *Ophiostoma tubicollis*-like. *Leptographium wingfieldii* was commonly isolated from jack pine at the three locations and made up 4.6% of the total ophiostomatoid fungi isolated from jack pine. *Leptographium wingfieldii* is a common blue stain fungus on pine in Europe and is believed to be closely associated with the pine-shoot bark beetle (*Tomicus piniperda* (L.)) (Solheim *et al.* 1993; Jacobs and Wingfield 2001; Jacobs *et al.* 2004). Coincidently, *T. piniperda* was introduced into North America at roughly the same times as *L. wingfieldii* (Jacobs *et*

al. 2004). Jacobs *et al.* (2004) felt that *T. piniperda* was responsible for introducing *L. wingfieldii* to new hosts, yet no specimens of *T. piniperda* were observed in the present study. As well, there have been no records of *T. piniperda* in the Thunder Bay region to date (CFIA 2008). In 2007, OMNR picked 83 sites across Northern Ontario, including the Thunder Bay region, to determine infestation status, which showed up negative to *T. piniperda* in Northwestern Ontario (CFIA 2008). However, the presence of *L. wingfieldii* could suggest that the bark beetle is in the area. The CFIA has listed *T. piniperda* as "one of the most destructive bark beetles of pine in its native Eurasia" and it is of great concern in North America (CFIA 2008).

Although *T. piniperda* poses serious threat to native pines in North America, *L. wingfieldii* could also be an economically important pest because it is considered a relatively pathogenic fungus in Europe (Solheim *et al.* 1993). Through mass inoculations, Solheim *et al.* (1993) observed that *L. wingfieldii* was capable of killing healthy young Scots pine (*Pinus sylvestris* L.) in Sweden. However, dosages were in high concentration, which is not representative of natural bark beetle attacks (Solheim *et al.* 1993). *Leptographium wingfieldii* has been found as an associate to many bark beetle species, including *Ips pini* (Jacobs *et al.* 2004), but it has never been recorded from *I. perturbatus* or *P. rufipennis* as in this study. To date, *L. wingfieldii* has never been reported on jack pine. This may be due to the lack of research on jack pine, since the fungus' primary host is pine. Solheim *et al.* (1993) suggested the pathogenicity of this fungus could be even more devastating by having the ability to enhance the performance of other bark beetle species not previously associated with the fungus due to its wide range of insect vectors. This is another fungus to be taken seriously in case of a bark beetle outbreak, especially that of *T. piniperda.*

Ophiostoma tubicollis was isolated once in the present study on jack pine at Q, but several isolates of a *Hyalorhinocladiella*-like fungus resembling the anamorph of *O. tubicollis* was observed at the same location on jack pine. Very little is known about *O. tubicollis*, which was the tentative name applied to the fungus based on the descriptions by Olchowecki and Reid (1974). Olchowecki and Reid (1974) placed the fungus under the genus *Ceratocystis*; however, it should belong to the genus *Ophiostoma* due to its *Hyalorhinocladiella* anamorph. Currently, the only records of *O. tubicollis* in North America are in Ontario (balsam fir) and Manitoba (jack pine, red pine and black spruce); however, the exact location in Ontario was not mentioned. The only other record of *O. tubicollis* was in Mexico on pine (Zhou *et al.* 2004). In Zhou *et al.*'s (2004) study, it was associated with the bark beetle *Dendroctonus valens* LeConte and has not been associated with any other bark beetle. It was not isolated from any of the bark beetles in the present study. No studies have examined *O. tubicollis*' pathogenicity to date. The fungus is slow-growing in culture and does not appear to be very common. Therefore, it is probably safe to suggest that *O. tubicollis* is not highly pathogenic.

STATISTICAL TRENDS

After running the linear model, it was found that the location of the forest properties had a significant impact on the abundance of the ophiostomatoid fungi observed. Species composition of ophiostomatoid fungi also differed between locations as well as the species of bark beetles observed in this study. It is not surprising that abundance and species richness will vary between locations, since site conditions can vary significantly between forest stands. Site conditions, such as canopy structure, plant composition, condition of trees, competition of other bark beetles/fungi and climatic

factors, can influence the behaviour and presence of bark beetles and ophiostomatoid fungi. Therefore, to get a good representation of the bark beetles and fungi present in a given area, it is important to sample from multiple sites.

Species composition of bark beetles between locations will also affect the abundance and occurrence of ophiostomatoid fungi. Some fungi have close associations to certain species of bark beetles and if a bark beetle is not present in an area, any closely associated fungi may not be either. However, many species of ophiostomatoid fungi are not closely associated with particular species of bark beetles and may be found associated with a large number of bark beetles.

This study suggests that by increasing the size of study area, the number of bark beetle and fungal species observed will also increase. This was illustrated after examining the results of the year one study. In total, there were four bark beetle species observed on the four selected trees in the year one study, while eight bark beetle species were observed on only two species of trees in the present study, seven of which were found on jack pine. This point was also illustrated when comparing the ophiostomatoid fungi isolated at the different locations as only a few fungi were present at all three locations and several fungi were observed at only one location. Therefore, if more trees were examined in the year two study and more areas on the bait trees were sampled from, the variety of fungi and bark beetles observed would most likely increase.

When testing the Null hypothesis that each section over the length of the tree has an equal chance of being colonized by the same ophiostomatoid fungi, all of the tests of the MANOVA found no significant statistical difference between the sections. This suggests that most bark beetles choose a place at random on a log and do not necessarily take preference to the top or bottom. Some species may have chosen thicker areas of the

tree (eg *Hylurgops rugipennis pinifex*) versus thinner areas, but it did not have a significant impact on the whole. Most species of bark beetles in the present study did not appear to take preference to one area of the bait tree over another. A second explanation could be that many of the ophiostomatoid fungi are generalists and do not depend on one particular bark beetle for dispersal. This way the fungus can be introduced anywhere along the length of the log.

A second linear model was developed to incorporate tree species as a factor for ophiostomatoid fungi observed on both species. When a MANOVA was performed on these data, tree species, location and their interactions were determined to be statistically significant. It had previously been determined that location had an impact on the abundance of ophiostomatoid fungi isolated from white spruce and jack pine, so it was not surprising to see that location had a significant impact on the fungi isolated from both tree species. Tree species had a significant impact on the abundance of ophiostomatoid fungi isolated, which could be because some fungi were more host specific to one tree species over the other, even though they were capable of infecting both hosts. Similarily, the bark beetles observed on jack pine may not have had the same amount of inoculum of each fungus as on the bark beetles observed on white spruce.

The results of Roy's Largest Root test were inconsistent with the other three tests of the MANOVA, because it was the only one that found every treatment to be significant. Olson (1976) stated that it is common to reject the Roy's Largest Root test because "it results in far too many false claims of significance".

CONCLUSION

Many new bark beetle and ophiostomatoid fungal associations were observed in the present study along with new records of hosts and geographic distributions for the identified ophiostomatoid fungi. Any new association, host or distribution mentioned in the year one study will not be reiterated in this section.

Many bark beetles have often been ignored due to the lack of interest by the public even though many bark beetles have pathogenic fungi associated with them. Due to the lack of research on these associations in northwestern Ontario, many new fungus-bark beetle associations have been observed in the present study. Unlike in the year one study, *O. abietinum* was commonly isolated from adult bark beetles in the present study. Since the fungus has never been reported in Canada or the United States, several new associations have emerged. This fungus is being reported for the first time on *Ips pini*, *I. perturbatus, Dryocoetes autographus*, *Polygraphus rufipennis* and *Pityogenes plagiatus plagiatus*. As well, *O. abietinum* has never been recorded on jack pine or white spruce.

Polygraphus rufipennis had several other new associations, including *L. wingfieldii*, *L. fruticetum*, *O. minimum* and *O. minus*. Several new associations have been observed with *I. perturbatus* in the present study, including *L. wingfieldii*, *O. ips* and *O. minus*. *Ophiostoma bicolor* was isolated for the first time from *Pt. plagiatus plagiatus* and *L. fruticetum* was observed as a new associate of *D. autographus*. *Dryocoetes autographus* has been found on a large range of conifers, but there were no records of this bark beetle occurring on jack pine. Other significant mentions include *L. wingfieldii* never being reported on jack pine or in the Thunder Bay region before and

this was the first record of *O. tubicollis* in the Thunder Bay region. *Crypturgus borealis* was the only species of bark beetle never previously reported in the Thunder Bay region; however, it has more than likely always been present in the region as it has been reported in several locations across Canada (Bright 1976).

It is difficult to say which bark beetles, if any, will be of economic concern in the future. One could examine their associated fungi to determine which species are highly pathogenic or the beetles' ability to successfully colonize healthy trees. In many cases bark beetles depend on pathogenic ophiostomatoid fungi to weaken the tree's defences for successful colonization. Since few studies have examined these associations in northwestern Ontario, this thesis provided information to fill in some of the gaps in the literature by identifying new associations and geographic distributions of ophiostomatoid fungi. Due to the high number of newly reported associations in the present thesis, it is apparent how little is understood about them and the destructive nature of these mutualisms. More often than not, it takes a large outbreak of bark beetles before these associations are revealed. Many species of bark beetles are capable of successfully colonizing healthy trees when beetle populations build up in numbers. Their ability to kill their hosts increases with the assistance of pathogenic fungi. In the present study, several potentially pathogenic ophiostomatoid fungi were observed that had never been recorded in this area (*e.g. L. wingfieldii* and *O. abietinum*). Bark beetles and their associated ophiostomatoid fungi are a natural part of our forest ecosystem. Although outbreaks of bark beetles will always be an issue, we can hopefully slow down the pace and prevent outbreaks from getting out of control by being proactive. This can be achieved by maintaining a healthy forest with vigorous growth and to understand the complex nature of bark beetles and their associated blue stain fungi.

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APPENDICES

APPENDIX I
FUNGI ISOLATED IN THE YEAR ONE FIELD AND LAB STUDY

Fungi isolated from Balsam Fir at Jack Haggerty Forest

Genus	Location*	Number of Isolates	Frequency
Ophiostomatoid Fungi			
Ophiostoma rectangulosporium	b f g l w	28	11.5%
Hyalorhinocladiella/Sporothrix-like	b f g l w	21	8.6%
Ophiostoma bicolor	b f g l w	21	8.6%
Pesotum-like #1	b f g l w	9	3.7%
Hyalorhinocladiella-like #1	b f g l w	8	3.3%
Ophiostoma ips	b g	6	2.5%
Non-Ophiostomatoid Fungi			
Unknown Hyphomycete #1	b f g l w	42	17.2%
Candida	b f l w	26	10.7%
Penicillium	f g l w	23	9.4%
Cytospora	b f l w	5	2.0%
Lecythophora-like	b f g l w	5	2.0%
Cladosporium sphaerospermum	l w	3	1.2%
Geomyces-like	l w	3	1.2%
Acremonium-like	f g	2	0.8%
Exophiala jeanselmei	l w	2	0.8%
Paecilomyces	l w	2	0.8%
Filobasidium-like	g	1	0.4%
Mortieriella	f	1	0.4%
Myxotrichum deflexum	w	1	0.4%
Phialophora-like	l	1	0.4%
Phoma	b	1	0.4%
Verticillium-like	g	1	0.4%
Unidentified fungi	b f g l w	27	11.1%
Wood decayer (+clamps)	l	1	0.4%
Wood decayer (-clamps)	b f w	4	1.6%
Total		**244**	

*b=fruiting body, f=frass, g=gallery/stained wood, l=larval washing and w=adult washing

Fungi isolated from Black Spruce at Jack Haggerty Forest

Genus	Location*	Number of Isolates	Frequency
Ophiostomatoid Fungi			
Ophiostoma ips	b f g	14	6.5%
Sporothrix-like	f g w	10	4.7%
Leptographium fruticetum	b w	7	3.3%
Pesotum-like #1	b g w	5	2.3%
Ophiostoma #2	b w	2	0.9%
Ophiostoma piceaperdum	w	1	0.5%
Pesotum-like #2	l	1	0.5%
Ophiostoma #1	b	1	0.5%
Non-Ophiostomatoid Fungi			
Candida	b f g l w	42	19.6%
Calcarisporium arbuscula	b f g w	34	15.9%
Penicillium	b f g l w	18	8.4%
Exophiala-like	b g w	5	2.3%
Rhizopus	b w	3	1.4%
Cytospora	f	2	0.9%
Hyphozyma-like	f w	2	0.9%
Phoma	b w	2	0.9%
Sphaeropsis	g	2	0.9%
Acremonium	l	1	0.5%
Coniothyrium	f	1	0.5%
Graphium #1	f	1	0.5%
Lecythophora	f	1	0.5%
Trichoderma	f	1	0.5%
Unidentified fungi	b f g l w	36	16.8%
Wood decayer (+clamps)	b f g w	16	7.5%
Wood decayer (-clamps)	b l w	6	2.8%
Total		**214**	

*b=fruiting body, f=frass, g=gallery/stained wood, l=larval washing and w=adult washing

Fungi isolated from Tamarack at Jack Haggerty Forest

Genus	Location*	Number of Isolates	Frequency
Ophiostomatoid Fungi			
Ophiostoma minus	b f g l w	23	12.3%
Leptographium abietinum	b f g w	16	8.6%
Hyalorhinocladiella-like #2	f g w	5	2.7%
Leptographium fruticetum	b f w	4	2.1%
Sporothrix	b w	4	2.1%
Ophiostoma pulvinisporum	b f g	3	1.6%
Ophiostoma abietinum	b f	2	1.1%
Pesotum-like #3	b f	2	1.1%
Ophiostoma piceae	b	1	0.5%
Ophiostoma #3	f	1	0.5%
Pesotum-like #1	w	1	0.5%
Non-Ophiostomatoid Fungi			
Penicillium	b f g l w	61	32.6%
Candida	b f l w	24	12.8%
Trichoderma	f g l	5	2.7%
Aspergillus	b f g	4	2.1%
Lecythophora	f w	3	1.6%
Phoma	g w	3	1.6%
Acremonium	b f	2	1.1%
Morteriella	f w	2	1.1%
Cladosporium sphaerospermum	g	1	0.5%
Coniochaeta-like	w	1	0.5%
Myxotrichum deflexum	w	1	0.5%
Paecilomyces	l	1	0.5%
Phialophora-like	b	1	0.5%
Sporotrichum	b	1	0.5%
Unidentified fungi	f g w	3	1.6%
Wood decayer (+clamps)	b f g l	10	5.3%
Wood decayer (-clamps)	b g	2	1.1%
Total		**187**	

*b=fruiting body, f=frass, g=gallery/stained wood, l=larval washing and w=adult washing

Fungi isolated from Jack Pine at Jack Haggerty Forest

Genus	Location*	Number of Isolates	Frequency
Ophiostmatoid Fungi			
Ophiostoma ips	b	2	2.3%
Ophiostoma minus	b	1	1.1%
Non-Ophiostomatoid Fungi			
Trichoderma	b f g w	38	43.2%
Verticillium	b f g	16	18.2%
Penicillium	b f g	12	13.6%
Exophiala jeanselmei	w	2	2.3%
Aspergillus	b	1	1.1%
Lecythophora	w	1	1.1%
Phialophora-like	w	1	1.1%
Rhizomucor	f	1	1.1%
Unidentified fungi	b g	5	5.7%
Cellular Slime Mould	b f g	8	9.1%
Total		**88**	

*b=fruiting body, f=frass, g=gallery/stained wood and w=adult washing

APPENDIX II
FUNGI ISOLATED IN THE YEAR TWO FIELD AND LAB STUDY

Fungi isolated from jack pine in the year two field and lab study

Genus	Location*	Tree Number JHF	SM	Q	Total	Frequency
Ophiostomatoid Fungi						
Hyalorhinocladiella-like #1	b f g l w	52	8	50	110	10.0%
Ophiostoma ips	b f g w	40	64	1	105	9.5%
Pesotum-like #1	b f g l w	55	32	-	87	7.9%
Ophiostoma minus	b f g l w	23	14	13	50	4.5%
Ophiostoma piceae	b f g l w	3	1	30	34	3.1%
Ophiostoma abietinum	b f g w	13	15	2	30	2.7%
Pesotum-like #2	b f g w	18	12	-	30	2.7%
Sporothrix #1	b f g l w	9	16	-	25	2.3%
Leptographium wingfieldii	b f g l w	5	9	10	24	2.2%
Ophiostoma #1	b f g	-	1	8	9	0.8%
Hyalorhinocladiella-like #2	b l w	-	-	8	8	0.7%
Hyalorhinocladiella-like #3	b f g l	-	-	5	5	0.5%
Ophiostoma minimum-like	f l w	-	-	5	5	0.5%
Ophiostoma bicolor	b w	1	2	-	3	0.3%
Ophiostoma #2	f	-	1	-	1	0.1%
Ophiostoma tubicollis-like	g	-	-	1	1	0.1%
Non-Ophiostomatoid Fungi						
Candida	b f g l w	24	19	42	85	7.7%
Rhizomucor	b f g l w	1	40	16	57	5.2%
Penicillium	b f g l w	9	28	17	54	4.9%
Dipodascus	b f g l w	37	2	13	52	4.7%
Trichoderma	b f g w	19	22	7	48	4.4%
Unknown Coelomycete #1	b f g w	29	-	16	45	4.1%
Phoma	b f g l w	11	3	2	16	1.5%
Coryne-like	g l w	5	6	-	11	1.0%
Tubercularia	b g l w	3	-	8	11	1.0%
Camarosporium-like	b f g w	-	10	-	10	0.9%
Unknown Coelomycete #2	b f g	-	-	8	8	0.7%
Coniotherium-like	b g l	-	-	7	7	0.6%
Lecythophora-like	b f g l w	1	5	1	7	0.6%
Hormonema dematioides	b f g w	2	4	-	6	0.5%

Alternaria	b f g	-	4	-	4	0.4%
Morteriella	b f g	1	3	-	4	0.4%
Epicoccum	f w	-	3	-	3	0.3%
Acremonium	b f	1	1	-	2	0.2%
Cladosporium sphaerospermum	b g	1	1	-	2	0.2%
Fusarium	b g	-	1	1	2	0.2%
Geotrichum-like	b f	1	-	1	2	0.2%
Graphium #1	b w	-	2	-	2	0.2%
Hyphozyma-like	l w	2	-	-	2	0.2%
Phialemonium dimorphosporum	l w	-	-	2	2	0.2%
Gliomastix	g	-	1	-	1	0.1%
Leptosphaerulina-like	g	1	-	-	1	0.1%
Paecilomyces-like	w	-	1	-	1	0.1%
Phialophora	w	-	1	-	1	0.1%
Pithomyces	b	-	1	-	1	0.1%
Rhinocladiella-like	w	-	1	-	1	0.1%
Scopulariopsis	g	-	-	1	1	0.1%
Verticillium	w	-	1	-	1	0.1%
Unidentified fungi	b f g w	24	31	28	83	7.5%
Wood decayer (+clamps)	b f g	3	-	4	7	0.6%
Wood decayer (-clamps)	b f g	17	-	2	19	1.7%
No Growth	b f g w	2	10	2	14	1.3%
Total		**413**	**376**	**311**	**1100**	

*b=fruiting body, f=frass, g=gallery/stained wood, l=larval washing and w=adult washing

Fungi isolated from white spruce in the year two field and lab study

Genus	Location*	Tree Number JHF	SM	Q	Total	Frequency
Ophiostomatoid Fungi						
Leptographium fruticetum	b f g l w	62	3	81	146	9.1%
Leptographium #1	b f g l w	18	3	27	48	3.0%
Ophiostoma abietinum	b f g l w	10	1	19	30	1.9%
Hyalorhinocladiella-like #2	b f g l w	-	-	24	24	1.5%
Ophiostoma piceae	b f g l w	1	-	14	15	0.9%
Hyalorhinocladiella-like #1	w l	-	-	13	13	0.8%
Ophiostoma minimum-like	l w	10	-	1	11	0.7%
Ophiostoma bicolor	b f g	-	-	9	9	0.6%
Ophiostoma #1	b f g w	2	-	4	6	0.4%
Ophiostoma minus	b l w	1	3	1	5	0.3%
Ophiostoma ips	b f	1	-	1	2	0.1%
Pesotum-like #1	g	1	-	-	1	0.1%
Non-Ophiostomatoid Fungi						
Candida	b f g l w	120	61	147	328	20.4%
Calcarisporium arbuscula	b f g l w	113	157	12	282	17.6%
Unknown Hyphomycete #1	b f g l w	89	69	119	277	17.3%
Penicillium	b f g l w	22	32	27	81	5.0%
Cladosporium sphaerospermum	b g l w	2	-	22	24	1.5%
Coniotherium	b f g	-	-	14	14	0.9%
Rhizomucor	b f g l w	7	1	2	10	0.6%
Phialophora	f g l w	1	1	7	9	0.6%
Scopulariopsis	f l w	-	3	5	8	0.5%
Sphaeropsis	f g	-	5	3	8	0.5%
Trichoderma	b f g w	-	-	8	8	0.5%
Cytospora	f g	-	5	1	6	0.4%
Phoma	g l	5	-	-	5	0.3%
Acremonium	f g w	1	-	3	4	0.2%
Alternaria	b f	1	-	2	3	0.2%
Geotrichum	f l	1	-	2	3	0.2%
Fusarium	b f	1	-	1	2	0.1%
Lecythophora-like	b l	-	-	2	2	0.1%
Thysanophora penicillioides	b	-	-	2	2	0.1%
Aspergillus niger	w	-	-	1	1	0.1%
Aureobasidium pullulans	w	-	-	1	1	0.1%
Exophiala jeanselmei	w	-	-	1	1	0.1%
Geomyces-like	w	-	-	1	1	0.1%

Graphium-like	f	-	-	1	1	0.1%
Morteriella	g	-	-	1	1	0.1%
Mucor	f	1	-	-	1	0.1%
Oidiodendron	l	-	-	1	1	0.1%
Paecilomyces-like	w	1	-	-	1	0.1%
Rhinocladiella-lke	f	-	1	-	1	0.1%
Unidentified fungi	b f g l w	32	17	38	87	5.4%
Wood decayer (+clamps)	b f g l w	18	5	52	75	4.7%
Wood decayer (-clamps)	g l w	7	-	20	27	1.7%
No Growth	b f l	7	4	9	20	1.2%
Total		**535**	**371**	**699**	**1605**	

*b=fruiting body, f=frass, g=gallery/stained wood, l=larval washing and w=adult washing

APPENDIX III
ISOLATION FREQUENCIES AND CONDITION OF TREE SECTIONS IN THE YEAR TWO FIELD AND LAB STUDY

Isolation frequencies performed on the jack pine sections at Silver Mountain

w	l	g	b	f	Tree/Section	Date Isolated	Diameter (cm)	*Ips perturbatus*	*Ips pini*	*Ips grandicollis*
2	0	5	5	5	SMPj1s1	October 22, 2007	23.4	1	1	0
0	0	5	5	5	SMPj1s2	October 22, 2007	15.6	0	0	0
0	0	5	0	0	SMPj1s3	October 22, 2007	10.1	0	0	0
2	0	5	5	5	SMPj2s1	October 24, 2007	21.4	2	0	0
1	0	5	5	5	SMPj2s2	October 25, 2007	15.9	1	0	0
1	0	5	5	5	SMPj2s3	October 25, 2007	8.5	1	0	0
0	0	5	5	5	SMPj3s1	October 26, 2007	22.3	0	0	0
0	0	5	5	5	SMPj3s2	October 26, 2007	15.1	0	0	0
5	0	5	5	5	SMPj3s3	October 26, 2007	9.2	5	0	0
5	0	5	5	5	SMPj4s1	January 8, 2008	22.2	4	0	1
5	0	5	5	5	SMPj4s2	January 16, 2008	18.0	5	0	0
5	0	5	5	5	SMPj4s3	January 16, 2008	14.7	5	0	0

Condition of the jack pine sections at Silver Mountain

Section	Bark Beetle Galleries	Wood Borers	Invasive Bugs	Stain
Pj1s1	low	moderate	moderate	80%
Pj1s2	low	moderate	low	70%
Pj1s3	none	moderate	low	90%
Pj2s1	high	moderate	moderate	70%
Pj2s2	high*	low	moderate	80%
Pj2s3	moderate	low	low	40%
Pj3s1	high*	high	high	70%
Pj3s2	low	high	high	50%
Pj3s3	high	low	low	40%
Pj4s1	moderate	moderate	high	40%
Pj4s2	high	moderate	low	50%
Pj4s3	high	moderate	low	50%

*only remnants of adult bark beetles remain

Isolation frequencies performed on the jack pine sections at Jack Haggerty Forest

w	l	g	b	f	Tree/Section	Date Isolated	Diameter (cm)	*Pityogenes plagiatus plagiatus*	*Ips pini*	*Polygraphus rufipennis*
0	0	5	5	5	JHFPj1s1	February 4, 2008	21.0	0	0	0
5	3	5	0	5	JHFPj1s2	February 5, 2008	15.7	4	1	0
5	0	5	5	5	JHFPj1s3	February 6, 2008	9.9	4	1	0
0	0	5	5	5	JHFPj2s1	February 5, 2008	23.2	0	0	0
2	0	5	5	5	JHFPj2s2	February 6, 2008	15.8	0	2	0
5	0	5	5	5	JHFPj2s3	February 6, 2008	10.2	0	5	0
5	5	5	5	5	JHFPj3s1	May 5, 2007	21.6	0	2	3
1	0	5	5	5	JHFPj3s2	May 5, 2007	16.8	0	1	0
4	0	5	3	5	JHFPj3s3	May 6, 2007	10.6	0	4	0
5	4	5	5	5	JHFPj4s1	May 6, 2007	18.2	0	4	1
5	5	5	5	5	JHFPj4s2	May 6, 2007	14.1	0	5	0
1	1	5	5	5	JHFPj4s3	May 6, 2007	8.1	0	1	0

Condition of the jack pine sections at Jack Haggerty Forest

Section	Bark Beetle Galleries	Wood Borers	Invasive Bugs	Stain
JHFPj1s1*	moderate**	moderate	low	40%
JHFPj1s2*	high	moderate	low	15%
JHFPj1s3*	high	moderate	moderate	10%
JHFPj2s1*	low**	high	high	20%
JHFPj2s2*	low	high	high	70%
JHFPj2s3	high	low	high	40%
JHFPj3s1	low	low	high	10%
JHFPj3s2	low	high	low	20%
JHFPj3s3	low	moderate	low	5%
JHFPj4s1	high	low	low	10%
JHFPj4s2	high	moderate	moderate	30%
JHFPj4s3	moderate**	low	low	20%

*fruiting bodies of *Stereum* species growing on outer bark

**only remnants of bark beetles remain

Isolation frequencies performed on the jack pine sections at Quackenbush Woodlot

w	l	g	b	f	Tree/Section	Date Isolated	Diameter (cm)	*Polygraphus rufipennis*	*Dryocoetes autographus*	*Hylurgops rugipennis pinifex*
5	5	5	5	5	QPj1s1	May 20, 2008	21.4	4	1	0
0	0	0	0	0	QPj1s2	May 20, 2008	15.1	0	0	0
0	0	0	0	0	QPj1s3	May 20, 2008	8.8	0	0	0
5	0	5	5	5	QPj2s1	May 20, 2008	22.2	0	2	3
1	0	0	0	0	QPj2s2	May 20, 2008	17.8	1	0	0
1	5	5	5	5	QPj2s3	May 21, 2008	13.4	1	0	0
0	0	0	0	0	QPj3s1	May 21, 2008	21.2	0	0	0
5	5	5	5	5	QPj3s2	May 21, 2008	17.7	0	5	0
0	0	0	0	0	QPj3s3	May 21, 2008	14.2	0	0	0
2	2	5	3	5	QPj4s1	May 21, 2008	19.1	0	2	0
1	0	1	0	2	QPj4s2	May 21, 2008	17.2	0	1	0
5	2	5	5	5	QPj4s3	May 21, 2008	12.9	2	3	0

Condition of the jack pine sections at Quackenbush Woodlot

Section	Bark Beetle Galleries	Wood Borers	Invasive Bugs	Stain
QPj1s1*	moderate	low	low	10%
QPj1s2*	none	high	none	80%
QPj1s3*	none	high	none	60%
QPj2s1**	moderate	low	low	30%
QPj2s2	low	high	low	30%
QPj2s3*	moderate	low	low	30%
QPj3s1	none	high	moderate	50%
QPj3s2	moderate	high	none	90%
QPj3s3	none	high	moderate	90%
QPj4s1*	low	moderate	moderate	30%
QPj4s2*	low	high	moderate	20%
QPj4s3*	moderate	moderate	low	10%

*mould problem (from thawing outside)

**bark difficult to pry off

Isolation frequencies performed on the white spruce sections at Silver Mountain

w	l	g	b	f	Tree/Section	Date Isolated	Diameter (cm)	*Polygraphus rufipennis*
5	5	5	5	5	SMSw1s1	June 3, 2008	19.5	5
2	0	2	0	3	SMSw1s2	June 3, 2008	14.1	2
5	5	5	0	5	SMSw1s3	June 3, 2008	8.5	5
5	5	5	0	5	SMSw2s1	June 4, 2008	21.6	5
5	5	5	0	5	SMSw2s2	June 4, 2008	16.8	5
5	5	5	0	5	SMSw2s3	June 4, 2008	11.2	5
5	5	5	0	5	SMSw3s1	June 4, 2008	20.8	5
0	5	5	0	5	SMSw3s2	June 5, 2008	13.7	0
1	5	5	0	5	SMSw3s3	June 5, 2008	8.2	1
1	5	5	0	5	SMSw4s1	June 5, 2008	20.1	1
5	5	5	0	5	SMSw4s2	June 5, 2008	14.7	5
5	5	5	0	5	SMSw4s3	June 5, 2008	8.2	5

Condition of the white spruce sections at Silver Mountain

Section	Bark Beetle Galleries	Wood Borers	Invasive Bugs	Stain
SMSw1s1	moderate	moderate	none	5%
SMSw1s2	low	high	none	5%
SMSw1s3	moderate	low	none	10%
SMSw2s1	high	low	none	20%
SMSw2s2	high	low	none	60%
SMSw2s3	high	none	none	10%
SMSw3s1	high	moderate	none	25%
SMSw3s2	high	low	low	5%
SMSw3s3	high	low	none	0%
SMSw4s1	moderate	moderate	none	40%
SMSw4s2	high	moderate	low	40%
SMSw4s3	high	low	none	20%

Isolation frequencies performed on the white spruce sections at Jack Haggerty Forest

w	l	g	b	f	Tree/Section	Date Isolated	Diameter (cm)	*Polygraphus rufipennis*	*Dryocoetes autographus*
5	4	5	0	5	JHFSw1s1	June 19, 2008	21.4	5	0
0	5	5	0	5	JHFSw1s2	June 19, 2008	16.6	0	0
0	1	5	0	5	JHFSw1s3	June 19, 2008	11.3	0	0
5	5	5	5	5	JHFSw2s1	June 19, 2008	19.2	5	0
5	5	5	5	5	JHFSw2s2	June 23, 2008	16.9	3	2
5	5	5	0	5	JHFSw2s3	June 24, 2008	13.6	3	2
5	5	5	5	5	JHFSw3s1	June 24, 2008	17.6	5	0
5	5	5	1	5	JHFSw3s2	June 25, 2008	14.2	3	2
5	5	5	2	5	JHFSw3s3	June 25, 2008	10.3	3	2
5	0	5	0	5	JHFSw4s1	June 25, 2008	14.9	5	0
5	1	5	0	5	JHFSw4s2	June 25, 2008	11.1	5	0
5	4	5	5	5	JHFSw4s3	June 26, 2008	7.7	5	0

Condition of the white spruce sections at Silver Mountain

Section	Bark Beetle Galleries	Wood Borers	Invasive Bugs	Stain
JHFSw1s1*	moderate	high	none	40%**
JHFSw1s2	low	high	none	30%
JHFSw1s3*	low	high	low	60%
JHFSw2s1	high	moderate	low	10%
JHFSw2s2	high	moderate	none	5%
JHFSw2s3*	high	low	none	20%
JHFSw3s1	high	moderate	none	20%
JHFSw3s2*	high	moderate	moderate	15%
JHFSw3s3	high	low	low	10%
JHFSw4s1	high	low	moderate	5%
JHFSw4s2	high	high	high	5%
JHFSw4s3	high	moderate	moderate	5%

*mould problem

**stain darker where wood borers are present

Isolation frequencies performed on the white spruce sections at Quackenbush Woodlot

w	l	g	b	f	Tree/ Section	Date Isolated	Diameter (cm)	*Polygraphus rufipennis*	*Dryocoetes autographus*	*Crypturgus borealis*
5	5	5	5	5	QSw1s1	August 21, 2008	21.2	3	2	0
5	5	5	5	5	QSw1s2	August 21, 2008	18.6	5	0	0
5	5	5	5	5	QSw1s3	August 21, 2008	14.1	2	3	0
5	5	5	2	5	QSw2s1	August 21, 2008	20.0	5	0	0
5	5	5	5	5	QSw2s2	August 21, 2008	16.4	3	2	0
5	5	5	5	5	QSw2s3	August 22, 2008	13.4	2	3	0
5	5	5	0	5	QSw3s1	August 22, 2008	20.7	3	1	1
5	5	5	5	5	QSw3s2	August 22, 2008	15.4	2	3	0
5	5	5	5	5	QSw3s3	August 22, 2008	10.5	3	2	0
5	5	5	3	5	QSw4s1	August 22, 2008	19.1	4	1	0
5	5	5	5	5	QSw4s2	August 22, 2008	14.6	3	2	0
5	5	5	3	5	QSw4s3	August 22, 2008	10.8	1	4	0

Condition of the white spruce sections at Quackenbush Woodlot

Section	Bark Beetle Galleries	Wood Borers	Invasive Bugs	Stain
QSw1s1	high	moderate	moderate	30%
QSw1s2	high	low	moderate	30%
QSw1s3	moderate	high	low	20%
QSw2s1	high	low	low	10%
QSw2s2	high	low	low	10%
QSw2s3	high	low	none	10%
QSw3s1	moderate	high	moderate	30%
QSw3s2	high	moderate	low	30%
QSw3s3	high	moderate	low	5%
QSw4s1	high	low	low	5%
QSw4s2	high	low	low	5%
QSw4s3	high	low	none	5%