Factors affecting the distribution of beech bark disease in two beech-maple forests in south-western Quebec

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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PREFACE

This thesis is composed of three chapters.

Chapter 1

This chapter is a general introduction and literature review.

Chapter 2

This chapter is a manuscript in preparation for submission to the *Canadian Journal of Forest Research*

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Chapter 3

This chapter is a general conclusion.

CONTRIBUTION OF AUTHORS

Overall, this research was conducted by T. Motchula. C. Buddle and J. Fyles assisted with project design and data analyses. First drafts of all material were written by T. Motchula, and C. Buddle assisted with editing and style. Tables and figures were prepared by T. Motchula.

ABSTRACT

Beech bark disease (BBD) was studied in two *Acer-Fagus* forests in Montreal, Quebec. Symptoms of BBD were used to compare the level of disease between the forests. Both beech scale (*Cryptococcus fagisuga* Lind.) and fungus (*Nectria galligena* Bres.) were found in the Morgan Arboretum, while only beech scale was found in the Molson Nature Reserve, signifying that these two forests are at the killing zone and advancing front, respectively. A multivariate approach was used in order to explore the factors affecting beech scale distribution. Bark nitrogen concentration had a positive association with beech scale population, while DBH and bark phenol and calcium levels had none. A model selection process using AIC showed that those models containing nitrogen, and DBH or phenols were the most likely to explain beech scale distribution. Beech scale populations were highest on the northern and eastern sides of the trees, and lowest on the southern side.

RÉSUMÉ

La maladie corticale du hêtre (MCH) a été étudiée dans deux forêts *Acer-Fagus* à Montréal, Québec. Les symptômes ont été utilisés pour comparer le niveau de la maladie entre les deux forêts. Les deux, la cochenille du hêtre (*Cryptococcus fagisuga* Lind.) et le champignon (*Nectria galligena* Bres.) ont été trouvé à l'arboretum Morgan, alors que seulement la cochenille du hêtre a été trouvée à la réserve de nature Molson. Cela signifie que les deux forêts sont au niveau du front meutrier et le front d'avancement, respectivement. Une approche multivariée a aussi été utilisée pour explorer les facteurs qui ont un effect sur la distribution de la cochenille. La concentration était associée avec la population de la cochenille, alors que le diamètre de l'arbre et les concentrations des phénols et du calcium ne l'étaient pas. Une approche de modèle (AIC) a montré que ces modèles qui contiennent de l'azote, et le diamètre de l'arbre ou les phénols ont été ceux les plus probables d'expliquer la distribution de la cochenille. Les populations de la cochenille les plus élevées du côté nord et est, et les plus basses au sud.

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

Introduction

Although beech bark disease (BBD) in North America has remained almost unknown to all but forest researchers for the past hundred years, it represents an important threat to the American beech (*Fagus grandifolia* Ehrh.). Similar to Dutch elm disease and oak wilt, BBD is characterized by high tree mortality when it first enters an area and reduced mortality as tree resistance becomes apparent (Houston 1975, Runkle 1990, Houston 1994a, Brisson and Le Sauteur 1997). For instance, when BBD first enters an area, beech mortality nears 90% (Griffin et al. 2003). However, although this disease was introduced into the Maritimes over a hundred years ago, less than 1% of trees are resistant (Houston and Houston 2000). Thus, researchers must improve on current measures to control the disease. Much of current research focuses on preventing the beech scale from reaching high populations, as this is what triggers the disease. However, in order to begin controlling BBD, we must first be aware of how this disease came to North America, as well as the mechanics of the disease complex. Only then can we fully appreciate the impact this disease will have on the beech forests of North America.

History in North America

BBD was first introduced into North America through Halifax in 1890, presumably from ornamental European beech *Fagus sylvatica* L. brought from Europe, where BBD was a major problem (Brown 1934). It is not known what caused BBD to spread throughout Europe, and a way had not been found to control the spread of the disease (Houston 1997). Until Mahoney et al. (1999) completed genetic testing on *Nectria* *coccinea* var. *faginata* Lohman and Watson, concluding that it likely originated from Europe, it was not a certainty (Houston 1980). Using phylogenetic analysis, Gwiazdowski et al. (2006) determined that beech scale, *Cryptococcus fagisuga* Lind., likely originated on *F. sylvatica orientalis* (Lipsky) Greuter and Burdet, in SW Asia and SE Europe. From Halifax, BBD subsequently spread westward and southward (Brown 1934). Today it encompasses the Maritime Provinces, southern Quebec and Ontario, and the northeastern United States (Houston and O'Brien 1983, Houston 1994a). The distribution of BBD is roughly one-third of the distribution of beech, although the disease is still spreading (Houston 1994a). Most maps of beech bark disease distribution are out of date, and researchers are presently trying to update these maps. In Quebec and Ontario, where the northern limit of beech occurs, researchers are also interested in whether BBD will survive in these northern limits, or whether it will be limited by environmental and climatic factors (Fernandez and Boyer 1988, Hopkin et al. 2000).

Disease complex

Two main organisms cause BBD: the scale insect *C. fagisuga* and the fungus *N. coccinea* var. *faginata*. These were both probably introduced from Europe, and as foreign species may impact North American ecosystems more than native species (Houston and O'Brien 1983, Le Guerrier et al. 2003). BBD has already been shown to be much more devastating to *F. grandifolia* than *F. sylvatica*, which is likely due to its recent introduction to North America and its longer period of coevolution with *F. sylvatica* (Wainhouse et al. 1988). However, there is a closely related North American species of fungus, *Nectria galligena* Bres., that may also be found in connection with BBD. The beech scale and the fungus form a commensalistic relationship, which causes this disease.

Without initial infection by the beech scale, *Nectria* spp. fungus cannot attack the tree (Erlich 1934, Houston 1997). The beech scale is a fluid feeder and inserts its specialized 2-mm long stylet through the beech bark and cork cambium, where it sucks up fluids (Gullan and Kosztarab 1997). This feeding causes little damage to the tree, and low populations of beech scale do not immediately cause BBD (Houston 1994a). Significant damage occurs after three to six years of population buildup and this makes beech susceptible to the invasion of the *Nectria* spp. fungus (Shigo 1972). As beech scale feed, they change the chemical composition of the bark around the insertion site, providing openings for the fungus to enter (Houston 1994a). They also prevent the host tree from fully using its natural defenses (Lonsdale 1980). Indeed, Perrin (1984) found that the severity of the *Nectria* spp. infection depended more on previous beech scale damage than to the tree's level of susceptibility to the fungus. The fungus takes advantage of the reduction in the trees' defenses and the openings provided by the scale insects to enter the tree and consume it from the inside. Once the fungus has invaded the tree, it is almost impossible to remove (Houston 1997). Furthermore, the beech scale can no longer feed there because the bark is dead and dry, so beech scale populations decrease. The arrival of this fungus establishes the onset of BBD (Shigo 1964). There are two main types of damage from the *Nectria* spp. fungus: invasion by isolated, separate spots on the bark, or invasion by long spirals that surround the tree, which are much more dangerous to the tree (Houston and O'Brien 1983).

Scale insects

The beech scale, *C. fagisuga*, is similar to other scale insects in that it is relatively small (1 mm) and has two life stages that differ in mobility. It is parthenogenic, so there are no males (Brown 1934, Ehrlich 1934, Houston and O'Brien 1983), evolving possibly due to the immobility of adult beech scale, making mate-selection nearly impossible (Gullan and Kosztarab 1997). Eggs are laid from June to September and hatch in 25 days. These first instar nymphs are mobile (often referred to as crawlers, (Ehrlich 1934 and Brower 1949)), unlike the second instar nymphs, which are sessile and overwinter (Ehrlich 1934, Gullan and Kosztarab 1997). Adults emerge in spring and summer (Ehrlich 1934). This is also one of the largest problems that researchers must overcome – adult beech scale are extremely sensitive to wind currents, and can be displaced 10 m with a wind current of 0.1 m/s (0.36 km/h) (Wainhouse and Deeble 1980, Houston 1994a). Once they land on a beech tree (2-10 m above the ground), beech scale remain there, feed and reproduce (Ehrlich 1934). They tend to inhabit areas on the bark previously colonized by beech scale (Wainhouse et al. 1988). They are usually found 2-3 m from the ground because deep snowdrifts shelter them during winter, depending on its severity (Brisson and Le Sauteur 1997). Beech scale are found in colonies, which become very dense as the population grows (Gullan and Kosztarab 1997). They have a higher survival rate if they are reintroduced to their original host tree than to another (Wainhouse et al. 1988). Adults secrete a waxy substance that serves as protection, which surrounds the scale insects, as well as their eggs (Gullan and Kosztarab 1997). Small populations of beech scale resemble tiny white dots on the bark, while large populations appear as large white patches. The mobile stage of beech scale exhibits positive phototaxis, so they move upwards on the tree (Ehrlich 1934). Thus, the colony gradually moves upwards, leaving the old waxy coating and dead scale insects behind (Houston et al. 1979b). The beech scale need high humidity to develop, so they are often found in humid microhabitats such as branch stubs, crevices in the bark and among certain lichens (i.e. *Lecanora* *conizaeoides* Nylander ex Crombie) and algae (i.e. *Desmococcus vulgaris* F. Brand) (Ehrlich 1934, Houston et al. 1979b, Wainhouse et al. 1988, Houston 1994a). *L. conizaeoides* is commonly found in England where heavy agricultural fertilizer is applied and in areas with high volumes of SO_2. Beech scale often colonize beech trees on steep slopes, as they are directly impacted by winds. Beech trees with low populations of beech scale may have small pits on their bark where they have fed. The beech scale may be transported by humans as well, as outbreaks have also been described in areas with much pedestrian traffic (Houston 1994a). Other animals such as squirrels and birds are also thought to spread the beech scale between trees and sites (Brown 1934, Ehrlich 1934, Houston 1994a).

Another scale insect, *Xylococcus betulae* (Perg.), is a secondary problem to beech trees. It damages beech trees in the same manner as *C. fagisuga*, but feeds on other trees as well. It further propagates BBD as the waxy secretions it produces act as shelter for *C. fagisuga* (Houston 1977). It is most often found on beech clones that have sprung from dying trees in the last stage of BBD (Houston 1975, Houston 1977, Houston et al. 1979a, Houston and O'Brien 1983). Fortunately, this scale insect may not be found in all areas ravaged by the disease and may be limited in the scope of damage it can cause to beech trees (Houston et al. 1979a).

Fungi

The two *Nectria* species are very closely related and difficult to differentiate, so they share many of the same characteristics (Shigo 1964). Like other fungus species, they have two stages: a sexual and asexual stage. The sexual stage is made up of tiny red perithecia, forming the outline of a lemon on the beech bark and which mature in the autumn (Houston and O'Brien 1983). The asexual stage appears as white clumps on the bark. The asexual stages of *N. coccinea* var. *faginata* and *N. galligena* are, respectively, *Cylindrocarpon faginatum* and *C. mali*. Both asexual stages mature in late summer and resemble colonies of beech scale (Houston and O'Brien 1983). Only *N. coccinea* var. *faginata* produce pectinases that enable them to infect the bark more easily (Houston 1994a). However, *N. coccinea* var. *faginata* is more of a threat to trees, perhaps because it is introduced, whereas *N. galligena* is native to North America and is less lethal, although it does cause a lot of damage to beech trees (Houston 1980, Houston 1994a,b, Plante et al. 2002). Trees are less likely to die from *N. galligena* than *N. coccinea* var. *faginata*. Also, *N. coccinea* var. *faginata* outcompetes *N. galligena*, likely because this introduced fungus is able to colonize areas quickly in the absence of natural predators (Houston 1994a). This is due to its aggressive colonization and infection patterns (Houston 1994b). Furthermore, Houston (1994b) found that *N. coccinea* var. *faginata* caused beech cankers three times larger than those from *N. galligena*, and also produced more perithecia. However, in Virginia, *N. galligena* is the most pathogenic fungus (Houston and O'Brien 1983). *N. galligena* also inhabits other hardwood trees as well (Houston 1994a,b). Houston and Mahoney (1987) recently found another species of fungus, *N. ochroleuca* (now *Bionectria ochroleuca* (Schwein) Schroers and Samuels), on beech dying of BBD (Houston 2004). However, this species of fungus has not yet been reported elsewhere in relation to this disease.

Secondary damage

After beech trees have been damaged by the BBD complex, they are extremely vulnerable to other pathogenic organisms that feed on dead or dying trees (Houston

1980). These include various species of fungi and beetles. The wood-boring beetles *Hylecoetus dermestoides* L. and *Xyloterus domesticus* L. are quite common in many areas. They feed off beech trees from the inside, and bore holes in the vascular cambium, accelerating tree death. Problem fungi include *Hypoxylon choaerensi* and *rubiginosum* (Pers.: Fr), and various species of *Stereum*, *Hymenochaete*, *Polyporus* and *Formes* (Shigo 1964).

Measuring the disease

There are a number of methods to categorize the presence of the beech scale, *Nectria* spp. fungus and BBD damage to beech trees. Brower (1949) counted the number of beech scale crawlers per square inch, while Fernandez and Boyer (1988) took ten 25 cm^2 wire grid samples at breast height and then counted the number of beech scale colonies this covered. Wiggins et al. (2004) used a similar method, but continued their observations over three seasons to document the change in beech scale populations over time. Forrester et al. (2003) estimated the percent cover of beech scale colonies and cankers on branch stubs, while Houston (1994b) isolated *Nectria* spp. perithecia from a wide region of trees in both Canada and the U.S. Griffin et al. (2003) focused on integrating canopy damage, bark damage, and beech scale and *Nectria* spp. presence, while Houston and O'Brien (1983) divided tree damage into four categories: (0) no damage; (1) small discrete legions, cambial tissue only affected locally; (2) obvious dead bark and bloody, sunken lesions, and (3) severe damage to vascular and cambial tissue with long vertical fissures and callus tissue. Griffin et al. 2003 reported that BBD was apparent in areas with category (2) damage. Gavin and Peart (1993) improved upon this model by dividing damage to the bark and fungal invasion into seven detailed categories.

Because there is no uniform method to examine BBD, it is difficult to compare different studies, and more research must be done to integrate the numerous factors affecting BBD.

The three stages of the disease

The advancing front

Shigo (1964, 1972) divided BBD into three stages: the advancing front, the killing front, and the aftermath zone. The advancing front is characterized by low populations of beech scale beginning to colonize beech trees. There is little to no damage to the trees themselves, save the little bark pits they produce in reaction to light beech scale infestation (Houston et al. 1979b). There is also no tree mortality. Enzymes injected into the beech trees by the beech scale change the chemical composition of the feeding site so *Nectria* spp. is better able to infect the tree (Houston 1994a, Brisson and Le Sauteur 1997). Feeding by the beech scale helps to limit the tree's ability to repel fungal invasion (Lonsdale 1980).

The killing front

The killing front only appears in an area three to six years after the advancing front because it takes this long for beech scale populations to reach levels high enough to allow the *Nectria* spp. to invade. However, severe winter temperatures damage beech scale populations and so may slow this progression (Ehrlich 1934, Shigo 1964, Houston and O'Brien 1983, Houston and Valentine 1988). Lonsdale (1980) found that a higher scale concentration caused larger *Nectria* spp. lesions on infected trees. The killing front is often apparent, with white beech scale wax and the white and red stages of the fungi covering tree trunks. On trees, dead black crevices oozing with brown slime called tarry spots or slime-flux indicate the beginning of *Nectria* spp. infection. These areas are often surrounded by perithecia as well (Ehrlich 1934, Houston and O'Brien 1983). They are also found in the later stages of the disease, surrounding insect holes and dead bark, and are breeding grounds for bacteria (Shigo 1964). With this stage begins the real damage to beech trees, as the disease complex provokes the tree to form cankers around the areas of damage. However, this only maintains populations of beech scale, as the cankers provide shelter for them (Houston and Valentine 1988, Houston 1994a). Tree mortality is highest in this stage, as *Nectria* spp. populations build up. Houston et al. (1979a) reported that 85% of beech trees die during the killing front, most of which are large trees (Houston 1975, Houston and O'Brien 1983). In severely infected areas, it may only take two years for trees to die (Le Guerrier et al. 2003). Dying trees are quite conspicuous, as they grow sparse, yellowed leaves in spring (Shigo 1972, Houston and O'Brien 1983).

The aftermath zone

In the aftermath zone, the forest composition has been significantly affected by BBD. Since most of the largest trees are dead, gaps in the canopy are prevalent and remaining trees may only grow at a rate of about 60% of that of healthy ones (Gavin and Peart 1993). When beech trees die, they produce clonal root sprouts, which spring up in large thickets, preventing other trees from taking advantage of the open space (Houston 1975, Runkle 1990). Thus, the forest is composed of mainly young trees making up a dense subcanopy (Houston 1975, Le Guerrier et al. 2003). Jones and Raynal (1988) found that stimulating the production of callus tissue on beech roots, especially during spring, produced large numbers of clonal root sprouts. However, since these clonal root sprouts are genetically identical to those trees that have died, they are also susceptible to

BBD and are usually cankered from *C. fagisuga*, *X. betulae* and *Nectria* spp. damage (Houston 1975, Houston and O'Brien 1983, Houston and Houston 2000). Unfortunately, despite ongoing research on the propagation of putatively resistant *F. grandifolia*, success has been very limited as no saplings have survived nonsterile conditions (Ramirez et al. 2007).

Tree mortality is low during this stage, as the most susceptible trees have already died. Those that have been severely weakened by the disease complex (as illustrated by the large, ugly cankers adorning the bark) are likely to live on for years in their weakened state (Gavin and Peart 1993). Both the external and the internal defects may be extensive, and beech snap is common as trees are further weakened by other organisms (Houston et al. 1979a). In aftermath forests in New York state, Krazny and DiGregorino (2001) found that although beech trees composed 26% of the canopy, they were responsible for 52% of gaps caused by falling trees. This scene is typical of aftermath forest distribution. Rhoads et al. (2002) studied forest composition following a severe ice storm in New Hampshire (US); here, a high proportion of large beech trees had snapped branches and trunks due to the ice load, compared to other tree species. They attributed this to severe BBD in the area, which had weakened the trees and allowed them to be further invaded by other organisms (Rhoads et al. 2002). They also found that beech trees were less likely to have recovered from the ice storm two years later, showing that BBD has long-lasting effects on an area (Rhoads et al. 2002).

Second-growth forests are more resistant to BBD than old-growth forests, presumably because the forest consists of more trees that have survived the initial wave (Latty et al. 2003). A model simulated by Le Guerrier et al. (2003) predicts that the beech tree population will decline 50 years after infection, but will then be stable for the next

300 years. At that point, the most abundant tree species will be the eastern hemlock (*Tsuga canadensis* L. (Carr)), which benefits from the shade provided by the beech subcanopy (Le Guerrier et al. 2003).

Other factors affecting beech bark disease

There are many other factors that affect the damage caused by BBD in an area. For instance, the age of the tree is extremely important. Forrester et al. (2003) studied a beech-maple forest where 99% of uninfected trees had a DBH smaller than 15cm. Although beech scale can infect any beech tree on which it lands, it has generally been established that larger trees are more likely to be infected (i.e. Mize and Lea 1979, Wainhouse and Deeble 1980, Fernandez and Boyer 1988, Houston and Valentine 1988, Morris et al. 2002, Griffin et al. 2003, Latty et al. 2003, Wiggins et al. 2004). It has been suggested that this is due to larger trees having more bark crevices where beech scale may shelter (Houston and Valentine 1988, Wainhouse et al. 1988, Houston 1994a). It may also be that larger trees provide a larger surface area on which to land (Fernandez and Boyer 1988). Latty et al. (2003) also suggest that larger trees have a larger canopy, and thus a larger surface area, on which more wind-blown beech scale may fall.

Furthermore, the older the stand becomes, the fewer the resources available to the trees, which may increase their stress (Gavin and Peart 1993). Older beech trees that are already stressed from other infections succumb to BBD more rapidly (Houston 1994a). Canopy trees are more susceptible to BBD than those in the understory because they grow faster. As previously mentioned, cracks in the bark can shelter the beech scale, propagating the disease, as well as allow them an easier point of entry, as thinner bark is easier to infect (Wainhouse et al. 1988). Thick bark can protect the tree from the beech scale because the insect's stylet may not be able to penetrate the thickest bark (Gavin and Peart 1993). A thicker layer of stone cells in the bark parenchyma also allows some beech trees a physical barrier to beech scale invasion (Wainhouse et al. 1988).

Genetic resistance to beech bark disease

The levels of certain chemicals in the bark also protect the tree from the beech scale. There has been much ongoing research as to the apparent resistance of some beech trees to beech scale invasion. It has been hypothesized that red beech are the most susceptible to the disease, white beech are intermediate and northern grey beech are best able to withstand beech scale invasion (Shigo 1964). This may be due to the differences in bark: for instance, red beech has the most crevices where beech scale can be sheltered (Shigo 1964). However, as yet there are no definite methods of differentiating between these three types of *F. grandifolia*, save their general geographic location. Gray beech are found from Nova Scotia to southern Ontario (Canada) and in the Appalachians (US), white beech are found on the southern Coastal Plain to the Piedmont (US) and red beech are found between these two distributions (Kitamura and Kawano 2001).

High levels of phenols have been associated with disease resistance in several tree species, and they are thought to be the tree's first level of defense toward infection (Ostrofsky et al. 1984, Wargo 1988). They are highest in areas nearest the vascular cambium, which is key to the health of the tree. Also, trees that are resistant to BBD may produce phenols faster than susceptible trees (Ostrofsky et al. 1984). Although trees with low phenol levels would be expected to be quite infected with BBD, one study found that if they had not previously been infected with beech scale, they were free of *Nectria* spp. infection (Houston 1994a). In another study, high populations of beech scale were found to be correlated with high concentrations of amino acids in beech bark, but not with bark nitrogen concentration (Wargo 1988). However, this study used a small sample size (n=15) and trees from the same stand, which likely affected the results.

Low levels of nitrogen in the bark produce smaller beech scale populations because nitrogen is a limiting factor for many scale insects (Wargo 1988, Houston 1994a, Krabel and Petercord 2000). Unfortunately, only 1% of beech trees show this apparent resistance (Houston 1994a, Houston 1997, Houston and Houston 2000). High levels of bark nitrogen may also be connected with the age and size of the tree, as canopy trees have more leaves, and so harbour more nitrogen than smaller trees (Latty et al. 2003). It has been suggested that areas with heavy nitrogen fertilization counteract any resistance that the trees may have because the nitrogen is taken up into the bark, ameliorating the environment for beech scale (Latty et al. 2003). This may worsen the situation until nitrogen levels in the soil decrease, and there is less available for the trees to absorb, which may in turn prevent most beech scale from feeding on the trees (Castello et al. 1995).

Resistant trees are often found in clumps because they originate as clonal root sprouts that are not affected by BBD, and may cause certain forests to harbour little disease due to the large population of resistant trees (Houston 1994a). BBD follows the principles of natural selection, as those trees that can withstand the disease are those that will survive in the forest. Those beech trees that show resistance also produce seeds with those not resistant, eventually generating beech trees with some sort of resistance (Houston and Houston 2000). Although the reproduction of these trees has long been postulated to curb BBD, new techniques for growing and planting those resistant must be tested, as current methods are not efficient (Houston and Houston 2000, Ramirez et al.

2007). Dr. J. Loo and her team at Natural Resources Canada are currently working on this potential to protect the American beech (Loo 2003). Also, the healthier the tree, the more prepared it is to withstand BBD (Castello et al. 1995). Trees that are able to quickly respond to beech scale feeding by producing cankers and scars are those that survive in the aftermath zone (Shigo 1964).

Abiotic factors affecting beech bark disease

There are a number of abiotic factors that can affect the severity of BBD in an area. Beech at high elevations may be weaker than those at low elevations because of stresses due to the environment (Griffin et al. 2003). If a tree already suffers from other pathogens, it is less able to devote energy to fighting the infection of the beech scale or the *Nectria* spp. fungus. Trees in areas with high wind speeds are more likely to be infected with BBD. High humidity microhabitats are the preferred habitats for beech scale, such as the surface of lichens such as *L. conizaeoides* and algae such as *D. vulgaris* (Houston 1977, Wainhouse et al. 1988). However, other lichens such as *Ascodichaena rugosa* Butin, found on moist slopes, *Graphis scripta* L. Ach. and *L. subfusca* L. Ach. prevent beech scale from establishing, although it is unknown whether this lichen is unpalatable to the beech scale or whether it is not the right microhabitat (Wainhouse et al. 1988). Also, low temperatures such as – 37°C kill the second instar nymphs during overwintering. Finally, heavy autumn rains may wash the beech scale off the trees, where the adults perish because they are immobile (Houston and O’Brien 1983, Houston and Valentine 1988).

Beech scale control and management

Most who have studied BBD and work on its control agree that the beech scale is the problem to target. The main limitation in this is finding its natural enemies, as the original distribution of beech scale is not known. The most recent research suggests that beech scale may originate from SW Asia and/or SE Europe (Gwiazdowski et al. 2006). If beech scale populations are reduced, the following year trees will show less cankering on the bark (Houston and Valentine 1988). Without the entrances provided by their feeding, the fungi cannot invade the tree (Shigo 1964). Although the native scale *X. betulae* damages the trees in the same manner as the beech scale, it is less common and it affects other trees besides beech. Even though it is preyed upon by native predators since it is not introduced, the facility with which it disperses does not offer any hope of eradicating it.

There are, however, a number of measures that offer some hope of limiting or slowing the spread of beech scale. When attempts were first made to control the beech scale, strong toxins such as kerosene were sprayed onto the trees, but this proved ineffective (Brower 1949). Spraying 5% lime sulphur did kill all beech scale, as well as moss and lichens. Foresters would also try to cut down and burn all affected trees, but the beech scale continued to spread (Brower 1949). Current measures work best at controlling the disease at the tree level, as beech scale colonies can be removed manually or with a high-powered water jet. An insecticide-fungicide mixture is sufficient to remove BBD from small groups of trees, although it is not a long-term solution (Brisson and Le Sauteur 1997). On beech trees in Ontario infected only with beech scale, a mixture of dormant superior oil and ferbam sprayed before bud flush, and subsequently with a sistox and ferbam mixture in mid-June was also sufficient to contain the population of beech scale, although, again, this is not a sustainable solution (Biessar et al. 1985).

There are a number of biological control measures that have been proposed to kill the beech scale on a local scale. Although most do not target the beech scale specifically (i.e. they are opportunistic generalists), they have been proven useful in lowering high populations (Houston 1997). Nevertheless, the key to limiting BBD is in keeping the populations of beech scale very low, as this does not give the fungi an opportunity to infect the beech tree. Like all scale insects, beech scale contain endosymbiont bacteria in their alimentary canal. Removing these bacteria would certainly kill the insect, as similar methods have been used to curb termite populations (Moran et al. 1982).

As wind dispersal is so essential to the spread of BBD, limiting its effect would be another way to control beech scale levels. Another species of scale insect, *Dactylopius austrinus* De Lotto prepare for dispersal by climbing to the top of thorny stalks in order to catch wind currents. If they are artificially dispersed, they no longer climb these stalks (Moran et al. 1982). If the life cycle of beech scale is similarly disturbed, they may remain where they are, preventing further spread of the disease. Furthermore, if a substance were developed that would dissolve the waxy protective coating of the beech scale, the insects would quickly die. This could also be accomplished if one could interrupt the production of the wax, which consists of lipids and resins, or its distribution to the wax glands (Gullan and Kosztarab 1997).

Mites (Arachnida: Acari) have been studied as a potentially important predator of beech scale, notably *Allothrombium mitchelli* Davis (Trombidiidae), which takes shelter in moss on the bark where beech scale is found (Wiggins et al. 2001). It is most abundant in July and August, depending on the location. This is also when *Nectria* spp. populations reach their highest levels, which may be correlated with the rise in beech scale population in late summer (T. Motchula, pers. obs.). This mite species has great potential in controlling populations of beech scale because it is native to northeastern North America (Wiggins et al. 2001). Thus, if high populations of these mites were released into an area, they would be able to survive the change in seasons, and would presumably reproduce as well. They overwinter in the adult stage, and so may feed on beech scale as they emerge. They are usually found on south slopes because these are warm, and they are only active at warm temperatures. They shelter among mosses that contain beech scale or aphids. However, this mite prefers altitudes of 1500 m or more, whereas BBD is not a problem at these high elevations as beech trees are more common at lower elevations. Like other mites, *A. mitchelli* only eats beech scale at low or moderate population levels, which does nothing to keep beech scale populations low (Wiggins et al. 2001). It only has one generation per year, and so is not able to reproduce in response to high beech scale levels. Furthermore, it is only found in areas with high canopy coverage, while severe BBD kills most of the canopy trees. Finally, this mite has limited dispersal as only the deuteronymph stage is active, and may carry the *Nectria* spp. fungus with it from tree to tree in search of beech scale (Wiggins et al. 2001).

Mites in the genus *Anystis* (Anystidae) behave much like *A. mitchelli* as they have been noted to eat all stages of beech scale, including the eggs and nymphs (Wiggins et al. 2001). This is of great importance because if researchers discover which mite species limit beech scale populations, it can be used to keep scale populations below the damage threshold needed for *Nectria* spp. to enter the equation. Mites of the genera *Tydeus*, *Abrolophus* and *Leptus* are also being looked into as potential solutions (Wiggins et al. 2001).

Other opportunistic generalist predators include the lightning beetle *Lucidota corrusca* L. and the ladybird beetles *Chilocorus stigma* Say and *Clavia* *quatuordecimguttata* var. *similis* Randall, which function similarly to mites (Brown 1934, Houston and O'Brien 1983). Both the larvae and the adults feed on the beech scale, although not on all stages (Shigo 1964, Houston 1997). They are excellent flyers, and travel from tree to tree in search of the beech scale. However, up to 80% also bring the *Nectria* fungus with them (Shigo 1964). They are also unable to keep up with the high reproduction rate of the beech scale (Houston 1997).

Beech trees may also be protected from BBD by other organisms that live on the bark. Although certain species of lichens may promote beech scale population growth, others prevent beech scale from inhabiting these trees. Beech trees whose bark is covered with the lichen *Ascodichaena rugosa* make unsuitable microhabitats for beech scale to live. This lichen is found in moist habitats, so beech trees in these habitats are less likely to be infected with BBD. Beech trees populated with large colonies of this lichen species have virtually no beech scale colonies. Unfortunately, *A. rugosa* does not flourish in North America as well as it does in Europe, and small, rough patches may offer shelter to beech scale (Houston 1997).

Fungi that are able to reduce levels of beech scale include *Cladosporium*, *Nectria*, *Verticillium* and *Paecilomyces* spp. (Vujanovic and Brisson 2001). Fernandez and Boyer (1989) found that high populations of the fungus *Aureobasidium pullalans* De Bary correlated with little or no beech scale.

Biological control of *Nectria* spp. fungus

The only known organism that may have the capability to significantly decrease the *N. coccinea* var. *fagisuga* population is the mycoparasite *Gonatorrhodiella highlei* AL Smith (Shigo 1964, Houston 1997). This, too, is an opportunistic generalist, but is able to prevent this fungus species from reproducing. It was first found in North America in 1933, in areas with BBD (Shigo 1964). It hooks onto the fungus with its hyphae. Unfortunately, *G. highlei* is usually only found in large populations of *N. coccinea* var. *faginata*, so would only be instrumental in decreasing these populations and not eradicating the fungus (Houston 1997). Thus, this mycoparasite could slow the advance of the killing front, while prolonging the advancing front. This could potentially allow more beech trees to wall off cankers made by *N. coccinea* var. *faginata* infection, decreasing the mortality in an area. Furthermore, this mycoparasite has also been found surrounding holes made by insects, so perhaps these decay insects are able to vector them from tree to tree (Shigo 1964). However, this would not benefit already infected beech trees. Other fungi that parasitize *Nectria coccinea* var. *faginata* and *N. galligena* include *Ustulina deusta* Hoffm., which is found near the base of trees (a common location of beech scale) and *Verticillium*, *Trichoderma* and *Cladosporum* spp. (Vujanovic and Brisson 2001). All fungi need the proper environmental conditions to survive, as changes in light, heat and humidity decrease a habitat's suitability (Vujanovic and Brisson 2001). To change these conditions would certainly decrease the amount of *Nectria* spp. infection in an area.

It has been suggested that the beech scale and both species of *Nectria* may live in an area without damaging beech trees until some environmental factors were to change them into aggressive pathogens (Shigo 1964, Lonsdale 1979). However, this hypothesis has not been further developed, and there are no studies that have found *Nectria* spp. in an area without damage to beech trees.

Consequences of beech bark disease

Depending on the stage of the disease and the percentage of beech in the forest, beech forests can remain the same or undergo profound change. Beech is one of the most important mast-producing trees in North America, especially since the decline of American chestnut (*Castanea dentata* (Marshall) Borkh.) due to chestnut blight (Castello et al. 1995, Wiggins et al. 2001, Faison and Houston 2004). Many animals, especially in the northern area of beech distribution, depend on beech mast production as this is the only hard mast in the area. Black bears, especially, forage for beech mast in the autumn, before denning, and their subsequent reproductive success depends on hard mast (Samson and Huot 1998, Faison and Houston 2004).

Also, some consequences of BBD are considered more important than others. In an area where aesthetics are important enough that they can elicit policy changes, the ugliness of the affected trees may be a large problem. A protected forest that has high beech mortality does not appeal to tourists. This is especially relevant in areas where beech makes up a significant proportion of the forest composition. Unfortunately, this is not a pressing concern for the majority of the population.

Beech wood is harvested mostly for furniture, flooring and firewood, since is dense and burns well (Houston 1983, Coladonato 1991, Gilman and Watson 1993, Barker et al. 1997). The value of a beech lot decreases dramatically if trees are severely damaged by BBD because the bark contains large cankers and the wood is weakened and snaps easily. Wood in this condition can only be used for firewood, which is worth much less than timber (Loo 2003).

Beech-maple forests constitute a large proportion of deciduous North American forests. Hane (2003) reported that in forests where beech mortality is high due to BBD, the survival rate of sugar maples (*Acer saccharum* Marsh.) in control stands is six times lower than that in stands where beech saplings have been removed. Canopy beech are more susceptible to damage during ice storms and windthrow than sugar maple, which may be due to their weakened state from BBD (Papaik et al. 2005, Takahashi et al. 2007). BBD can completely alter forest dynamics, as eastern hemlock (*Tsuga canadensis* L. Carr) can also take advantage of openings in the canopy to flourish (Runkle 1990, Griffin et al. 2003). This increases the humidity in the forest, which promotes beech scale, furthering the disease (Gavin and Peart 1993). When canopy beech die, they stimulate beech clones growth and the age cohort of the forest is completely altered. Furthermore, the death of many large trees in a short period of time adds much nitrogen to the soil, which may reach nitrogen saturation, causing more nitrogen to leave the area. This can have great effects on forest nutrient cycling, as beech trees making up 35% of the forest biomass still take up 50% of soil nitrates (Latty et al. 2003).

Conclusion

BBD is currently being studied by many scientists because it has the potential to affect forest ecosystems in many different ways. It is also important to examine the BBD complex because it looks at the impacts of an introduced insect and pathogen. Although it has been studied in North America for over eighty years, there are still many gaps in our knowledge of this disease, and in particular, our ability to control it (Houston 1980, Houston 2004). Most North American research on BBD has been based in the US and has practically ignored its consequences in Canadian forests. Although it is devastating complete stands in Quebec and Ontario, it is still unknown how far north and west it will travel. More work on biological control of the beech scale is needed to curtail its constant spread across this continent. Although research is ongoing on breeding beech seemingly resistant to BBD, to date no sprouts have survived nonsterile conditions (Barker et al. 1997, Ramirez et al. 2007). More success might be had by stimulating resistant trees to produce clonal root sprouts in natural settings.

General objectives and research questions

My research was conducted at two forests in the greater Montreal area of south-western Quebec: the Morgan Arboretum and the Molson Nature Reserve. The two study forests were chosen because they are located relatively close to one another and offer different forest structures that may affect their BBD distribution. They also provided the opportunity to collect data on BBD in its early stages, which can later be used to follow the course of the disease.

This thesis has three objectives. The first objective of this study is to determine the level of BBD in the Morgan Arboretum and the Molson Nature Reserve. My aim was to explore plot-wise characteristics that might help to explain the distribution of BBD within these two forests.

Using a multidisciplinary approach is essential to understanding all of the factors that affect BBD because this disease involves an insect, two species of fungus, and the health of the infected tree. BBD infection is very variable in that each tree reacts differently to both the initial infection and prolonged exposure to the disease. Therefore, both soil and tree data were used to narrow down those factors that might directly affect the population of beech scale. Thus, the second objective was to examine the plot- and tree-wise characteristics to explain the abiotic and biotic factors that affect the distribution of BBD at the two study forests.

Because it is chiefly dispersed by wind and is immobile once it lands on a beech tree, the microhabitat in which the beech scale lives is very important to its survival. The bark on the beech tree is not uniform in its entirety, and so each side of the tree can harbour different populations of beech scale. The population of beech scale on a tree can also change greatly over time, depending on the favourability of the substrate. This, in turn, directly affects the level of disease on the tree. Thus, the third objective of this study was to examine direction-wise characteristics on individual trees within a stand to see how beech scale populations change over time. This will allow more focus on microhabitat factors that directly affect beech scale populations, and may permit researchers to predict the future level of disease in a forest.

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Connecting statement

The epidemiology of BBD was outlined in Chapter 1. To fully understand the distribution of the disease, it is important to undertake a multifaceted approach that involves the infected forest on a plot-, tree-, and directional-level. In the study presented in Chapter 2, all of these factors are taken into account to present a full picture of BBD in the two study forests in Montreal, Quebec.

CHAPTER 2: FACTORS AFFECTING THE DISTRIBUTION OF BEECH BARK DISEASE IN TWO BEECH-MAPLE FORESTS IN SOUTH-WESTERN QUEBEC

Abstract

Beech bark disease (BBD) was found to be present in two urban forests near Montreal. The disease is documented to be at the killing front in the Morgan Arboretum, located on the western edge of the Island of Montreal, and at the advancing front at the Molson Nature Reserve, located 3 km away on the island of Île-Perrot. The infective fungus found at the Morgan Arboretum was *Nectria galliena* Bres., and neither this nor *N. coccinea* var. *faginata* (Lohman and Watson) was detected at the Molson Nature Reserve. Beech scale population sizes for the forests were initially similar, but diverged after 12 months. Populations were significantly lower at the Molson Nature Reserve, and increased over time at the Morgan Arboretum. Beech scale population size was positively correlated with bark nitrogen content. Soil factors that affected beech scale populations included pH, calcium and nitrogen. Beech scale populations were found to be lowest on bark facing south. This study provided an initial framework for further studies on BBD in south-western Quebec.

Introduction

Beech bark disease is an important tree disease in north-eastern North America, causing high tree mortality, and thus altering forest composition with dramatic outcomes similar to oak wilt and Dutch elm disease (Brisson and Le Sauteur 1997). This disease is caused by an initial infestation of the beech scale (*Cryptococcus fagisuga* Lind.) and the subsequent infection of fungus (*Nectria coccinea* var. *faginata* or *N. galligena* to

American beech trees (*Fagus grandifolia* Ehrh.) (e.g. Ehrlich 1934, Houston 1994a). The disease can be documented in three distinct stages (Shigo 1964): 1) The advancing front, characterized by low beech scale populations and little damage to beech trees; 2) the killing stage, characterized by high beech scale and *Nectria* spp. populations, and high tree mortality; and 3) the aftermath zone, with most remaining trees growing at a reduced rate, and large stands of beech clones present.

Beech scale and *Nectria* spp. are present in various regions of Quebec (Brisson and Le Sauteur 1997, Vujanovic and Brisson 2001, Takahashi et al. 2007), and are thought to have been in Quebec for at least forty years (Brisson and Le Sauteur 1997). The distribution of the disease in this province represents the northern limit of BBD in North America (Houston et al. 1979b, Houston and O'Brien 1983, Brisson and Le Sauteur 1997). However, since few forests in the province have been studied intensively, detailed data are lacking about the stage of the disease in most regions of Quebec, and little is known about factors that may influence the distribution of the disease at its northern limits.

Most of the research done on BBD has taken place in Europe because it probably originated there, and was first described there (Ehrlich 1934). Although it was once believed that BBD might wipe out European beech (*F. sylvatica* L.), it is now thought that BBD is endemic to Europe and will continue to cause no more than low-level damage (Houston et al. 1979a, Aldhous 1981, Houston 1994a). The chalk soils of England cause the beech canopy to yellow prematurely (chlorosis), which emulates disease progress and may predispose the tree to BBD infection (Lonsdale and Pratt 1981). Also, the yellowing of the canopy is one of the first symptoms of BBD, as the tree weakens, and may indicate trees that are already infected with *Nectria* spp., even before there are physical signs on the bark (Houston and O'Brien 1983). It is important, therefore, to differentiate between chlorosis due to BBD, or soil composition. The health of the beech tree naturally affects its ability to resist the disease, although this may not always be apparent (Castello et al. 1995). Soil contents that affect the distribution and health of beech should also affect their ability to resist, or be susceptible to BBD. These factors include nitrogen in the form of NO_3- and NH_4+ (Templer and Dawson 2004), pH, calcium, and sand, clay and silt content (Houston et al. 1979a). However, it is unknown how strongly this relates to the distribution of beech scale, the initiator of the disease. This study will examine the connection between beech scale populations and soil chemistry, to understand the current distribution of the disease within two forests in southwestern Quebec.

The size of the host tree is a well-studied factor in the establishment of BBD in a forest, as the larger trees are able to sustain larger populations of beech scale (i.e. Wainhouse and Deeble 1980, Latty et al. 2003). It is believed this is due to their larger surface area and the tendency for larger trees to have more crevices in which the beech scale can shelter (Houston and Valentine 1988, Gavin and Peart 1993). However, most factors affecting BBD are unknown, or have only been studied singly, without taking into account the interaction between potential causal factors of the disease. For example, the chemical composition of the bark may affect the distribution of BBD. Bark containing a high concentration of nitrogen has been shown to be a better food source for beech scale, and thus trees with lower levels of nitrogen in the bark may be resistant to the disease (Wargo 1988). Ostrofsky and his colleagues (1984) illustrated that high levels of phenols in the bark act as protection against *Nectria* spp. fungus. However, these studies have not been confirmed elsewhere, and there are no studies examining the effect of both factors (nitrogen and phenols) on beech scale populations. Thus, this study will explore the role of nitrogen and phenols on beech scale populations near the northern limit of beech distribution.

Other factors, such as temperature and wind, directly affect the populations of beech scale and *Nectria* spp. fungus on beech bark. Beech scale is thought to be unable to survive at temperatures of less than -37^{o}C, and both beech scale and *Nectria* spp. fungus are distributed by even slight wind, as well as animal and insect vectors (Houston 1979b, Houston and O'Brien 1983, Houston 1994a). As winter temperatures in Quebec nearly reach these temperatures (especially when the wind chill is taken into account), the beech scale population may be severely limited in this respect and allow the tree time to defend itself. This contrasts highly with BBD-infested areas in the United States and Europe, as temperatures are usually not low enough to kill off whole beech scale colonies (Houston et al. 1979b). Strong winds can also desiccate both the beech scale and the *Nectria* spp. fungus. Wiggins et al. (2004) found that the beech scale populations on northern and southern sides of beech trees varied independently of each other. Their study was conducted in the Great Smokey Mountains National Park in Tennessee (USA), where winter temperatures are not effective at controlling beech scale populations. Understanding how beech scale populations function at the northern limit of beech is essential to predicting how BBD will progress in the future. Thus, research at the tree level is required to understand beech scale populations.

This study has three research objectives: 1) to examine plot-wise characteristics to explain the distribution of the BBD complex at two study forests in SW Quebec; 2) to examine whether various tree-level abiotic and biotic factors can explain patterns of BBD at the two study sites; and 3) to examine how beech scale populations change, spatially and temporally, on individual beech trees. Completed together, these three objectives will serve to explore the factors behind the presence of BBD in southern Quebec, and may help us understand the spatial and temporal distribution of BBD in our study forests.

Methods

Study sites

This research was conducted between May 2004 and May 2006, at the Morgan Arboretum and the Molson Nature Reserve, both protected forests owned by McGill University. These forests were chosen for the study because their proximity to each other allowed BBD to be studied in two completely different forests (with different forest structures, and populations of American beech) that contain the same disease. They also share the same meteorological factors and so their results could easily be compared. Furthermore, these forests are located near the northern limit of American beech, where BBD has spread slower than in warmer, more southern areas (Brisson and Le Sauteur 1997, Davis et al. 2000). The Morgan Arboretum is a 240 ha. forest located in Ste-Anne-de-Bellevue (45^{o}25N, 73^{o}57W), on the western side of the Island of Montreal. Within the Arboretum, I selected 88 plots for study. The plots all contained living American beech but also contained an overstory of sugar maple (*Acer saccharum* Marsh.), red maple (*Acer rubrum* L.) and white ash (*Fraxinus americana* L.). The understory consisted of American beech, striped maple (*Acer pennsylvanicum* L.), sugar maple and red maple. The plots had been laid out in a grid pattern 50m apart in 1998.

The Molson Nature Reserve (63 ha.) is located on Île-Perrot, west of the Island of Montreal (45^{o}23’41.79”N, 73^{o}58’32.45”W), about 3 km from the Morgan Arboretum. The plots chosen for this study are located in the western half of the forest and had also been previously laid out in a grid pattern. The plots (17 in total) were spaced 25m apart.

The forest overstory in the study area consisted of American beech, sugar maple, red oak (*Quercus rubra* L.), with an understory of striped maple and American beech. For both forests, every second plot was used to prevent overlap between plots (i.e. plots at the Morgan Arboretum and the Molson Nature Reserve were 100m and 50m apart, respectively).

Plots were used for data collection if they contained at least one living beech tree with a diameter at breast height (1.4m, DBH) greater than 10cm. Therefore, of the 105 aforementioned plots, 33 at the Morgan Arboretum and 17 at the Molson Nature Reserve were the focal plots for data collection. All plots were surveyed for surrounding vegetation in a 5m radius of the centre of the plot. Beech trees were chosen using a #2 wedge prism ($2m^2$/ha) at the centre of the plot. The use of the prism ensured an even distribution of large beech trees farther away, and smaller ones closer, to the centre of the plot (EC-FAO 2003). Beech trees were tagged and numbered with flagging tape, beginning with trees north of the centre and moving clockwise. The DBH of each tree was recorded, as were the presence of beech scale and *Nectria* spp. fungus.

Measuring BBD symptoms

To assess the status of beech scale populations for each tree in the study forests, photographs of beech scale populations were taken using a frame method: frames were set up 1m high, at each cardinal direction (N,E,S,W) of each beech tree with a DBH over 10cm. Frames were square cardboard windows with holes at the corners that were placed on the bark. The holes were marked with spray paint to ensure that the photographs would be taken at the same place in subsequent seasons. For trees with a DBH less than 30cm, a

5x5cm (25cm^2) frame was used. For trees with a DBH over 30cm, a 10x10cm (100cm^2) frame was used (Fig. 2.1).

The two different frame sizes were used to prevent the curvature of the tree from influencing the photographs. Photographs were taken of each frame in autumn of 2004, spring of 2005 and autumn of 2005. The camera (Canon PowerShot S400) was stabilized on a tripod to prevent blurriness, and photos that were unsuitable for analysis were retaken within a week. The photographs were analyzed by counting the number of beech scale insects per frame and noting the characteristics and texture of the bark. Each frame was counted twice to minimize human error, and the average count was used in the analysis. Because the removal of the scale insects' waxy covering would have affected the beech scale population of the following seasons, the insects were not disturbed in this manner. Instead, the approximate number of insects under the wax was estimated, based on the size of the wax covering the scale insect. Frames were lightly sprayed with water to enhance the view of the scale. Although bark characteristics such as lichen and cankering were included in the photographs, none significantly obscured the scale.

The bark on the trunk of beech trees was surveyed in summer 2005 to note the level of BBD damage on the tree, and aid in determining the overall status of BBD in these forests. Cankers are defensive wounds on the beech bark that were produced by the tree's reaction to invasion. Two kinds of cankers were noted: pits (slight damage produced by feeding beech scale) and large cankers (evidence of *Nectria* spp. infection that was contained by the tree). Tarry spots, areas on the bark that have been infected by *Nectria* spp. fungus, and appear before the perithecia of the fungus, were also noted, as they are signs of early BBD infection (Houston and O'Brien 1983). These bark characteristics were recorded for all trees, from ground level as far up the trunk as possible (approx. 3m).

Samples of *Nectria* spp. fungus were collected from each living beech tree infected with live fungus in order to determine the species of *Nectria* present in the study areas. To collect the fungus, circles of bark 2.5 cm in diameter were punched out from the tree. Each bark sample contained high densities (at least several dozen) intact red *Nectria* spp. perithecia (bruised perithecia do not release spores, which are essential for the species determination). Each bark sample was then placed in a clean, labelled glass jar lined with dampened paper towel to provide a moist environment and stimulate the release of spores. After approximately 24 hours, a few perithecia had released spores. The spores were removed from the perithecia using a sterilized needle and transferred to malt agar plates under a laminar flow hood. The colour of the resulting spore growth determined the species of *Nectria* fungus present on the tree.

Canopy data were collected on June 30th and July 7th, 2005. Data were taken by photographing the canopy of all living trees (living trees were categorized as having green leaves in the canopy) from the north and south sides of the tree with a digital camera housed with a fish eye lens. Data were analyzed using GLA (Gap Light Analyzer) software (Version 2.0, Simon Frasier University 1999). Data from north and south sides were averaged to obtain a final percentage of living canopy.

Chemical analyses of bark samples

To test whether scale populations were related to the concentrations of phenols, nitrogen and calcium in the phloem of the tree, I collected bark from each living beech tree with a DBH greater than 10cm for chemical analysis.

Methods for bark removal were adapted from Ostrofsky et al. (1984) and Dübeler et al. (1997). To collect the bark, one bark circle from each tree of 20mm in diameter, <10mm thick (thickness of outer bark) was removed as close to north as allowable. All bark removed was free from any infection/disturbance, such as beech scale, *Nectria* spp. fungus, cankering and lichen. This ensured that the chemical composition of the bark had not been altered by any of these external sources (Latty et al. 2003). Bark was removed using the same methods as bark collected for *Nectria* spp. analysis. Any sapwood was discarded, with the bark kept for chemical analyses. Each bark sample was then massed prior to further manipulation. Samples were air-dried in paper envelopes before being ground up and undergoing chemical extraction. Extraction was performed using methodology from Parkinson and Allen (1975) for nitrogen and Martin and Martin (1982) for phenols (Allen 1989). Calcium was read on a flame atomic absorption spectrophotometer (Perkin Elmer model 2380, the Perkin-Elmer Corporation, Norwalk, CT, USA) after a 10-fold dilution, where lanthanum and lesium were added to counteract interferences.

Characterization of soil parameters

To assess whether BBD was related to the underlying soil characteristics, I relied on previously collected soil data, taken from the 33 plots at the Morgan Arboretum in 1998 by G. Larocque (Dept. NRS, McGill University). Soil elements and compounds that were extracted include: potassium, phosphorus, magnesium, calcium, pH, nitrate, ammonium and mineralization and nitrification at depths of 0-15cm, and percentage of sand, silt and clay at depths of 15-30cm. All of these elements and compounds were used in statistical analysis to search for correlations with beech scale populations. All chemical analysis was performed using methods outlined by Carter (1993). Unfortunately, soil data from the Molson Nature Reserve were unavailable.

Data analyses

All correlations were performed using SAS for Windows, version 8.02 (SAS Institute, Cary, NC, US). Data did not meet the assumptions required for parametric statistics, so analogous non-parametric tests were completed. Scale population data were standardized against the size of the grid used. All other data were not transformed. Significance was marked at 5%.

To complete the first objective, the Kruskal-Wallis test was performed to compare soil characteristics between plots in the Morgan Arboretum, and tree and bark characteristics between plots from the Morgan Arboretum and the Molson Nature Reserve. Variables compared between the two study forests were tree characteristics (DBH, percentage of living canopy) and bark characteristics (nitrogen, phenols and calcium concentration).

Once Spearman-rank correlations between beech scale populations and abiotic and biotic factors that may have influenced them were completed, those factors with the highest correlation coeffients were chosen to create models. These models were made to explain the populations of autumn 2005 beech scale on trees. The parameters for each model were placed together in a multiple regression (SPSS 10.0.5 for Windows (SPSS Inc., Chicago, IL, USA)) and the residual sum of squares was used to calculate the AIC (Aikake's Information Criterion). AIC estimates the Kullback-Leibler distance to determine the maximum likelihood estimates of models given in the analysis (Hobbs and Hilborn 2006). AIC is used to test the likelihood of models explaining the hypothesis using parameters selected from preliminary analysis (Burnham and Anderson 2001; e.g. Ishihama and Washitani 2007, Squires et al. 2007, Venier and Pearce 2007, Vinarski et al. 2007).

To complete the second objective, correlations were performed (using Spearman's correlation constant) to compare soil characteristics with total autumn 2005 beech scale population per site. This allowed any effect of soil characteristics on beech scale population, or on the development of BBD, to emerge. Correlations were also estimated between total autumn 2005 beech scale population per tree and extracted bark nutrients (nitrogen, phenol and calcium concentrations per tree), DBH, and average living canopy per tree. Thus, all measured sources of variation were tested against beech scale populations, in order to explore the factors that affect both beech scale populations and BBD in Quebec.

To complete the third objective, correlations were performed between beech scale populations on the northern, eastern, southern and western sides of the trees for each season. The total beech scale population per tree was compared between each seasonal count (autumn 2004, spring 2005 and autumn 2005).The directional beech scale counts were also correlated over each field season separately for each forest to document the changes in beech scale populations over time. Thus, changes in beech scale populations over the length of the study were effectively examined.

Results

Distribution of trees and BBD in the two study forests

There were 205 trees sampled at the Morgan Arboretum; of these, 184 were living and 21 dead. Of the living trees, 6 were without beech scale or *Nectria* spp. fungus, 161 contained only beech scale, and 17 had both beech scale and living populations of *Nectria* spp. fungus (Table 2.1). Out of these 17 trees, all fungus samples were found to be *N. galligena*. There were 67 American beech trees sampled at the Molson Nature Reserve, with 52 living and 15 dead trees. Of the living trees, 3 trees were found to be free of both beech scale and *Nectria* spp. fungus, 49 trees were found to harbour only beech scale, and no trees contained *Nectria* spp. fungus (Fig. 2.2).

Scale counts did not differ significantly between the two study forests in autumn 2004, but were significantly different by spring 2005 and in autumn 2005; by then, scale populations were significantly higher at the Morgan Arboretum compared to the Molson Nature Reserve.

The Molson Nature Reserve was found to have twice the percentage of dead standing trees than the Morgan Arboretum (Table 2.1). More dead trees (52.3%) in the Morgan Arboretum had DBHs larger than 40cm, whereas most (86.7%) of the dead trees at the Molson Nature Reserve were less than 40cm across (Fig. 2.2). At the Molson Nature Reserve, all of the trees free from both beech scale and *Nectria* spp. fungus were smaller than 20cm across, whereas at the Morgan Arboretum these trees were evenly distributed among diameter classes of 0-20cm and 20-40cm (two and four trees, respectively). The majority (70.6%) of *Nectria*-infected trees at the Morgan Arboretum were larger than 40cm across, but there were some in smaller diameter classes as well (Fig. 2.2). There was no significant difference between the DBHs or bark phenol concentrations of trees between the two study forests (Table 2.1). Significant differences were found with respect to the percentage of living canopy, bark nitrogen concentration and bark calcium concentration (Table 2.1).

Factors affecting BBD

Autumn 2005 scale counts were correlated (using Spearman-rank correlation) against various abiotic and biotic parameters. Total beech scale count was significantly positively correlated with nitrogen (r = 0.19, *P* = 0.007), and negatively associated with percent living canopy (r = -0.17, *P* = 0.01). Scale count was not significantly correlated with phenols (*P* = 0.2843), calcium (*P* = 0.9602) or DBH (*P* = 0.2028). To further test the role that each of these parameters played in beech scale population dynamics, AIC analyses were done using the models presented in Table 2.2. None of the models were particularly strong, but the most likely models for tree-wise scale distribution all contained nitrogen (i.e., models 2 and 3, Table 2.2).

To understand the presence and distribution of beech scale in the Morgan Arboretum, soil characteristics from sampling areas that contained beech scale were correlated to the total population of beech scale (autumn 2005) for each plot. Significant positive correlations were found between beech scale population per tree for sand concentration. Significant negative correlations were found for silt concentration, clay concentration, pH level, magnesium concentration, calcium concentration, mineralization rate, nitrate concentration, ammonium concentration and nitrification rate (Table 2.3).

Beech scale population dynamics in time and space

Beech scale populations from different sides of the tree correlated with those from other trees from the same field season, and with the total scale population for that tree. The highest correlations were between east and west scale populations with total tree scale counts, during spring and autumn 2005 ($0.80<r<0.84$). The lowest correlations were found for all directions during autumn 2004 ($0.38<r<0.70$). All correlations were extremely significant (*P*<0.0001), and correlation coefficients ranged from 0.38 to 0.84 (Table 2.4).

Directional beech scale populations were compared within field seasons to see how beech scale populations varied among directions of the same tree. During autumn 2004, the north and east directions were similar at the Morgan Arboretum, and significantly higher than those of the south and west (Fig. 2.3a). This shifted slightly during spring 2005 (Fig. 2.3b), when the population at the southern direction decreased. During autumn 2005 (Fig. 2.3c), this trend continued, as overall beech scale populations decreased slightly but remained in the same distribution. In the Molson Nature Reserve, beech scale populations in the southern direction were consistently lower than the other directions for all three field seasons.

The overall levels of beech scale for the two study forests were similar in autumn 2004. In the Morgan Arboretum, beech scale populations increased during 2004 and 2005 (Fig. 2.4) (Kruskal-Wallis: $\chi^2 = 53.4987$, d(f) = 2, *P*<0.0001). In 2005, there was no significant difference between spring surveys and autumn surveys for the scale populations. At the Molson Nature Reserve, scale populations were significantly higher in the spring of 2005 than those of autumn 2004 and autumn 2005 (Fig. 2.4) (Kruskal-Wallis: $\chi^2 = 8.5885$, d(f) = 2, *P* = 0.0136). Beech scale populations were lower at the Molson forest in 2005 compared to the Morgan forest (Table 2.1).

Discussion

Status of BBD at the Morgan Arboretum and the Molson Nature Reserve

This research has quantitatively documented the distribution of BBD in two forests located near a major urban centre along the St Lawrence Seaway. Although it is known that beech scale has been in the Montreal area for many years (Brisson and Le Sauteur 1997, Vujanovic and Brisson 2001), we now have well-documented information about the specific level of infestation of this insect. The Morgan Arboretum and Molson Nature Reserve contained different stages of BBD in their forests. Both contained the beech scale, but *Nectria* spp. was only present at the Morgan Arboretum.

It appears that the Morgan Arboretum is at the killing front of BBD since a high population of beech scale, as well as *Nectria* spp., was present. In contrast, the Molson Nature Reserve is considered to be at the advancing front since scale populations remain relatively low, and *Nectria* spp. was not detected. However, the presence of equal numbers of tarry spots at the two study forests indicate that *Nectria* spp. might be present at both forests, even though there is no superficial sign of the fungus on the tree. Tarry spots indicate an infection (possibly that of *Nectria* spp.) under the bark, which erupt at the surface when bacteria populations explode (Houston and O'Brien 1983).

Since only the native fungus (*N. galligena*) was found at the Morgan Arboretum, it is at the beginning of the killing front. The first *Nectria* fungus to appear in an area has been found to be the native species (Cotter and Blanchard 1981). Later, when it is well-established, the introduced fungus (*N. coccinea* var. *faginata*) is believed to outcompete the native species (Cotter and Blanchard 1981, Houston 1994a, Houston 1994b). Finding only *N. galligena* strongly implies that the American beech forest in the Morgan Arboretum will be highly altered in the near future. It will be interesting to document the progress of the disease at these two forests in the future, and to compare that with other nearby studied forests, such as the Gault Reserve (Takahashi et al. 2007).

There may be several reasons to explain the different levels of BBD in our study forests. First, the distribution of American beech is different in each forest. The Morgan Arboretum contains a large tract of pure beech forest, whereas the American beech at the Molson Nature Reserve are all found in mixed deciduous forest. Beech scale that are dispersed from infected trees in mixed forests can land on other trees besides American beech, and so may slow the spread of the disease (Moran et al. 1982, Wainhouse and Gate 1988). However, large American beech forests allow a higher percentage of favourable landing sites on nearby trees, which aids in the spread of the disease, and these forests may even act as initial spots of infection (Houston et al. 1979b). It is difficult to compare the level of BBD at our study forests with those of other forests because there are so many different methods to study the presence and distribution of the disease (i.e. Houston and O'Brien 1983, Gavin and Peart 1993, Forrester et al. 2003, Griffin et al. 2003). However, we used a similar method to Fernandez and Boyer (1988), and so can compare BBD levels of trees in Toronto (ON) with ours. They found that most of the trees in their study forests had either no beech scale at all, or less than 25 beech scale colonies per $25cm^2$. (However, they removed the waxy coating from the insects, which we were unable to do since our populations were sampled repeatedly over time.) Furthermore, they found no signs of *Nectria* spp. infection on any of their trees. These results are similar to those we found for the Molson Reserve. However, since this study took place in 1982, it would be prudent to resample these forests to document their change in BBD infection. This might also suggest how BBD at the Molson Reserve might change over the coming years.

A second explanation for the difference between the study forests may be due to the age and size distribution of American beech at the two locations. While the majority of dead trees in the Molson Nature Reserve are small and unmarked by cankers, perhaps caused by normal mortality in less-than-ideal conditions, most of the dead trees at the Molson Arboretum are larger, well-established trees that have evidence of BBD infection (severe cankering and *Nectria* spp.). This indicates that BBD has been present at the Morgan Arboretum for a number of years. Furthermore, these results are found in two forests that have a similar distribution of living trees (Fig. 2.2, Table 2.1).

Another sign of different BBD levels between the two forests is the trees' overall health, which aids in determining their ability to resist BBD (Shigo 1964, Castello et al. 1995). The yellowing of the canopy is a widely-recognized symptom of BBD, as the trees divert their energy to fighting infection (Houston et al. 1979a). As the Morgan Arboretum had less living canopy than the Molson Nature Reserve, it is likely that this was caused by a higher level of BBD. Lonsdale (1980) found that high populations of beech scale helped inhibit the tree's defense against *N. coccinea* infection in Europe. When comparing the status of the trunks of the trees, both forests had similar levels of bark pitting (from low levels of beech scale damage) and tarry spots (from underlying infection). It is no surprise that the two forests harbour similar levels of pitting, as only 3% of all trees were completely free of beech scale. Furthermore, the number of trees with tarry spots at the Morgan Arboretum was understandable, as *N. galligena* was found there. However, it was unusual to find tarry spots in the Molson Nature Reserve, as they have long been recognized as indicators of underlying *Nectria* spp. infection. It is highly unlikely that *Nectria* spp. rings were overlooked in the bark surveys, or that only other trees outside of the plots harboured the fungus. It is much more probable that the tarry spots noted were caused by other tree infections (Houston et al. 1979b.) The American beech trees at the Molson Nature Reserve will have to be carefully monitored for signs of *Nectria* spp. infection, especially trees with tarry spots present. The only difference in the level of bark damage found between the two forests was in the percentage of trees with large cankers. Cankers are signs of *Nectria* spp. infection that have been at least partially blocked by the tree (Houston 1975). This fungal infection may be present only at the Morgan Arboretum because of the large American beech population, which offers more sites for infection. Also, whereas the American beech trees at the Molson Nature Reserve are scattered, those at the Morgan Arboretum are contained in a pure beech, or beech/maple forest. Neighbouring trees act as sources of infection for both the beech scale and *Nectria* spp. (Houston et al. 1979b).

We are unable to explain the absence of *Nectria* spp. at the Molson Nature Reserve. The two study forests are less than 3km apart, which is negligible considering the disease has been found in isolated pockets in North Carolina and Tennessee (US), more than 400 miles (643km) away from the nearest infected forests (Houston 1994a). It is therefore quite possible that the American beech trees at the Molson Nature Reserve have been exposed to the *Nectria* spp. fungus. Although it was not found in this study, *N. coccinea* var. *faginata* has already spread to nearby forests in Ontario (Davis et al. 2000, Sajan 2001). The low populations of beech scale found in the Molson Nature Reserve indicate that although *Nectria* spp. may have entered the forest, beech scale populations are not high enough to predispose trees to fungal attack. (It is important to note that the three stages of BBD described by Shigo (1964, 1972) might require revision in northern climates due to different meteorological and environmental conditions.) When beech scale feed from the bark, their stylet changes its chemical composition, making it more susceptible to infection by *Nectria* spp. (Houston 1994a). It is therefore likely that *Nectria* spp. fungus will be found in the Molson Nature Reserve within the next few years, as beech scale become more prevalent. It is unlikely that climate affected the distribution of *Nectria* spp. between the two forests, as they are located so close together.

Abiotic and biotic factors affecting BBD at a stand level

Studying the relationship betweens soil compounds and BBD also presents an opportunity for understanding potential causal mechanisms for the disease complex at both the tree and plot level. Beech scale populations were found to be positively correlated with soil sand concentration (Table 2.3), which is predicted by the literature (Gilman and Watson 1993). It is unclear why beech scale populations were negatively correlated with silt, clay and calcium concentration, when American beech trees are known to favour acidic soils with high concentrations of silt, sand and clay (Gilman and Watson 1993). Beech scale populations may be correlated to nitrate concentration due to the insect's nutritional requirements (Houston 1994a). Low soil calcium levels may affect the ability of the tree to produce healthy cells that can withstand the disease (Schaberg et al. 2001). However, it is unclear why the other characteristics affect beech scale populations because there have not been any studies describing soil influences on beech scale populations.

Abiotic and biotic factors affecting BBD at a tree level

Overall, we documented relatively few strong relationships between biotic (e.g., DBH and bark chemistry) and abiotic (e.g., soil chemistry) factors and populations of beech scale. It was surprising to find that DBH did not significantly correlate to the beech scale population, as it is well-documented in the literature that larger trees harbour more beech scale and have higher mortality (i.e. Mize and Lea 1979, Wainhouse and Deeble 1980, Fernandez and Boyer 1988, Houston and Valentine 1988, Morris et al. 2002, Griffin et al. 2003, Latty et al. 2003). *Nectria* spp. fungus is only able to infect a tree after populations of beech scale have grown significantly so their feeding chemically alters the bark, which takes several years (Houston 1994a). High populations of beech scale also decrease the efficacy of the tree's natural defenses against *N. coccinea* in Europe, and possibly against other *Nectria* spp. as well (Lonsdale 1980). Thus, *Nectria* spp. is usually found in larger trees. In this study, it was found even in trees with DBHs of 7.5cm, and since these trees were already infected at such a small size, this suggests that the Morgan Arboretum will have very high American beech mortality (where even small trees are infected).

As the level of phenols in the bark was similar between both forests, it is unlikely that it affected BBD levels between the two forests. Phenols are believed to present a level of defense for the infection, although a few studies have shown that *N. coccinea* var. *faginata* is unaffected by high phenol levels (Wargo 1988, Houston 1994a). However, Wargo (1988) reported higher concentrations of bark phenols in trees heavily infested with beech scale, suggesting this to be an effect of beech scale feeding. While Ostrofsky et al. (1984) found that the inner bark contained a higher phenol concentration than the outer bark, Wargo (1988) reported the opposite result: outer bark had a higher concentration of phenols than inner bark. Since both of the sample sizes of these two studies were so small (n=8, n=5, respectively), it is impossible to conclude with any certainty how phenol levels are concentrated in beech bark. They may even be distributed differently in different geographic areas, perhaps due to environmental conditions or tree health. Further studies must be completed in order to resolve this issue, especially since bark phenol concentrations may be instrumental in developing resistance to BBD.

In the two forests studied here, both the levels of nitrogen and calcium differed. Nitrogen is an essential nutrient for scale insects and nitrogen-rich bark will harbour higher populations of scale insects (Wargo 1988, Houston 1994a). Furthermore, trees with little or no beech scale infection contain lower levels of bark amino acids, although few relationships have been found between the level of beech scale infestation and bark nitrogen concentration (Wargo 1988, Krabel and Petercord 2000). Calcium is essential for the maintenance of cell walls (Schaberg et al. 2001)**,** thus directly affecting the strength of the tree and perhaps its ability to resist the disease. Perrin and Garbaye (1984) found that trees with lower concentrations of bark calcium had higher levels of cankering. As the trees at the Morgan Arboretum have more nitrogen and less calcium than those at the Molson Nature Reserve, they may be both better food sources for beech scale and less able to withstand the infection of beech scale and *Nectria* spp. This would make them more susceptible to BBD, and put them in a better position to have a higher level of disease than those at the Molson Nature Reserve.

Only nine out of two hundred and seventy-two trees were found to be uninfested by beech scale (3.3%). It is unclear whether these trees were resistant to BBD or had not yet been in contact with beech scale. Given the ease with which beech scale spreads, and its widespread distribution in both study forests, it is likely that these trees present a natural resistance to BBD. This percentage is much higher than the estimated percentage of resistant trees (i.e., < 1%) (Houston and Houston 2000, Loo 2003). Although beech scale has been present in Quebec since 1965 (Brisson and Le Sauteur 1997), beech scale populations on the trees in this study remain quite low compared to trees from other studies (i.e. Morris et al. 2002, Latty et al. 2003). In Toronto (ON), Fernandez and Boyer (1988) found trees with a DBH of 11cm to be completely covered in *C. fagisuga*, whereas in NY state, Munck and Manion (2006) reported that most trees with a DBH <7.6cm were free of beech scale. Thus, close to the northern limit of the disease, the scale insect may be distributed differently than in the rest of its range. Furthermore, large trees have been observed as centres of infection to other, younger trees (Houston et al. 1979b). However, the majority of these studies took place in the north-eastern U.S. (i.e. New York, New Hampshire) where environmental and meteorological factors may differ from those in more northern locations. Houston and Valentine (1988) noted that mild winters resulted in higher beech scale populations. Fernandez and Boyer (1988) suggested that very large trees may have lower beech scale populations because they have developed a tolerance to beech scale over time. Low winter temperatures may have resulted in killing both beech scale and *Nectria* spp. fungus, or perhaps since the level of BBD in southern Quebec is lower than reported elsewhere (e.g., Houston 1975, Morris et al. 2002), there is no effect of DBH on beech scale populations because conditions are not optimal, resulting in a slower rate and progression of infection. In Maine in 1975, the beech forests were in the last stage of BBD, whereas at that time, the disease was just entering Quebec (Houston 1975). In New Hampshire, Morris et al. (2002) found that 100% of young beech trees with a DBH greater than 3cm were infected with beech scale; in the present study, I only found beech scale on trees larger than 7.5cm DBH. It is unclear whether beech scale found on the smaller trees in this study were *C. fagisuga* or *Xylococcus betulae* (Perg.), which feeds on small trees (Houston 1977). The latter scale insect may also influence the survival of *C. fagisuga* as its presence provides shelter (Fernandez and Boyer 1988).

Unfortunately, researchers have not yet been able to successfully clone surviving *F. grandifolia* putatively resistant to BBD (Barker et al. 1997, Ramirez et al. 2007). However, if the roots of seemingly resistant or tolerant beech trees are scraped to produce callus tissue, successful root sprouts emerge (Jones and Raynal 1988). This may be a more viable solution to laboratory work on resistance. Furthermore, in the Catskill Mountains (NY), Griffin et al. (2003) found that all trees sampled were infected with BBD, 33% with *Nectria* spp. and 28% were dying or dead. These trees were in the aftermath stage of BBD, which occurs several years after initial *Nectria* spp.-induced damage. Thus, although the study forests have not yet reached the dire levels of BBD shown in these studies, it is possible that it will occur soon, unless environmental factors such as cold winter temperatures slow its progression. However, this will not prevent BBD from killing trees. In one of the most diseased plots in this study where *N. galligena* was found, half of the trees were already dead or dying, or were “whitewashed” with beech scale so that death was inevitable (data not shown). Individual trees with BBD need to be monitored over a long period of time in order to catalogue the progression of BBD in its northern range.

The AIC analysis resulted in two of the four models being considered likely models for beech scale population. Unfortunately, none of these models were particularly strong, although two were much stronger than the others. Bark nitrogen figured in each of the likely models, so there is probably a strong link between nitrogen and beech scale population, a finding confirmed by correlation analysis. High bark nitrogen levels are an important limiting factor for the beech scale (Wargo 1988, Latty et al. 2003). Wargo (1988) and Latty et al. (2003) found that DBH was directly related to bark nitrogen concentration, which was also found in this study (data not shown). Although limited in his conclusions by a small sample size, Wargo (1988) observed that higher beech scale populations lived on trees with higher bark amino acid concentration. However, bark nitrogen concentration was not found to effect, or have an effect on, beech scale populations. Areas with high nitrogen fertilization have larger populations of beech scale because the ground is so saturated with nitrogen that all American beech trees become good food sources (Latty et al. 2003). Bark nitrogen levels will only decrease once the soil nitrogen is depleted, which may then decrease beech scale population (Castello et al. 1995). However, beech scale are found in so many areas with varying nitrogen availability that other factors must jointly be involved in its distribution and success. Unfortunately, due to the short duration of this study, it was impossible to determine whether the phenol and nitrogen concentrations were affected by the presence of the beech scale or whether they were unrelated.

Another factor used in the model selection was phenol concentration, which figured highly in the most probable models from the AIC analysis, although it was not significantly correlated independently with beech scale population. Thus, it may interact with one of the other factors to affect beech scale population. Ostrofsky et al. (1984) demonstrated that bark phenols were higher in trees susceptible to beech scale infestation. Furthermore, some trees were thought to be resistant to beech scale as they produced phenols faster than other trees (Ostrofsky et al. 1984). Although Wargo (1988) did not find an effect of bark phenol scale population, he did conclude that beech scale increased the trees' bark phenol concentration. Unfortunately, neither of these conclusions were confirmed in the present study, as phenols were not well correlated with beech scale populations. Similar to the results from tree DBH, this could be due to low levels of beech scale, which may not be subject to the same limiting factors as high populations. Also, the phenol concentrations found in the current study were low and occupied a small range of values (< 10mg/g tissue), compared with the two studies here (<90mg/g tissue).

Spatial and temporal dynamics of beech scale populations

Beech scale populations were at the same density in both forests in the autumn of 2004. However, by the spring of 2005, they had increased threefold at the Morgan Arboretum, and almost doubled at the Molson Reserve (Table 2.1). This trend continued into autumn 2005: populations remained high at the Morgan Arboretum, but decreased significantly at the Molson by the end of the research period (Fig. 2.4). It is likely that weather caused the beech scale populations to change so dramatically over the course of one year. Harsh winter temperatures may have killed many beech scale, as average temperatures in January 2004 were lower than they had been since 1994 (-15.1°C vs. average of -10.4 °C, Env. Can. 2006). Beech scale are known to be killed by temperatures of -37 °C, but can also be desiccated by harsh winter winds if they are not sheltered by snowbanks (Brisson and Le Sauteur 1997). Also, less snow fell in January 2003 and 2004 than had fallen since 1996, which may have influenced the amount of snow cover available to the overwintering beech scale (Env. Can. 2006).

The spike in beech scale population in spring 2005 can be attributed to the emergence of the 2nd instar larva, and its subsequent wind distribution (Brown 1934, Ehrlich 1934). The similar population levels in autumn 2005 indicate that there was low beech scale mortality during the summer, at least at the Morgan Arboretum. The higher beech scale populations in the northern and eastern directions were likely caused by the spread of the insect by wind. Houston et al. (1979b) found that higher levels of beech scale were found on these sides of the tree, which correlated with wind direction.

However, it is unknown why beech scale populations decreased in autumn 2005 at the Molson Nature Reserve.

During the last two field seasons, beech scale populations on the south side of trees in both forests remained significantly lower than all of the other directions. It is unclear why beech scale did not survive on the southern sides of the trees. Perhaps meteorological factors are the cause: adult immobile beech scale are known to be washed off trees by heavy autumn rain (Houston and Valentine 1988). This may also have affected both autumn and subsequent spring beech scale population. Beech scale prefer areas with a high amount of moisture as well (Ehrlich 1934). Another reason for the discrepancy in beech scale populations in the two forests could be in the distribution of lichen such as *Lecanora conizaeoides* that provide humid microhabitats and shelter for the beech scale (Houston 1977, Houston et al. 1979b). Other lichen such as *Ascodichaena rugosa* in England have smooth surfaces and prevent the long-term survival of beech scale (Houston et al. 1979a); since beech scale movement is limited (generally <1m from the landing site), they might not be able to relocate to a more habitable area on the bark (Houston et al. 1979b). As lichens were not measured in the course of this study, it is impossible to determine whether lichen coverage on the beech bark significantly affected the distribution of beech scale. However, this would be a profitable course to follow in the future.

Higher beech scale populations were consistently found in the northern and eastern directions in the Morgan Arboretum. It is likely that wind strength is responsible, as beech scale nymphs move from tree to tree only by wind and animal vectors (Houston 1979b, Wainhouse and Deeble 1980, Houston 1994a). Because the main path in the Morgan Arboretum cuts through the beech forest, it may have acted as a wind tunnel to further the spread of BBD, or the beech scale may have entered by increased human traffic (Houston 1994a). Furthermore, the beech forest is located on a slope, which increases the amount of wind, beech scale and *Nectria* spp. fungus entering the forest (Ehrlich 1934, Houston et al. 1979a, Munck and Manion 2006). Unfortunately, wind pattern data were not collected during the course of this study, but would be valuable in the near future.

Only one other study has compared beech scale populations on different sides of beech trees. Wiggins et al. (2004) used similar research methods, and found that the northern and southern directions of trees harboured higher beech scale populations. This differed greatly from the current study, as the lowest beech scale populations were found in the southern direction, which was constant over the last two field seasons. These results are not surprising when taking into account that their study took place near the southern limit of BBD where the climate and forest structure differ greatly from that of Quebec. Wiggins et al. (2004) also found that there was a direct link between beech scale populations in the autumn and those of the previous spring. However, their study took place over the course of two years, whereas the current study lasted only a year. Unfortunately, it is not possible to determine whether there is a definite link between the spring and autumn 2005 beech scale populations over such a short period of time. Because temperature is so important in the winter survival of beech scale, it would be prudent to undertake further study of the beech scale populations in southern Quebec over the course of several years to monitor these populations. With more information, a direct link could be derived between beech scale populations and local weather patterns, and could be used to model the future spread of BBD at its northern limit.

One great difference between Wiggins et al. (2004) and the current study was that their seasonal and directional correlations were weak at best ($0.003<R^2<0.25$) and those in the current study were much stronger ($0.38<R^2<0.84$). The high correlations between different directions indicate that during studies with time or personnel constraints, it would be fairly reliable to collect data from only one side of the tree instead of each cardinal direction. However, it would be prudent to continue collecting data over each of the four cardinal directions for follow-up studies to determine whether there are significant year effects on these correlations. Further research on beech scale populations in Quebec will improve our knowledge of the spread of the disease in this province.

Conclusion

Since BBD is present both at the Morgan Arboretum and the Molson Nature Reserve, two forests with different tree distributions and levels of the disease, they present ideal places to study such a variable disease. Unfortunately, since this study only occurred over one year, many questions still remain regarding the BBD complex. There are many factors that affect the presence of beech scale, none of which have been fully explored. Furthermore, *Nectria* spp. populations are difficult to characterize as they may be present in a forest without producing perithecia. BBD will continue to spread westwards and southwards, following the distribution of beech, and it must be better understood if is to be slowed, if not stopped. Furthermore, a better understanding of the distribution of beech scale, the initiator of the disease, will allow forestry workers to develop practices to prevent all American beech trees from dying.

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Table 2.1. Comparisons between the Morgan Arboretum (MA) and the Molson Nature Reserve (MNR) in south-western Quebec for tree characteristics, beech bark disease symptoms and bark chemistry. Means ± SE presented where applicable. All tests are non-parametric (1 degree of freedom), performed using Kruskal-Wallis test, or using Fisher's Exact Test, indicated by *. Seasons are noted as follows: Aut = autumn, Spr = spring, 04 = 2004, 05 = 2005. Letters indicate significant differences between values.

Site/tree factor	MA	MNR	Significance	Chi square
Percentage of dead trees*	10.2 a	22.4 b	**0.02**	6.48
DBH of all trees (cm)	33.8 ± 1.07 a	30.7 ± 1.79 a	0.16	1.95
DBH of living trees (cm)	36.1 ± 1.01 a	37.2 ± 1.76 a	0.55	0.36
DBH of dead trees (cm)	43.0 ± 2.71 a	26.6 ± 2.96 b	**0.0005**	12.2
Percentage living canopy	77.2 ± 1.05 a	80.3 ± 2.13 b	**0.05**	3.96
Percentage of trees with bark pitting*	77.6 a	77.6 a	1.00	$1.0E^{-4}$
Percentage of trees with bark cankers*	82.9 a	53.7 b	**$5.14E^{-6}$**	23.4
Percentage of trees with tarry spots*	14.6 a	16.4 a	0.70	0.13
Bark phenol concentration (mg/g tissue)	5.35 ± 0.11 a	5.13 ± 0.17 a	0.28	1.18
Bark nitrogen concentration (mg/g tissue)	6.06 ± 0.07 a	5.79 ± 0.11 b	**0.04**	4.42
Bark calcium concentration (mg/g tissue)	33.0 ± 0.48 a	36.2 ± 0.97 b	**0.003**	8.99
Mean number of beech scale per tree (Aut04)	23.4 ± 3.77 a	14.6 ± 3.04 a	0.37	0.8
Mean number of beech scale per tree (Spr05)	60.2 ± 8.74 a	27.2 ± 5.71 b	**0.014**	6.1
Mean number of beech scale per tree (Aut05)	56.7 ± 7.65 a	17.6 ± 4.78 b	**0.0001**	24.2

Table 2.2. Akaike's information criterion (AIC) of the correlation models of growth of beech scale populations, depending on tree-wise factors. Data were taken at the Morgan Arboretum and the Molson Nature Reserve in south-western Quebec in 2005. [N] = bark nitrogen concentration, [phenols] = bark phenols concentration. N = 200.

Model	Model ID	No. parameters (K)	Residual sum of squares	AIC	Delta AIC (Δ*i*)	Akaike weight (*wi*)
[N] [phenols] DBH	1	3	1558424	784.33	1.95	0.15
[N] DBH	2	2	1559252	782.37	0	0.40
[N] [phenols]	3	2	1563776	782.62	0.25	0.35
[phenols] DBH	4	2	1611234	785.22	2.84	0.096

Table 2.3. Correlations among plot soil chemical composition and total autumn 2005 scale count of beech from the Morgan Arboretum, in south-western Quebec. All correlations performed using Spearman's correlation coefficient. Nonsignificant correlations are denoted by NS. (K = potassium, P = phosphorus, Mg = magnesium, Ca = calcium, NO3 = nitrate, NH4 = ammonium).

	Correlation coefficient (r)	Significance
%Sand	0.40	0.02
%Silt	-0.34	0.05
%Clay	-0.43	0.01
pH	-0.52	0.002
K(μg/g)	NS	0.08
P(μg/g)	NS	0.27
Mg(μg/g)	-0.47	0.006
Ca(μg/g)	-0.50	0.002
Mineralization rate	-0.40	0.01
NO_{3-}	-0.44	0.01
NH_{4+}	NS	0.06
Nitrification	NS	0.06

Table 2.4. Correlation strengths of beech scale populations from different sides of the same tree, compared with the the other sides of the same tree, and with the total scale population for that tree for three field seasons (Aut = autumn, Spr = spring, 04 = 2004, 05 = 2005, N = north, E = east, S = south, W = west). Beech scale population data were taken at the Morgan Arboretum and the Molson Nature Reserve in south-western Quebec. All correlations were significant (p<0.0001).

	Autumn 2004			
	N	E	S	W
N	-	-	-	-
E	0.51	-	-	-
S	0.42	0.38	-	-
W	0.47	0.38	0.52	-
total	0.72	0.68	0.70	0.70

	Autumn 2005			
	N	E	S	W
N	-	-	-	-
E	0.58	-	-	-
S	0.52	0.55	-	-
W	0.63	0.53	0.52	-
total	0.80	0.80	0.75	0.80

	Spring 2005			
	N	E	S	W
N	-	-	-	-
E	0.64	-	-	-
S	0.55	0.59	-	-
W	0.58	0.54	0.60	-
total	0.77	0.84	0.77	0.81

Figure 2.1. Photograph taken at the Morgan Arboretum (Montreal, Quebec) during autumn 2005 of the frame used to standardize beech scale count during data collection. Frame is 25cm x 25cm. White beech scale are present. Credit Tania Motchula.

Figure 2.2. Comparison of beech bark disease status on individual trees at the Morgan Arboretum (MA) and the Molson Nature Reserve (MNR) in south-western Quebec separated into diameter classes. N = 205 (MA) and 67 (MNR). Y-axis depicts percentage of trees in both forests.

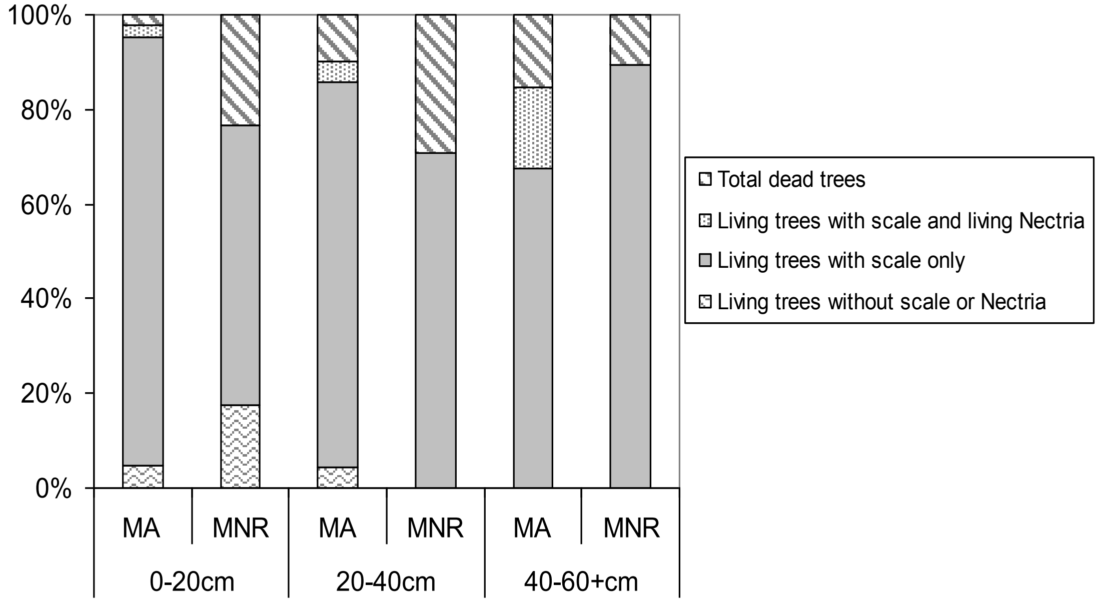


Figure 2.3. Total beech scale population for all trees on different sides (N,E,S,W) of beech trees in the Morgan Arboretum (MA) and the Molson Nature Reserve (MNR) in south-western Quebec during a) autumn 2004, b) spring 2005 and c) autumn 2005. N = 205 (MA) and 67 (MNR). Letters indicate significant differences between directions of the same forest. Note different scale of Y axes.

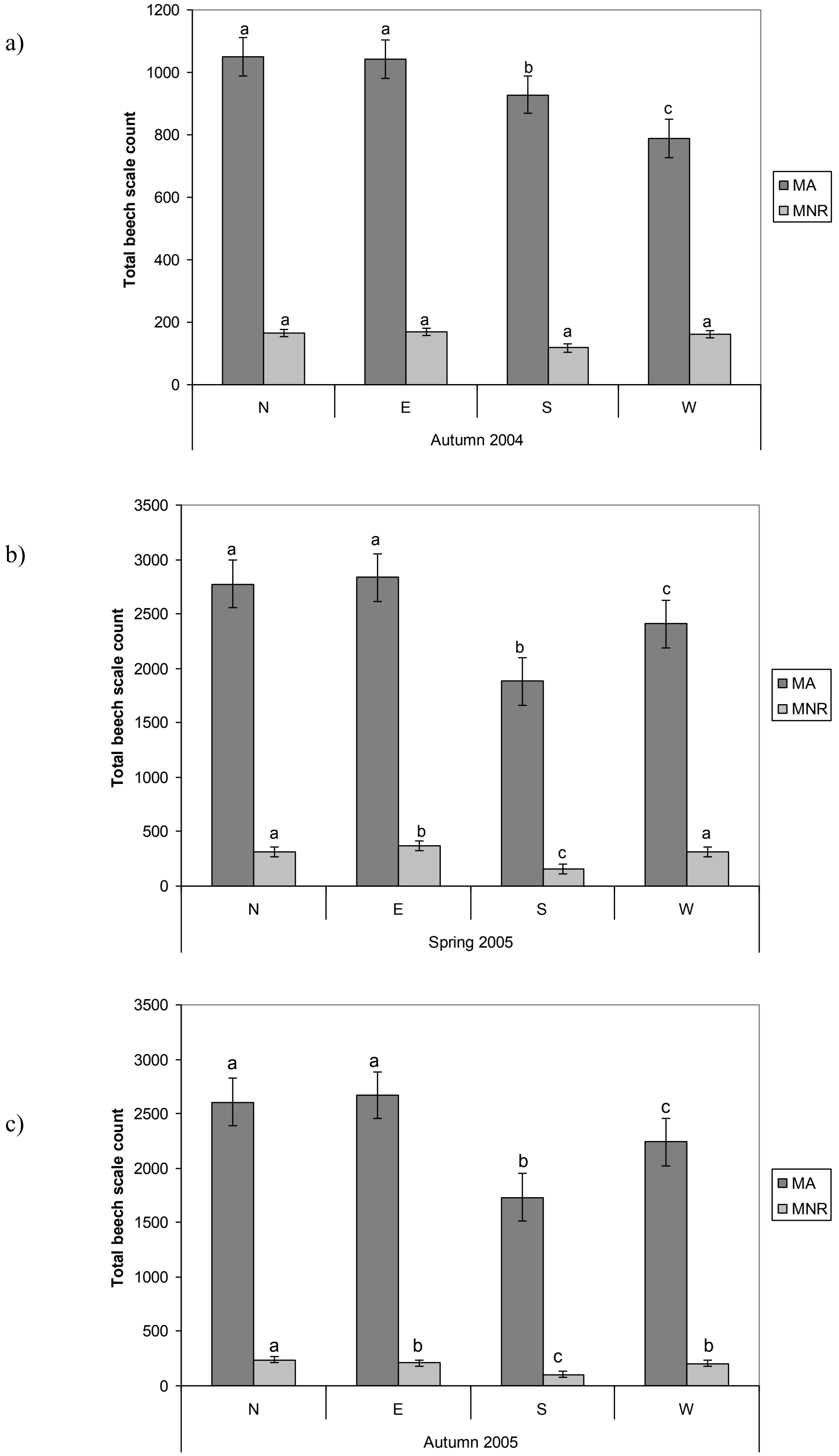
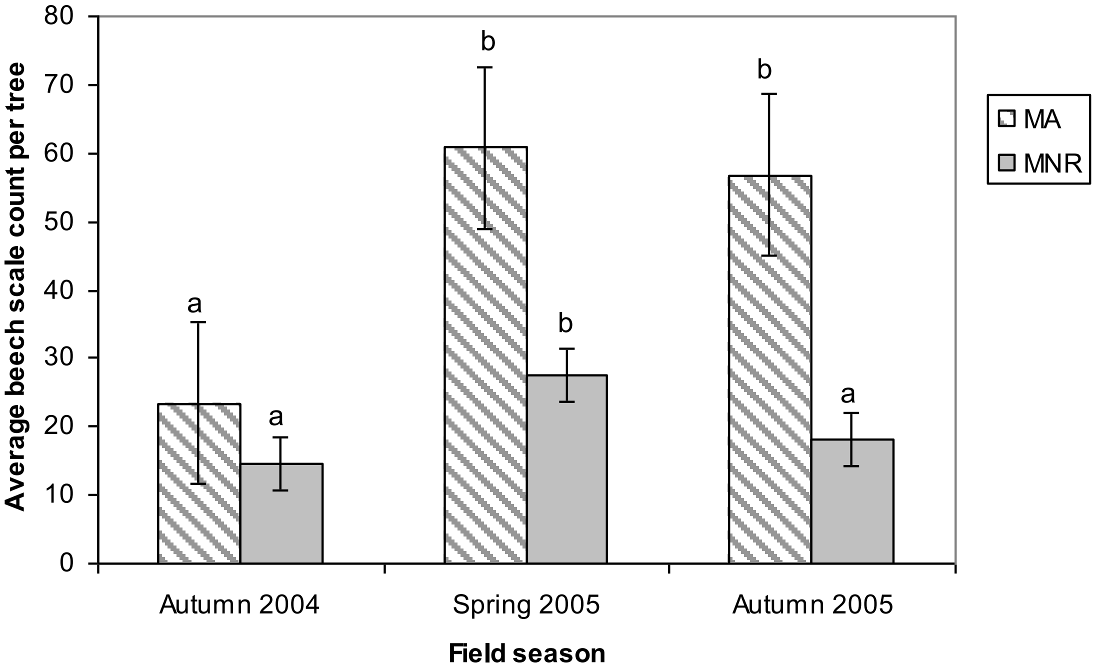


Figure 2.4. Mean beech scale populations (± SE) per tree at the Morgan Arboretum (MA) and the Molson Nature Reserve (MNR) in south-western Quebec during autumn 2004, spring 2005 and autumn 2005. Letters indicate significant differences between the same forest by season.



CHAPTER 3: GENERAL CONCLUSION

BBD has been studied ever since it was brought to North America in 1890 (e.g. Ehrlich 1934, Shigo 1972, Houston and O'Brien 1983, Wainhouse et al. 1988, Houston 1994, Gwiazdowski et al. 2006). Since then, numerous studies have been completed on the distribution of the disease in different areas, and fewer exploring the resistance of American beech (*Fagus grandifolia*) to BBD and the effect of BBD on forest dynamics (e.g. Houston 1975, Latty et al. 2003, Le Guerrier et al. 2003). Only one study has explored populations of beech scale on different sides of a tree (Wiggins et al. 2004). The present study aimed to involve all of these factors affecting the level of BBD in different forests, because only by including all of the possible causes can we hope to see how they interact with each other.

This study found differing levels of BBD at the Morgan Arboretum (killing front) and the Molson Nature Reserve (advancing front). Comparing the two forests, it is clear that soil and tree characteristics, and forest dynamics are responsible for these different states of BBD in forests in such close proximity to each other.

Soil and bark characteristics were compared to the population of beech scale for each tree, and it was found that high nitrogen levels in the bark increased the population of beech scale because nitrogen is a good nutritional source for beech scale (Houston 1994). Bark phenol and calcium concentration, and tree DBH were found to have no effect on beech scale population. Soil characteristics that provided a substrate for healthy trees were found to also be beneficial to beech scale populations.

Populations of beech scale were found to be highest on the northern and eastern sides of the tree, and lowest on the southern side. The reason for this is unclear, as the only paper dealing with directional beech scale populations reported that the highest populations of beech scale were found on the northern and southern sides of the tree (Wiggins et al. 2004). It is likely that local environmental climatic conditions were at least partly responsible for the different results, as Wiggins et al. (2004) studied forests approx. 1,600 km south of the present study area.

More multidisciplinary studies must be done on BBD in different areas of its North American range in order to identify the qualitative factors that affect its distribution. It would be prudent to continue long-term beech scale population studies in forests affected with all stages of BBD to follow the course of the disease and identify the different factors affecting the different stages of BBD. Since at present BBD cannot be controlled, it is vital to the maintenance of our remaining American beech forests to understand how they will change in the future.

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