Microhabitat Selection and Characterization of the Woolly Beech Scale, *Cryptococcus fagisuga,* in Southern Quebec.

Sean Boyce
Department of Natural Resource Sciences
McGill University, Montreal
January 2009

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science in Renewable Resources

Short Title

Microhabitat Selection and Characterization of the Woolly Beech Scale

Acknowledgements

I would like to thank my supervisors Dr. Benoît Côté and Dr. Chris Buddle for their advice and support throughout this project, and NSERC for providing financial assistance through Dr. Côté's operating grant. I would also like to thank the Centre d'Étude de la Forêt (CEF) for advice concerning the choice of statistical analysis, and for allowing me to share the preliminary results of my experiment at their annual colloquium in 2008. Finally, I would like to thank Jonathan Maisonneuve for his invaluable assistance in the field, as well as Joey Bowden, Andrea Dechene, Kathleen Aikens, Carol Frost, Amy Moores, Zachary Sylvain, Tara Sackett, and Gregory Hum for feedback on the early drafts of this thesis.

Table of Contents

List of Figures

List of Tables

Abstract

Beech bark disease is a widespread and fatal disease of the American beech, (*Fagus grandifolia* Ehrh.*)*, initiated by an infestation by the woolly beech scale insect, (*Cryptococcus fagisuga* Lind.*)*. This infestation is characterized by spatial patterns in the number of beech scale colonies within trees by both aspect and height. I studied these patterns by monitoring differences in microhabitat quality within trees over four months (June-September) at two forests in Montreal, Canada. Spatial patterns in bark temperature, bark resistance to puncture, moisture content, and roughness were compared to patterns in beech scale colonization as determined by colony counts at the start and end of the study. Additionally, stemflow collars were attached to some trees to experimentally determine the effect of stemflow on beech scale colonization patterns. Beech scale colonies were found to be significantly more numerous on the north side of trees, as well as higher up (2.5-2.8 m aboveground) the bole of trees. Seasonal bark temperature was 0.5^{o}C higher at 2.5 m aboveground. Seasonal bark moisture content was significantly higher further up the bole of trees by an average of 3%. The north aspects of tree boles were found to be significantly more resistant to puncture. Diverting stemflow moisture away from the north aspect of trees did not change the bark moisture content, but did cause a significant reduction in scale colonization. The effects of transient stemflow moisture as a source of moisture or as a dispersing agent for beech scale nymphs may explain the higher scale populations on the north side of trees. My results demonstrate that beech scale populations vary spatially with microhabitat quality.

Résumé

La maladie corticale du hêtre est une maladie répandue et mortelle du hêtre à grandes feuilles (*Fagus grandifolia Ehrh.).* Une infestation par le puceron laineux du hêtre, (*Cryptococcus fagisuga Lind*.) représente la première étape dans la progression de la maladie (Ehrlich 1934). Sa propagation sur l'arbre est affectée par l'exposition et la hauteur. Nous avons caractérisé ces patrons spatiaux en mesurant la température, la résistance, la rugosité et l'humidité de l'écorce des arbres. Ces mesures ont été prises dans deux forêts de la région de Montréal, Québec entre Juin et Septembre à deux hauteurs différentes (1.3 et 2.5 m) et du côté nord et sud des arbres. Nous avons également installé des colliers aux arbres pour empêcher l'écoulement sur le tronc du côté nord des arbres. Les pucerons étaient plus nombreux sur le côté nord et plus haut (2.5-2.8 m du sol) sur l'arbre . La température moyenne de l'écorce était 0.5^{o}C plus élevée à 2.5 m. Le contenu en eau de l'écorce était 3% plus élévé à la hauteur de 2.5 m. Le résistance de l'écorce était plus haute sure les côtés nord des arbres. Les collets ont diminué la colonisation par le puceron laineux pendant l'été d'environ 80%. Nos résultats suggèrent que l'écoulement sur le tronc est une source d'humidité importante pour l'insecte, ou une méthode de transport, qui peut expliquer les plus grandes populations observées du côté nord des arbres. En bref, nos résultats démontrent que les populations du puceron laineux du hêtre varient avec la qualité du microhabitat.

1. Introduction

The effects of insect pests have been catastrophic in North American forests and plantations, both in terms of economy and ecology. The areas devastated by insect outbreaks can be enormous; for example the recurring spruce budworm (*Choristoneura fumiferana* Clen.) epidemic has defoliated millions of hectares each time it occurs (PMRA 2005). This represents a loss of hundreds of millions of cubic meters of wood (PMRA 2005). In Christmas-tree plantations, the pine needle scale (*Chionaspis pinifoliae* Fitch.) causes significant damage to the needles of the host trees, to the point where they cannot be sold (Bishop et al. 1994). The scales secrete a white wax that enables them to resist pesticides (USDA 1998). Predation and parasitism normally limit the population of this scale insect, but the application of insecticide strongly affects its natural enemies, hampering effective management. Presently, an epidemic infestation by the mountain pine beetle (*Dendroctonus ponderosae* Hopk.) in British Columbia threatens to destroy up to 80% of merchantable pine over the next five years, in the southern and central regions of the province (Province of British Columbia MFR, 2007). The pine beetle is able to overcome the defenses of the host tree due to the fungi that it carries, which suppresses the normal wound response of pine trees. This response would ordinarily prevent the pine beetle's eggs from hatching (Safranyik et al. 1974). The severity of the disease is amplified by the association between the fungal and insect agents.

Some of the most disastrous epidemic tree diseases involve invasive species. When the European elm bark beetle, (*Scolytus multistriatus* Marcham) was transported to North America in the 1930s, it initiated the spread of another insect-fungal disease complex known as Dutch elm disease. Since then, Dutch elm disease has been responsible for the mortality of an estimated 35 million ornamental elms in the United States and Canada (Ip 1992). What makes diseases such as Dutch elm disease particularly serious is that it represents a disturbance caused by an invasive species, allowing rapid proliferation unchecked by local biological control agents. When the causative agent is an invasive species, it can cause permanent changes to the disturbed ecosystem (Drake et al. 1989), which may impede the recovery of the host species. A current threat of major concern is the Asian longhorned beetle, (*Anoplophora glabripennis* Motschulsky). This beetle, native to China, Korea and Japan, is perceived as a major threat to the North

American forest industry (Kimoto and Duthie-Holt 2006). It is known to aggressively infest maple and poplar species among others, and so has the potential to impact both the lumber and sugarbush industries.

Invasive species are responsible for irreparable harm to ecosystems, if allowed to propagate uncontrolled in their new habitat. The woolly beech scale, *Cryptococcus fagisuga* Lind., was introduced to North America over 100 years ago (Ehrlich 1934), and has become established throughout the Northeastern United States and Canada (Houston et al. 1979). This insect allows the infection of American beech trees (*Fagus grandifolia* Ehrh.) by one of several fungal agents, initiating a disease complex known as beech bark disease. Beech bark disease represents an ongoing threat to late-successional temperate forest ecosystems in northeastern North America.

1.1 Beech Bark Disease

Beech bark disease is a widespread and fatal disease of the American beech. The disease is caused by an infestation by the invasive woolly beech scale insect, which facilitates a subsequent infection by a *Nectria* fungus (Ehrlich 1934). While the rapid spread of the disease was a source of concern when it was first noticed in the early twentieth century, it was quickly neglected due to the low economic importance of beech as a lumber species (Shigo 1972). More recently, concern over the disease has grown as we begin to more fully understand the ecological roles of the American beech (Jakubas et al. 2004, Storer et al. 2004). This renewed interest in the subject has led to a better understanding of beech bark disease, and the primary causative agent, the woolly beech scale.

1.2 History and Spread of the Disease

The spread of the beech scale began when it was first introduced to North America in the mid-nineteenth century, on ornamental beech saplings destined for public gardens in Halifax, Nova Scotia (Houston 2004). It was noticed in the area around Halifax by the late 19th century, and by 1935 had been a known cause of beech mortality within most of the Canadian Maritimes, as well as small patches along the eastern coast of the United States (Houston 2004). Currently, the range of the disease encompasses

most of northeastern North America, from the northern limit of the American beech to areas just south of New York State. Small patches extend further south, into Kentucky and Virginia.

1.3 The Woolly Beech Scale

The woolly beech scale (Hemiptera: Eriococcidae) is a sessile colony-forming scale insect native to Europe. Its life cycle consists of three stages. In the first stage, it hatches from an egg in late summer as a small nymph with fully developed legs and antennae. It then migrates to an appropriate patch of beech bark, and inserts its stylet-shaped mouthpiece to feed on the nutrients present in the inner bark. The second stage begins as it loses use of its limbs and becomes sessile, excretes a white woolly wax, and overwinters at its chosen site. It begins the third stage by molting in the spring to become an adult about one millimeter long, and lays numerous pale yellow eggs in midsummer. This results in the death of the insect. A good description of the lifecycle and characteristics of the beech scale insect is provided by Shigo (1972).

The beech scale reproduces parthenogenetically, resulting in the potential for explosive population growth. However, the spread of the beech scale to new hosts is limited by the fact that nymphs do not disperse to new trees on their own, but rely on passive wind dispersal (Wainhouse 1980). This has limited the spread of the beech scale to an average rate of 6-8 kilometers a year (Houston et al. 1979). On a single beech trees the spread is exceptionally slow, as nymphs on average do not migrate far from their parent colony. In an artificial infestation experiment, dispersion was limited to an average of a few decimeters per year (Houston et al. 1979), with almost all scales remaining within one meter even after three years. This demonstrates that the beech scale tends not to move far from where it hatches before selecting a location to become immobile and begin feeding. This lack of mobility may cause beech scale colony density over different areas of a tree to reflect microhabitat quality. Houston (1982) attempted to create foam enclosures to trap parasites of the woolly beech scale, and while no parasites were observed, beech scale insects showed a strong preference for the area underneath the foam pads. A later experiment confirmed that attaching foam pads to trees was an excellent way to inoculate trees with the woolly beech scale, or to promote the local

growth of existing colonies (Houston 1982). This may have been in part due to the pad reducing the mobility of scale nymphs. Nevertheless, it still suggests that the number of colonies in an area may be a strong indicator of the suitability of the local microhabitat.

The beech scale has few predators in North America. Among them are the mite *Allothrombium mitchelli* Davis, and the coccinellid beetle *Chilocorus stigma* Say (Wiggins et al. 2001, Mayer and Allen 1983). Unfortunately, neither of these species are effective biological control agents of the beech scale or the disease as a whole (Houston 1994, Wiggins et al. 2001). No parasitoids of the beech scale are known.

1.4 The *Nectria* Fungi

Once beech bark has been perforated by the beech scale, it becomes susceptible to a fungal infection. Originally it was thought that an invasive European fungi, *Nectria coccinea* var. *faginata*, was the primary agent responsible for the disease outbreak, with several native species of *Nectria* playing a lesser role (Houston 1994). However, more recent genetic analyses of *Nectria* fungi have placed these varieties under a new genus, *Neonectria* (Mantiri et al. 2001). Furthermore, a recent phylogeny constructed from genetic analysis of these *Neonectria* species strongly suggests that the fungus responsible for the majority of the disease outbreak in North America is native, and is given the name *Neonectria faginata* (Castlebury et al. 2006). While the North American *Neonectria* fungi are in many ways similar to their European counterparts (now named *Neonectria coccinea*), they are a distinct sister species. Either species of fungi will cause large target shaped cankers on the bark, often killing tissue outright and leaving a telltale vertical strip of dead bark that becomes heavily infected. These areas of dead bark are often characterized by white or bright red fruiting bodies, and become inhospitable to the beech scale. The spread of the *Neonectria* infection slowly kills the host tree, either by girdling it, by increasing its susceptibility to windthrow (Papaik et al. 2005), or by allowing other infectious agents to colonize the tree such as varieties of the root fungus *Armillaria* (Houston 1994).

Very few organisms feed on or are able to exclude *Neonectria* fungi. The mycopathogen *Nematogonum ferrugineum* Pers. is able to reduce the spread of the fungi by inhibiting sporulation (Shigo 1972), but is not sufficient to stop or reverse the spread

of the disease. While it has few antagonists, *Neonectria* fungi are not particularly infectious under normal conditions. They are unable to infect the bark of healthy beech trees, even when levels of bark phenols are particularly low (Houston 1994). This may be because the high moisture content and low nutrient value of hardwood sapwood makes it hard for mycelia to become established unless the normal function of sapwood is compromised (Boddy and Rayner 1983). It has been suggested that the mycelia of several fungal species, including *Neonectria*, begin to establish themselves in beech once the wood begins to dry out (Chapela and Boddy 1988). Generally, large-scale *Neonectria* infection in North America is limited to forests already infested by the beech scale.

1.5 The American Beech

Fagus grandifolia is a shade tolerant, late successional species that has been widespread along the northeastern coast of North America for approximately the last 7000 years (Loach 1970, Bennett 1985). Beech bark is known to have significantly drier bark than many other hardwood tree species (Billings and Drew 1938), and is smooth, hard, and impermeable. This presents a challenge for any insect parasites. However, low bark moisture content has been studied as a factor that may affect susceptibility to fungal diseases in the American beech (Chapela and Boddy 1988, Chapela 1989). Beech trees are normally very resistant to infection by *Neonectria* fungi, except when injured or under conditions of stress, such as drought, nutrient imbalance, or other diseases (Houston 1994). When the protective qualities of beech bark are reduced by perforations made by the feeding beech scale or other wounds (Houston 1994); the dry bark is very susceptible to infection by the *Neonectria* fungi. Additionally, the combined pectinase activity of the beech scale and *Neonectria* may play a role in overcoming the defenses present in beech bark (Perrin 1983, Perrin 1984). However, the only factor that is known to completely prevent the course of the disease is the existence of rare beech populations that exhibit nearly complete resistance to infestation by the beech scale.

Less than 1% of American beech exhibits resistance to infestation by the beech scale. These resistant trees tend to occur in groups, and since resistance is strongly heritable (Koch and Carey 2004), are commonly closely related. Unfortunately, attempts to discover a marker gene for beech bark disease resistance in the American beech have

failed so far (Koch and Carey 2004). There is strong evidence that resistant trees exhibit a slightly different nitrogen (N) metabolism (Wargo 1988, Koch and Carey 2004), which combined with the heritability of resistance, suggests that resistance involves one or more polymorphisms in genes controlling N metabolism. Variations in bark anatomy (such as bark thickness) have not been successfully demonstrated as significant factors in determining disease resistance of the American beech (Leaven and Evans 2004). This suggests that bark N content is likely the primary factor that determines the overall level of beech scale infestation present on a given tree.

In Europe, beech bark disease resistance in the European beech has been primarily associated with variations in bark anatomy rather than bark chemistry (Houston 1994). However, some studies also demonstrated that the presence of certain alleles at the isocitrate dehydrogenase and peroxidase loci also have a negative effect on populations of the beech scale (Krabel and Petercord 1998, Gora et al. 1994). This suggests that there are several factors controlling resistance to beech bark disease in the European beech, contrary to the American beech.

Promoting the spread of trees resistant to beech bark disease has been suggested as a way to reduce the impact of beech bark disease on forest ecosystems. It has been suggested that encouraging the simple management practice of cutting down all root sprouts surrounding a tree that has succumbed to the disease will significantly promote the spread of resistant trees (MacKenzie and Iskra 2005). However, this practice is not likely to stop or significantly slow the spread to the disease on susceptible trees; the beech scale will colonize a stand even if the source of infestation is distant (Houston et al. 1979). The benefit is an accelerated replacement of susceptible beech trees with resistant ones as the disease progresses, creating a smoother transition towards a forest of resistant trees. It also serves to eliminate the potential barrier to new growth presented by the root sprouts. This practice may reduce the ecological impact of beech bark disease on North American hardwood forests.

1.6 The Ecological Importance of American Beech

The American beech is a common dominant species in North American hardwood forests due to its extreme shade tolerance, and its ability to exclude other trees in a

number of ways. A good history of the American beech as it became prominent throughout eastern North America is provided by Bennet (1985). American beech is known to acidify the soil with its slowly decomposing litter (Peterson 2002), as well as reduce the success of maple seedlings by producing many offspring during periods of stress (Hane 2003). In addition, its litter decomposes slowly, reducing nutrient. In a mature beech forest, most of the nutrients in the environment are locked up in the beech trees as a result of these strategies, with only a few other shade tolerant species such as sugar maple (*Acer saccharum* Marsh.) able to remain competitive (Cogbill 2005). In addition, beech trees may reproduce vegetatively via root sprouting under conditions of stress. In the context of beech bark disease, this means that diseased trees often have enough time to produce many root sprouts before death. These sprouts often have a high incidence of the disease, and represent a significant barrier to succession (Evans et al. 2004). These thickets of diseased root sprouts have been aptly named "Beech Hell".

Beech is also known to produce frequent masts of beechnuts, an important food for many forest animals. Any reduction in beechnut production would likely affect the survival and behavior of many forest species (Storer et al. 2004). A reduction in beechnut production has been shown to affect patterns of reproduction and behavior in black bears and martens (Jakubas et al. 2004). Given the potential repercussions of a widespread decimation of beech populations on both forest quality and wildlife populations, understanding beech bark disease is an important step in preserving the value of North American forests.

1.7 Knowledge Gaps

Despite the fact that the infestation has become epidemic in large areas of northeastern North America, relatively little is known about the behavior of the woolly beech scale, the initiating agent of the disease. This is largely due to its cryptic behavior. While significant work has been done in determining patterns of beech scale infestation among trees (Leaven and Evans 2004, Latty et al. 2003, Koch and Carey 2004), patterns of scale establishment within trees have not been as extensively studied (Houston et al. 1979). Much of the available information is a result of casual observation (Houston et al. 1979), and processing of qualitative data (Wiggins et al. 2004). One of our major

interests has been to confirm and quantify these patterns by counting individual scale colonies over different areas of host trees.

Aspect, height, and stemflow have been observed to affect patterns of beech scale distribution (Houston 1982, Wiggins et al. 2004), although attempts to determine the cause of these patterns have not been successful (Wargo 1988). The woolly beech scale has been observed to colonize the north sides of trees at higher densities, and some observations suggest that the beech scale exhibits higher densities at a height somewhere above 1.5 meters (Houston 1982, Wainhouse 1980), although this has yet to be determined conclusively. Precipitation (as stemflow) may have an adverse effect on the establishment of new colonies (Houston 1982), although it may also be an important factor in preventing desiccation, which is a risk factor for scale insects as demonstrated by the adaptations used to mitigate this risk. The main adaptation is the secretion of white, waxy wool to shelter the sessile adult insect and eggs. A good summary of the adaptations specific to scale insects that reduce the risk of desiccation has been produced by Gullan and Kosztarab (1997). Understanding how the patterns of beech scale infestation develop within trees is an important step in advancing our understanding of the epiphytology of beech bark disease (Houston et al. 1979).

1.8 Microhabitat Selection by the Beech Scale

Given that the beech scale is immobile after site selection, dies after producing eggs, and does not typically move more than a few decimeters during its mobile stage, we would expect that any patterns of bark microhabitat quality would be strongly reflected by patterns in scale colony distribution and success. In aphids, which are considered to have similar nutritional requirements to scale insects (Wargo 1988), there are several known cases where such strong site-selection patterns develop, among much more mobile species. The pine woolly aphid, *Pineus boerneri*, is known to preferentially colonize canopy exposed to more sunlight, forming a horizontal distribution pattern (Chilima and Leather 2001). This is presumed to be because these areas of the tree are likely to have higher amino N concentrations (Field 1983). Similarly strong patterns are observed with the aphid *Uroleucon caligatum* on goldenrod, also caused by nutrient availability (Hartnett and Bazazz 1984). Overall, aphid populations are often limited by total N or

specific amino acid availability (Wargo 1988, Dadd and Krieger 1968), although the availability of overwintering refuges has also been suggested to be limiting (Wade and Leather 2002). The beech scale is suspected to be N limited, as increased bark N content has been shown to increase disease severity (Latty et al. 2003), and American beech fully resistant to beech bark disease have been shown to have lower bark N content (Wargo 1988). Bark N content has been well established as a factor limiting colonization of new trees by the beech scale (Wargo 1988, Latty et al. 2003). Bark thickness and soil moisture have been studied as potential factors that also affect the distribution of the beech scale among trees but no significant effect was found (Leaven and Evans 2004), although it was determined that site moisture had a positive effect on bark thickness.

Currently it is unknown whether physical or chemical factors affect the distribution of the beech scale within an American beech tree. Wargo (1988) tested for differences in bark N content between the north and south aspects of American beech trees, as well as comparing shaded and unshaded areas of bark. No significant differences were found, which demonstrates that while lower bark N content has a negative effect on scale populations, the effect is most useful for explaining population differences between trees. On a single tree, beech scales appear to prefer to colonize north aspects and locations above 1.5 meters, without any gradients in bark N content playing a role. As a result, these patterns in scale colony distribution within single trees must be explained by different factors than those explaining the scale distribution between trees. Patterns in bark anatomy are good candidates, since these are already known to affect scale colony distribution on the European beech.

Bark roughness is thought to have a positive influence on scale colony formation on the European beech by facilitating the colonization of new trees (Houston et al. 1979, Houston 1982), although it may provide overwintering refuges as well. Bark roughness is thought to be related to the diameter at breast height (DBH), and older, larger trees have rougher bark (Leaven and Evans 2004). Layers of lignified bark in the European beech have also been observed to decrease the incidence of beech scale colonies (Houston 1994). Exposure to temperature fluctuations is also thought to be harmful (Witter et al. 2005). Finally, since aphids exhibit adaptations to minimize moisture loss (Gullan and Koszstarab 1997, Spiller et al. 1990), extremes in either the local moisture content of

living bark, and/or the environmental exposure to incident moisture may be important factors affecting microhabitat quality. Houston (1982) demonstrated that the number of scale colonies on a single tree can be locally increased by modifying the microhabitat using foam blocks pressed against the bark, providing shelter. His treatments appear to have increased the local moisture content, in that the bark under the treatment pads appears dark and moist in photos. However, it was also noted that in areas where the treatment pads were exposed to high amounts of stemflow, scale colonies failed to develop, indicating that either extreme of moisture exposure is detrimental to the beech scale. In the present study, we aim to determine how different aspects of beech bark microhabitat affect the distribution of scale colonies on individual beech trees.

2. Research Objectives

The main objective of this study is to investigate the underlying causes for the various within-tree patterns of beech scale infestation that are noted throughout the primary literature. I attempted to determine the patterns in colony distribution, and how they are influenced by bark microhabitat characteristics: bark moisture, temperature, roughness, and resistance to puncture. In addition I am interested in how stemflow, height, and aspect influence these bark properties. Finally, I attempted to determine how site selection affects the success of individual scale colonies.

3. Hypotheses

The following statements represent alternative hypothesis to the null state of no difference being detected, and these are based on what knowledge exists in the primary literature:

1. Beech scale colonies will exist at a lower density on the south aspect of trees, and the bark will be warmer and drier on this side of the trees.
2. Beech scales will exist at higher densities 2.5-2.8 meters up the tree, when compared to scale density 1.0-1.3 meters from the ground.
3. Colony density will be greater on areas of moister, rougher bark, as well as bark that is less resistant to puncture.
4. Diverting the stemflow away from the north aspect of trees will reduce surface

bark moisture, increase bark resistance, and result in lower scale populations.

5. Colony success, measured as the number of live insects per colony, will be greater on the locations demonstrated to be superior microhabitats.

4. Materials and Methods

4.1 Study Sites, Experimental Groups, and Treatments

4.11 The Morgan Arboretum

McGill University's Morgan Arboretum is about 260 ha in area, and is the larger of the two sites sampled in the present study. It is located on the island of Montreal in southern Quebec (45^{o}25'N, 73^{o}57'W, 30 meters above sea level). Most of the Morgan Arboretum is composed of natural forest stands that range from pioneer to climax forests typical of the Great Lakes-St. Lawrence forest (Rowe 1972). The study site was located in a mature stand composed mainly of American beech and red maple (*Acer rubrum*). The soils have developed on a fluvial sand and are classified as Ferro-Humic Podzols (Millette 1948). A good description of the soils, vegetation, and topography of the area has been provided by Lajoie and Baril (1954). Stand density was estimated at 256 trees/ha, with a basal area of 26 m^2/ha (Table 1). The average age of the trees in the stand is around 150 years old (Trofymow and CIDET Working Group 1998, Fyles 1997).

4.12 The Molson Reserve

The Molson Reserve is a 51-hectare forest located on Ile Perrot near Montreal (45^{o}40'N, 73^{o}98'W). The soils in the area are acidic podzols (pH 5 to 5.5), classified as stony sandy loams derived from sandstone. A smaller area is composed of moderately drained brown-grey marine clay free of rocks. The topography ranges from level to gently rolling, with an elevation of 30 to 75 meters. A good description of the soils, vegetation, and topography of the area has been provided by Lajoie and Stobbe (1950). Although the Molson reserve is characterized by several swampy areas, our study site was at a higher elevation and was relatively free of standing water, and was located on stony sandy soil. The vegetation immediately around the study site is primarily composed of hardwoods with sugar maple and American beech being most common. Nearby, some coniferous species exist on areas where shallow soils occur over bedrock, these areas

include stands of red pine (*Pinus resinosa* Aiton) and white pine (*Pinus strobus*). DBH, stand density, and basal area at the Molson Reserve was similar to those at the Morgan Arboretum (Table 1).

Table 1 The average DBH (+/- standard error) of sampled trees, and stand characteristics of the two sites.

Site	DBH of sampled trees (cm)	Stand density (trees ha^{-1})	Basal area (m^2 ha^{-1})
Morgan Arboretum	36.1 $\pm$ 1.3	256	26
Molson Reserve	39.3 $\pm$ 2.0	225	24

4.13 Selection Criteria

Within each site trees were selected within a single stand. This served to minimize differences in wind and moisture exposure, as well as canopy structure. Selected trees were free of graffiti or other evidence of human disturbance (nails, paint, etc). They were at least 20 cm in Diameter at Breast Height (DBH), and had no deformation at a height of 1.5 meters that would interfere with attaching a stemflow collar. The selected trees were alive, and exhibited obvious signs of beech bark disease. No trees having signs of any other disease were selected, since this may have resulted in areas of dead bark that the scale insect could not colonize, which would reduce the quality of the data collected. Forty trees were selected at the Morgan Arboretum, and twenty trees were selected at the Molson Reserve. A lower incidence of the beech scale insect at the Molson Reserve led me to use this reduced sample size and collect fewer data at this site.

4.14 Assigning Trees to Experimental Groups

Trees were randomly assigned to one of four experimental groups. At both sites,

control and stemflow collar equipped trees were used for complete bark microhabitat characterization using 10 trees per treatment at each site. Bark microhabitat characterization required frequent periodic measurements including the use of a penetrometer to measure bark resistance. Repetitive bark puncture over the growing season was thought to have the potential to affect scale colonization. Therefore, the colony counts were done only on sub-groups of trees (control and stemflow collar-equipped) of the Morgan Arboretum that were not used for monitoring of bark resistance but otherwise subjected to all other bark microhabitat measurements. Additionally, the bark roughness of all trees at the Morgan Arboretum was recorded.

4.15 Installation of Stemflow Collars

Stemflow collars were attached to divert stemflow away from the north side of trees. Stemflow collars made of flexible polyethylene tube approximately 1 m long (depending on the circumference of the tree) were used. They were wrapped around trees in a spiral pattern such that the end of the spiral was 1.3 m from the ground and on the south side of the tree. Small holes were cut in the bottom of the tube on the south side, in order to allow some stemflow to drip through the collar and be distributed on the south sides of trees. This also served to reduce the amount of water that exits the end of the tube during heavy rain, which may otherwise have splashed back on the north side to some extent. Finally, the stemflow collars were carefully sealed to the tree using silicone sealant (GE Sealants Silicone II). Since silicone sealant contains high concentrations of acetic acid, these collars were attached on a fairly dry day so that the acetic acid evaporated and did not leach onto the tree due to incident precipitation. The weather also had to be warm enough to allow the silicone to dry properly. This design of stemflow collar was chosen over the polyurethane foam design (Likens and Eaton 1970) out of concern that unreacted chemicals in the polyurethane foam may impact the beech scales and affect their colonization patterns.

4.2 Colony Counts

In early June, colonies between 1 and 1.3 m from the ground were counted on each aspect (north, south) of all trees at the Morgan Arboretum. On control trees at this

site, colonies were also counted between 2.5 and 2.8 m from the ground. The count was performed by wrapping a 30 cm wide grid made of plastic mesh around the tree at the height of interest. The size of the grid squares formed by the plastic mesh was 1.5 cm. In early September, the final colony count, which only includes the non-destructive sampling groups, was conducted at the Morgan Arboretum. This count was performed in the same manner as the first count. Comparing the colony counts by location between the start and end of the field season identified the amount and approximate location of new colonies. This allowed us to measure new colony formation over the season by location, on both stemflow collar and control groups. No colony counts were performed at the Molson Reserve as the population of the beech scale on trees at this location was insufficient.

Afterwards, the average number of live insects per colony was recorded for each aspect of every tree within the final colony count, by a random sampling of three colonies. Previous studies have directly or indirectly used the number of visible scale colonies as an indicator of the amount of beech scales present (Wargo 1988, Houston et al. 1979). This count was performed as a test of whether the colony counts at the Morgan Arboretum are a reasonable indicator for actual beech scale populations. The colonies were selected randomly on the counting grid, on trees not exposed to destructive sampling. All colony counts had been completed at this point, so the non-destructive sampling groups no longer needed to be protected from destructive measurement.
The nearest colony to a randomly determined location was chosen. Stemflow collar equipped trees were sampled only between 1 and 1.3 meters, whereas control trees were sampled both at 1 to 1.3 meters and between 2.5 and 2.8 meters. Sampling was done by removing the colony along with the small piece of the bark it was directly attached to, storing it in an air-tight tube, and searching for the live insects with a pin under a stereoscopic microscope the next day. Only live insects were counted. Dead insects within the colonies were easily identified, as they appeared darker, misshapen and desiccated.

4.3 Bark Microhabitat

4.31 Sampling

Measurements were recorded on a bi-weekly basis at each site between June and September 2007. Measurements were therefore taken every week, alternating between sites. Depending which group a tree was part of, it was subject to different measurements. Temperature and moisture were recorded at both aspects on all trees at 1 to 1.3 m aboveground and on all control trees at 2.5 to 2.8 meters aboveground. As previously discussed, only a sub-group of trees (control and stemflow collar equipped trees) at both the Morgan Arboretum and the Molson Reserve were used for a complete bark characterization including penetrometer measurements. Additionally, we quantified the bark roughness of all trees at the Morgan Arboretum (See Appendix I).

Sampling began at approximately 10 :00 each week, for logistical reasons. Temperature was taken at two arbitrary points on each aspect and at both heights on the trees to create an average for each location. An infrared thermometer (Fisher Scientific High Temperature Infrared Thermometer, model 15-077-970) was used for expediency, and to avoid affecting the tree bark when sampling. Bark moisture was measured with a non-destructive wood moisture meter (Exotek MC-300WS) by placing it on two arbitrary (but reasonably flat) locations on each aspect and height of interest to create average moisture reading for each area. This meter determines wood moisture by measuring the dielectric constant of the wood using an oscillating electric field, and so does not puncture the bark as many wood moisture meters do. This was important, because it allowed me to measure moisture in both the destructive and non-destructive sampling groups. Next, bark resistance to penetration was measured at both aspects and heights at two arbitrary points using a penetrometer.

4.32 The Bark Penetrometer

The penetrometer was constructed by using a wooden rod inserted into a steel pipe approximately half its length (see Appendix II). The wooden rod has a fine carbide steel drill bit affixed to its end. The wooden rod has a metal bar driven through the other end. A spring is attached to each end of this bar, as well as to the steel tube. Applying pressure against the drill bit while holding the steel tube causes the wooden rod to be

pushed through the tube, extending the springs. A vernier caliper accurately measures this extension. To measure bark resistance, the penetrometer is rested horizontally on the palm of one hand such that the drill bit just touches the bark. While holding the steel tube, the device is slowly pressed until the drill bit is inserted all the way (2 mm) into the bark, then the distance the caliper has extended is recorded. For additional details, see Appendix II: The Bark Penetrometer.

4.33 Bark Roughness

Bark roughness was measured at the Morgan Arboretum between 1 and 1.3 meters on stemflow collar trees, and at both heights and aspects on control trees. These measurements were taken throughout the season because we did not expect many new bark fissures to be created over the summer. These measurements were taken by attaching the colony counting grid at the height of interest, and measuring the width of every visible bark fissure along a randomly selected horizontal grid line. These fissures ranged from about half a millimeter in width on healthy bark, to several centimeters wide on severely scarred bark. The sum of these measurements was recorded, as well as the DBH of the tree in question. Using this data, the roughness of the bark at each location of interest on each tree was expressed as a percentage of a tree's circumference. This method is similar in concept to that used by Glitzenstein and Harcombe (1979) to quantify bark texture in southern red oak *(Quercus falcata*).

5. Data Analyses

Many of the data collected in this experiment involves the repeated measurement of subjects (trees) over time. As a result, a repeated measures ANOVA was selected as the main analytical tool.

When ordinary ANOVAs were required, PROC GLM was used for the analysis. PROC UNIVARIATE was used for all tests of normality before conducting analyses with PROC GLM. PROC MIXED was used to test for homoscedasticity or sphericity. Given the large variance expected due to the small sample sizes within some groups (N=10 for some scale count data), we used a threshold for significance of p=0.1 rather than p=0.05

for most analyses. A previous study on the insect resistance of ponderosa pine (*Pinus ponderosa* var. *scopulorum* Engelm.) used this approach to resolve issues due to few replicated plots (Kolb et al. 1998). For normality, homoscedasticity, and sphericity tests, we used p=0.05 (See Appendix IV).

5.1 Spatial Patterns in Scale Colonization

5.11 Effect of Location on Scale Populations

Scale population counts were conducted at the start of the experiment on all trees at the Morgan Arboretum, and on all control trees at the Arboretum at the end of the experiment. Repeated measures analysis was used to determine the effects of aspect and height on scale counts. Additionally, the effect of time was included in the model to test whether scale populations had changed significantly over the season. The model was:

Count=time aspect height aspect*height aspect*time height*time

Where:

Count is the number of scale colonies observed.

Time is the time of measurement: either the start or end of the experiment.

Aspect is either the north or the south side of trees.

Height is either low (1 to 1.3 meters) or high (2.5 to 2.8 meters).

5.12 Effect of Stemflow Collars on Scale Populations

The effect of treatment on scale populations was judged in two ways. The first method was to determine the difference between scale populations on the north and south aspects of control trees at the end of the season, and compare that to the north-south difference of trees with stemflow collars. This was done using an ANOVA, with data for the lower parts of control and stemflow collar trees at the Morgan Arboretum, using the model:

deltaNS=treatment

Where:

DeltaNS is the difference in scale populations between the north and south aspects.

Treatment is either control or stemflow collar.

The second method used to test for treatment effects was to determine the scale population change on each location on each tree, by subtracting the initial count values from the final values. An ANOVA was performed on this data set to determine if the north sides of stemflow collar trees experienced a different seasonal population growth than the north sides of control trees. The model was:

delta=treatment

Where:

Delta is the population growth over the season.

Treatment is either control or stemflow collar.

5.2 Bark Microhabitat

5.21 Location Effects

Temperature, moisture, and bark resistance data were collected every week, alternating weekly between two sites, for four months. These data were collected from four different areas on each control tree: north aspect high, north aspect low, south aspect high, and south aspect low. The model was:

Property= location date location*date

Where:

Property is the bark property of interest: The model was run separately for temperature, moisture, and bark resistance to puncture.

Location is the area of bark measured: North high, north low, south high or south low.

Date is the day of measurement: There are twelve dates in this group.

An equipment failure in the field occurred on July 19th. The tip of the penetrometer broke and required replacement. The new tip may have been sharper, so an ANOVA was performed comparing the mean penetrometer readings before and including July 19th to later readings. The model was:

Resistance= Date

Where:
Resistance is the measured bark resistance

Date is either up to and including July 19th, or after July 19th.

5.22 Stemflow Collar Effects

Tests for stemflow collar effects on bark microhabitat were also done using repeated measures analysis. The repeated measures analysis was identical in principle to the one performed to determine the effect of location. The data used was limited to the north-low location on stemflow collar and control trees, because the treatment involved a redirection of stemflow away from this location. Our models were:

Property= treatment date treatment*date

Where:

Property is the bark property of interest: This model was run separately for temperature, moisture, and bark resistance.

Treatment: this is either stemflow collar or control.

Date is the day of measurement: There are twelve dates in this group.

5.23 Site Differences

The differences between the two experimental sites were determined using repeated measure analysis. However, since on any particular week only one site was sampled, we divided the twelve sampling weeks into 6 two-week periods. Within each two-week period, each site was sampled once, allowing us to determine habitat differences between the sites throughout the field season. Only control groups were used for this analysis. Our models were:

Property = location date site location*date site*date location*site*date

Where:

Property is the bark property of interest: This model was run separately for temperature, moisture, and bark resistance.

Date is the time of measurement, there are six two-week periods in this group.

Site is either the Morgan Arboretum or the Molson Reserve.

5.24 Bark Roughness

The effect of DBH on bark roughness was determined performing a linear regression with PROC GLM in SAS. Our model was:

DBH = roughness

Where:

DBH is the Diameter at breast height (1.3m).

Roughness is the percent linear bark roughness (See Appendix I: Determining Percent Bark Roughness).

The effect of location on bark roughness was determined using an ANOVA using PROC GLM in SAS. Our model was:

Location = roughness

Where:

Location is the area of bark measured: North high, north low, south high or south low.

Roughness is the percent linear bark roughness.

The effect of bark roughness and tree DBH on scale colony density were determined by performing a linear regression with PROC GLM in SAS. Our models were:

Scalecount = property

Where:

Scalecount is the number of scale colonies on a particular location on a particular tree.

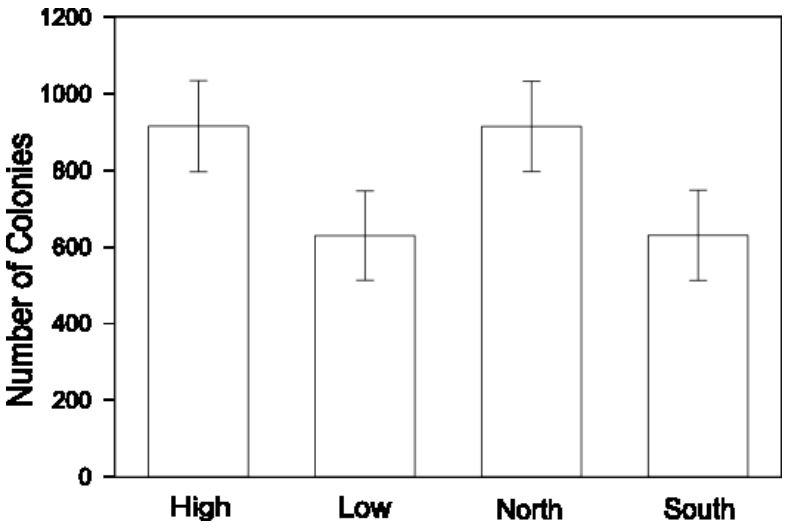
Property is DBH or percent bark roughness (the analysis is run once for each)

6. Results

6.1 Spatial Distribution of Scale Colonies

The number of scale colonies was significantly higher on the north aspects and higher up the trees (Figure 1). Scale colony numbers over the 30 cm high sampling grid ranged from 650 colonies on the lower position of the south aspects to more than 1000 colonies on the higher position of the north aspects.

Figure 1 The number of beech scale colonies at different locations on sampled beech trees in the Morgan Arboretum. Error bars show the standard error, N=10.



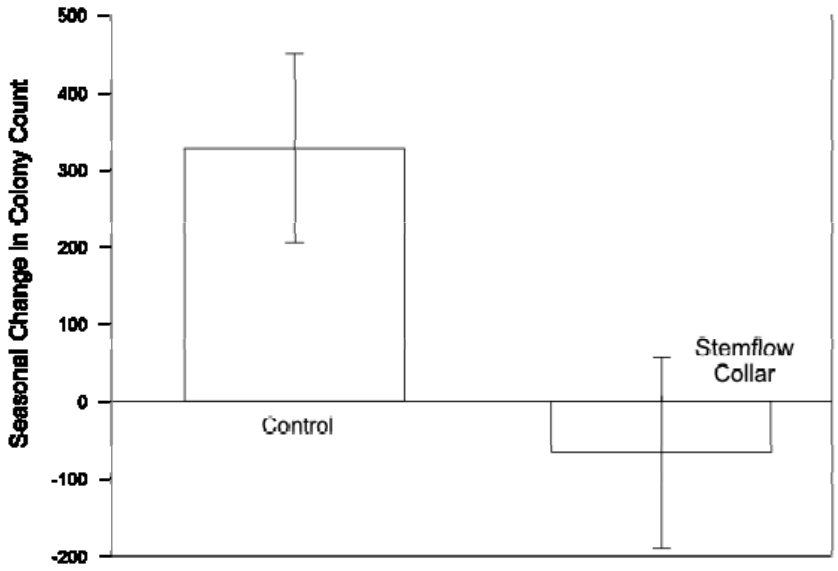
There were no significant differences in the number of live scales per colony by aspect, height, or treatment (Table 2). Furthermore, there were no significant correlations between the number of live scales per colony, and bark roughness and the number of scale colonies as determined at the end of the field season (September 2007). Over 90% of sampled colonies contained at least one live scale, with a mean of 2.4 scales per colony and a standard deviation of 1.3.

6.2 Temporal Change in Scale Colony Numbers

The number of beech scale colonies increased significantly over the sampling period, from an average of 522 colonies per location on each sampled tree, to an average of 1023 colonies. The effect of stemflow collars on scale colony distributions was significant. The difference in the number of colonies between the two aspects was significantly different between stemflow collar trees and control trees (Figure 2), and the stemflow collars significantly reduced new colony formation on the lower northern locations of sampled trees (Figure 3).

Figure 2 The difference between the north and south colony counts for trees with a stemflow collar and control trees at the end of the field season (September 2007. Error bars show standard error, N=10)

Figure 3 Change in the average number of colonies on the north sides of stemflow collar trees and control trees from June 2007 to September 2007. Error bars show standard error, N=10.



6.3 Effect of Location and Date of Sampling on Bark Characteristics

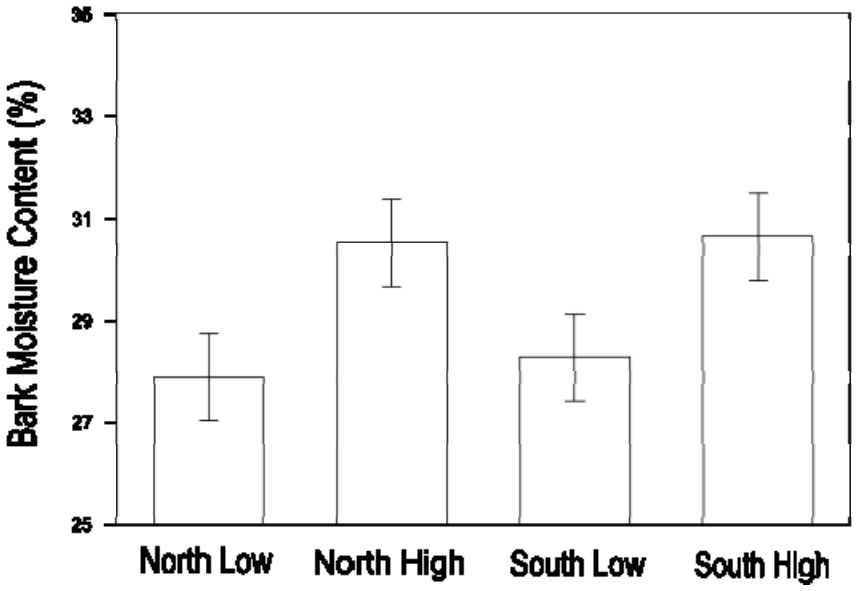
All measured bark characteristics were observed to vary significantly among locations and time. The interaction between bark characteristics and time did not vary significantly (Table 2). The date of measurement explained most of the measured variation in each of the bark characteristics, with the difference being maximum for bark temperature (Table 2). All of our measured bark characteristics varied significantly with either aspect or height, but never both.

Table 2 ANOVA of the effect of location and date of sampling on bark moisture, resistance to penetration and temperature.

Variable/Factor	DF	F value	Prob > F
Moisture			
Location	3	2.91	0.04
Date	4	6.94	<0.0001
Location X Date	12	0.56	0.87
Resistance to penetration			
Location	3	3.27	0.02
Date	10	4.91	<0.0001
Location X Date	33	1.12	0.30
Temperature			
Location	3	2.75	0.04
Date	10	223	<0.0001
Location X Date	33	0.28	1

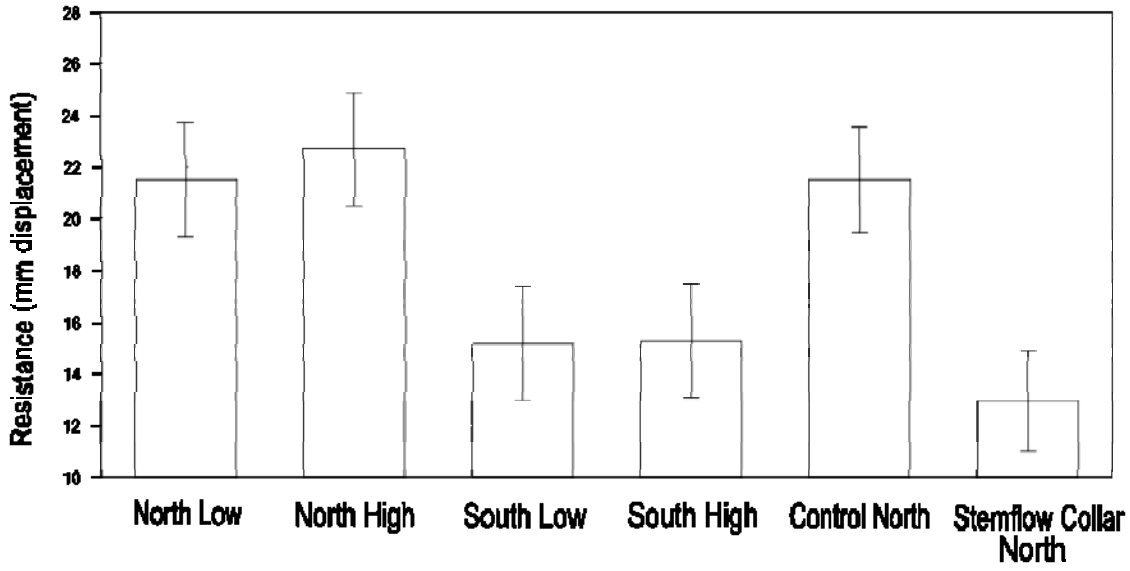
Bark moisture was found to be significantly higher at the highest location on the tree (Figure 4) ranging from about 28% on the north/low position to almost 31% on south/high position. This relationship remained true on both the north and south aspects of trees. There was no significant effect of aspect or of stemflow collars ($p = 0.10$) on bark moisture levels.

Figure 4 The average moisture content of bark at different locations on beech trees. Error bars show standard error, N=60.



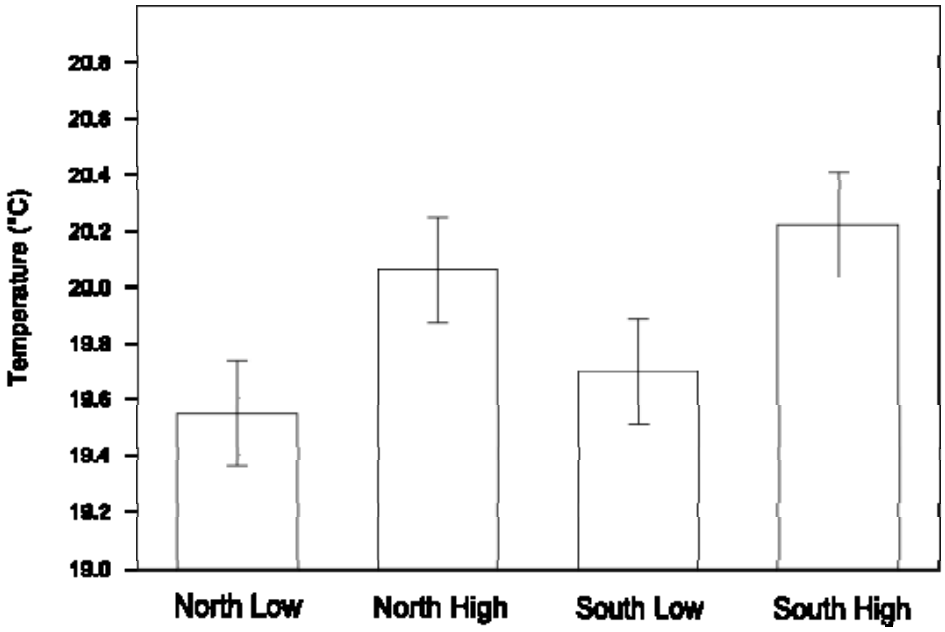
Bark resistance to penetration was significantly affected by aspect: bark was approximately 50% more resistant on the north side of trees (Figure 5). This remained true at both measured heights, and height did not significantly affect bark resistance. Bark resistance responded significantly to stemflow diversion. By comparing the lower north sections of control and stemflow collar trees, it was found that the stemflow redirection collars significantly reduced bark resistance over the field season (Figure 5).

Figure 5 The average resistance of bark to puncture at different tree locations, as well as on the lower north sides of treatment and control trees. Error bars show standard error, N=15 for Stemflow Collar North, N=30 otherwise.



Bark temperature was found to be significantly higher at the highest location on the tree with an average difference of 0.5 °C between the two measured heights (Figure 6). There was no significant effect of aspect and of stemflow collars (p= 0.70) on bark temperature. Bark temperature averaged ca. 20 ^{o}C, which was less than the average air temperature.

Figure 6 The average daytime bark temperature at different locations on the tree bole. Error bars show standard error, N=60.



Bark roughness was not found to vary significantly by aspect ($p=0.18$) or height ($p=0.64$). Furthermore, bark roughness and DBH did not exhibit any clear linear relationship, despite an apparent peak in bark roughness between 35 and 40 cm DBH (Figure 7).

6.4 Effect of Bark Characteristics on Overall Colony Density

The distribution of the data (Figures 7 and 8) suggest that the trees with the highest colony densities appear to fall within certain ranges of DBH, temperature, roughness and moisture. However, no linear relationship was found between DBH, bark moisture, temperature and roughness, and colony density.

Figure 7 Scatter plot of DBH and bark roughness, showing the high variance in the data. N= 120 locations across 40 trees.

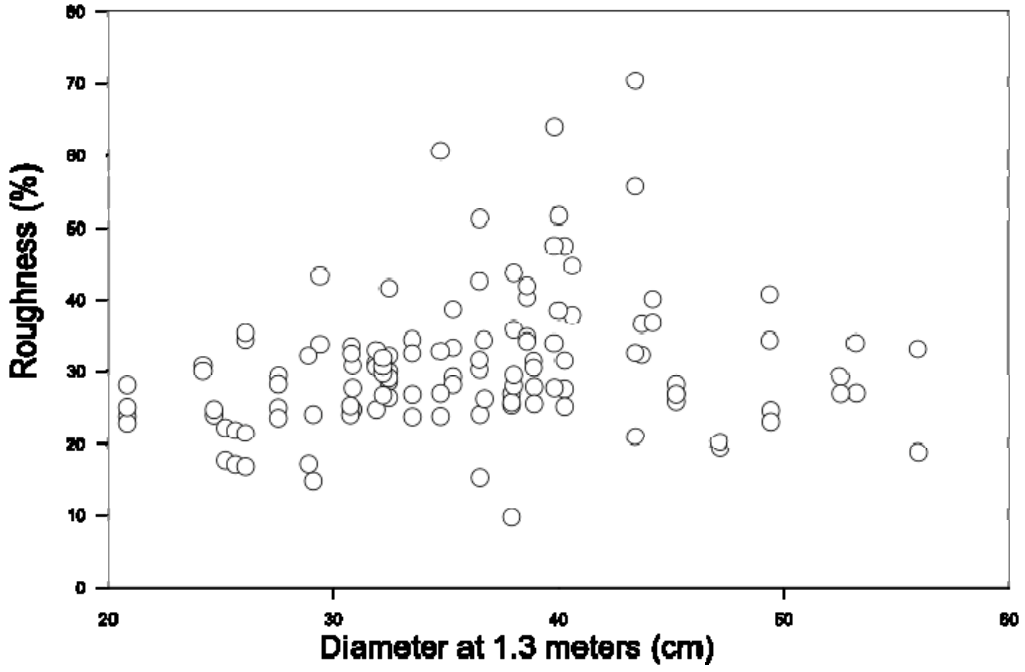
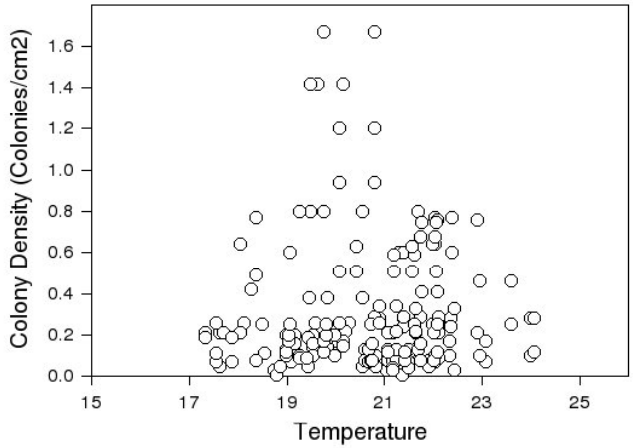
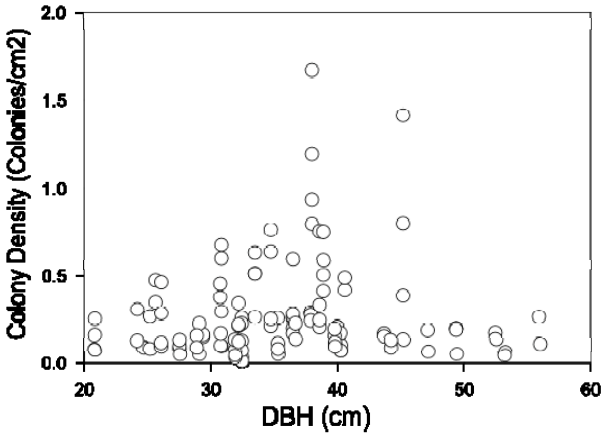
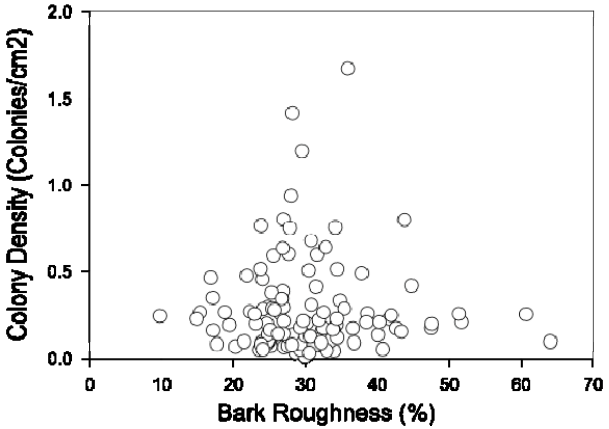
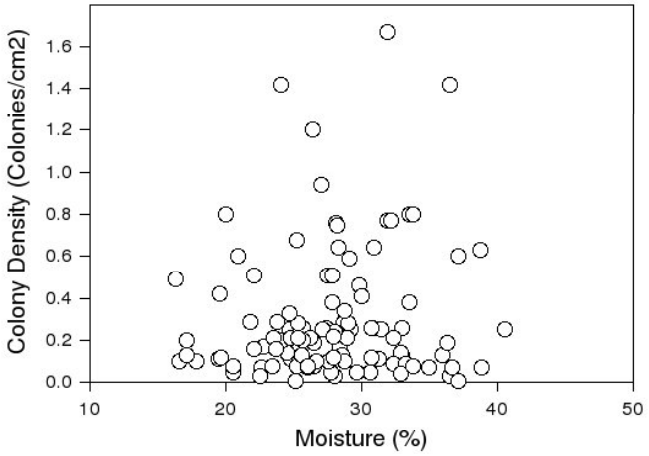


Figure 8 Scatter plots of colony density against bark characteristics and DBH, showing the high variance in the data. N= 120 for all plots.









6.5 Site Effects

On average, the trees at the Morgan Arboretum had significantly warmer bark that was more resistant to penetration, but had a lower moisture content than the trees at the Molson Reserve (Table 3). The Molson Reserve has far fewer beech scale colonies than the Morgan Arboretum, to the point that it was not considered relevant to perform beech scale colony counts at the Molson Reserve.

Table 3 Average bark temperature, resistance to penetration and moisture content of trees at the Morgan Arboretum and Molson Reserve.

Bark characteristic	Morgan Arboretum	Molson Reserve
Temperature (^{o}C)	21.2 $\pm$ 0.1	18.6 $\pm$ 0.1
Resistance (mm)	22.7 $\pm$ 1.5	14.7 $\pm$ 1.4
Moisture (%)	26.9 $\pm$ 0.5	30.4 $\pm$ 0.7

7. Discussion

7.1 Spatial Patterns in Bark Properties and Colony Counts

7.11 General Patterns

My results support our hypothesis that scales are more common on the north sides of trees. This is in agreement with the current consensus in the literature (Houston 1982, Witter et al. 2005, Wiggins et al. 2004). Conversely, the vertical distribution of the woolly beech scale has not received as much attention as the effect of aspect. Only one study mentioned that beech scales may migrate up the beech bole to form higher densities at a height somewhere above 1.5 meters (Houston 1982). The pattern we observed is consistent with a scale count performed by Wainhouse (1980) on a single tree. Vertical patterns in the distribution of another beech scale species, the New Zealand sooty beech scale insect (*Ultracoelostoma assimile* Maskell) are also known to occur. The bark at

higher locations on the Southern Beech (*Nothofagus* spp.) supports higher densities of this insect. Bark thickness was the primary hypothesis invoked to explain this pattern (Wardhaugh et al. 2006). In our study, we hypothesized that microhabitat temperature, moisture content, bark roughness and bark resistance to penetration would play roles in defining the observed patterns in the within-tree distribution of the woolly beech scale. We observed differences by height or aspect in each of these properties except bark roughness, and they form the basis of our explanation for the observed patterns in beech scale colony distribution.

7.12 Temperature

We found that temperature was significantly higher further up the tree, with an average difference of 0.5 °C between our two measured heights (Figure 6). We expected temperatures to be significantly higher on the south sides of trees as well, due to increased exposure to sunlight, however no significant difference was observed. Extensive shade provided by the canopy is a likely explanation. In a less densely populated forest or field, it is possible that aspect would have a significant effect on temperature. It is also possible that on rainy days, stemflow made the temperature more uniform over the bark surface. Since temperature measurements were taken at mid-day (+/- 2 h around noon), they are not necessarily representative of the average daily temperatures of the stem *per se*. Under a closed canopy where direct solar radiation is primarily from sunflecks, air flow/circulation during the day likely favored air mixing and decreased the vertical and aspect gradient of temperature. If all measurements were taken at night, we may have observed that the vertical temperature gradient was much stronger. Locations further up the bole of the tree would be much warmer due to absorption of radiation given off by the canopy. This effect has been demonstrated experimentally by comparing the temperature gradients along the boles of trees in clear-cut, 50% cut, and normal forests (Heilman and Zasada 2000).

Since measurements were taken in the morning and early afternoon, it is possible that any vertical temperature gradient that existed the previous night would persist enough for us to observe it during the day. The vertical temperature gradient has three possible causes. It could be attributed to the residual effect of a nocturnal temperature

gradient caused by the absorption of radiation from the canopy, increased exposure to sunlight from incomplete canopy coverage, or a cooling effect from the ground. However, a previous study in a beech forest (Christy 1952) showed that while the mediating effect of the ground can be strong underneath the leaf litter, air temperatures at or near the litter surface are approximately the same as they are higher up the tree during the summer season. Having found that air temperature during the summer is uniform between the canopy and litter surface during the day and night, they concluded that the canopy effectively controls the climate beneath it. This suggests that both of our heights of measurement were too far from the ground for any significant mediating effect of the ground on bark temperature to take place. Higher temperatures higher up the bole are therefore likely linked to their proximity to the canopy, which radiates heat towards the ground at night. Alternatively, the same gradient that exists at night could be maintained by solar radiation passing through incomplete canopy cover during the day. Areas further up the bole of the tree would be more exposed to solar radiation as they would be less shaded by understory vegetation.

These higher temperatures during the day are probably not high enough to present a risk of desiccation for the beech scale. The woolly beech scale shares many adaptations with other scale insects that represent strategies used to avoid desiccation (Gullan and Kosztarab 1997), including waxy secretions and the use of eggs. We may note that the "crawler" stage of the beech scale lifecycle is rather short, possibly indicating that at this stage the insect is at particular risk of desiccation if it does not begin feeding. However, since most crawlers do not migrate far from their parent colony, their exposure to desiccation is limited. The fact that beech scale colonies were more frequent at our higher measurement points indicates that either the higher daily temperatures are a factor which promotes scale infestation, or that these patterns exist despite, rather than as a result of, patterns in daytime bark temperature.

As cold-blooded organisms, the development of aphids (as with other insects) is heavily influenced by the temperature of their surroundings (Campbell et al. 1974). As a result, there is a possible positive effect of higher temperatures. Given that the beech scale can counteract the minor desiccating effect of these higher temperatures through feeding as some aphids do (Spiller et al. 1990), scales present at our higher locations

would enjoy a small boost in their developmental rates. However, since the average magnitude of the effect during the day is not very large (0.5 °C), it is unlikely that this temperature difference can account for the full disparity between the populations at high and low locations in the present study. However, if this temperature difference is maintained or even amplified during the night, it is possible that it significantly increases the rate of scale development on areas higher up the tree bole. The temperature gradient present on beech bark may be one of several factors that make the higher locations on the tree bole more habitable to the woolly beech scale.

On the other hand, if we consider the effect of temperature on the scale colony density between trees (Figure 8), we can see that the trees with the highest scale colony density appear to have mean temperatures of approximately 20 °C. Since these temperature readings are used as an indicator for exposure, trees that departed significantly from the mean may have been exposed to greater extremes of temperature. This suggests that beech scale colony density can be impacted by exposure to at least some degree. However, it is also likely that the trees with the highest scale densities are more likely have this approximate temperature simply because the number of samples is greater around this point. As a result it seems inappropriate to make any specific conclusion given the data collected in the present study.

Finally, it is possible that high populations of the beech scale alter the temperature variation of the bark microhabitat. As beech bark disease progresses, the crown of the infected beech tree may become more transparent because of a decrease in foliage density. It has been demonstrated that as the canopy is opened up, the temperature gradient that increases with height along the bole of a tree becomes much steeper during the day (Heilman and Zasada 2000). At night, the canopy cover radiates heat towards the ground, producing higher temperatures higher up the tree bole. In this way, the canopy acts as a buffer, increasing temperatures during the night and maintaining a more even temperature gradient during the day. Removing this buffer results in colder nocturnal temperatures as well as more variable temperatures during the day (Heilman and Zasada 2000). This could negatively impact beech scale populations, as temperature fluctuations and direct solar radiation are detrimental to the wooly beech scale (Witter et al. 2005).

7.13 Roughness and DBH

It has been clearly demonstrated that the severity of beech bark disease tends to be higher on trees of larger diameter both qualitatively (Griffin et al. 2003) and quantitatively (Fernandez and Boyer 1988). Leaven and Evans (2004) reasoned that this may be due to increased bark roughness among larger beech trees. Bark roughness was found to favor scale colony formation on the European beech by facilitating the colonization of new trees (Houston et al. 1979, Houston 1982). In the present study, we found no convincing linear relationship between scale colony density and bark roughness or tree DBH. This may be because large trees with very high scale populations have died off at our study sites, removing them from the population. On the other hand, bark roughness reached its highest values on trees of 35-40cm DBH (Figure 7), and scale densities also had their peak values within this DBH range (Figure 8). The coincidence of these two maxima suggests that rough bark can ameliorate the habitat of the woolly beech scale. If this was the case, we would expect there to be a clear relationship between bark roughness and scale colony density. However, in Figure 8 we can also see that the beech scale exhibits the highest populations on moderately rough bark, with no convincing linear trend. It is possible that bark roughness improves the habitat of the wooly beech scale, but that extremely high bark roughness indicates that a tree is in poor health and is a less suitable habitat for the woolly beech scale.

7.14 Resistance to Puncture

In the present study, we measured the resistance of the bark to puncture at various locations in order to assess the resistance of the bark to insertion of the scale stylet. We found that the north aspects of beech trees were significantly more resistant to puncture than the south aspects, at both sampled heights. No significant effect of height was detected on bark resistance (Figure 5). Despite the increased resistance of the bark on the north sides of the American beech, greater amounts of scale colonies form on this aspect. Based on the evidence that increased bark lignification in the European beech restricts scale populations, we would expect the opposite to be true. To resolve this apparent contradiction, we need to consider the defensive response of the American beech to fungal agents.

The American beech forms cankers and areas of resistant bark in response to pathogens such as the *Neonectria* fungi. On trees afflicted by beech bark disease, these cankers can become so severe that they girdle the tree, or weaken the bole structurally to the point where it will snap under heavy winds (Papaik et al. 2005). During the course of the present study, we observed that areas of cankered bark were very resistant to puncture. Since infestation by the beech scale promotes infection by the canker-causing *Neonectria* fungi, the increased bark resistance of the north sides of beech trees probably represents an effect of the higher local severity of beech bark disease. This would also help explain the differences in bark resistance between our two sites (Table 1). The Morgan Arboretum has more resistant bark, and also much higher scale populations than the Molson Reserve. However, to conclusively demonstrate any effect it would be necessary to compare the bark resistance of beech trees within forests infested with the beech scale to forests that have not yet been infested, which is beyond the scope of the present study.

Since scale populations remain higher on the north side of trees (Figure 1), the increased bark resistance does not appear to inhibit the beech scale. This may be because colonies become well established before the cankers develop, because the negative effects of the harder bark are negated by better habitat provided by the cankered bark, or simply because the beech scale can easily feed on the bark tissues despite the increase in resistance. Witter et al. (2005) suggested that during the early stages of infestation, the north aspects of beech trees provide a superior microhabitat, but the increased activity of *Neonectria* on that aspect due to the higher local densities of the beech scale kills the bark. This forces the beech scale to colonize other locations on the bole. This is consistent with our observation that the north sides of trees are more resistant to puncture; the higher densities of the beech scale could cause more severe cankering due to *Neonectria*, which in turn would increase the resistance of the local bark to puncture.

7.15 Moisture

The dry, hard and impermeable bark of beech trees leave the scale insect exposed and present a risk of desiccation to the scale insect in some stages of its lifecycle. Generally, adult scale insects produce secretions as an adaptation that protects them from

desiccation (Gullan and Kosztarab 1997). In some species, adult insects may also rehydrate themselves during dry periods by switching feeding from the phloem to the xylem, which has a higher water content (Spiller et al. 1989). This specific behavior is probably absent in the woolly beech scale, because it feeds on intracellular nutrients present in the bark parenchymal tissue (Leaven and Evans 2004), its short stylet cannot reach deeper bark tissues. Nonetheless it is likely that dietary moisture is the sole moisture intake of adult insects.

As a consequence of the importance of dietary moisture to the response to desiccation in aphids, we reasoned that patterns in bark moisture content may play a role in the distribution of the woolly beech scale. Our results indicate that the bark is significantly moister 2.5 meters up the tree as compared to the region 1 to 1.3 meters from the ground (Figure 4). We suspect that the reason for this difference is that the bark further up the tree is thinner (Wardhaugh et al. 2006), and that active phloem and xylem tissues are closer to the bark surface, resulting in a higher volume of available moisture. Beech stemflow contains small concentrations of nitrogen and organic matter as well (Eaton et al. 1973), and may be a minor source of nutrients. The non-destructive moisture meter uses oscillating electric fields to measure capacitance (Water has a high dielectric constant, and so this is a good measure of moisture content) to a depth of approximately five millimeters, a depth that is relatively good match to the two mm stylet of the beech scale. Higher moisture availability is therefore a likely contributing factor to the increased beech scale colony counts higher up the tree. Such relationships cannot however be established for the differences in colony counts between the north and south side of trees.

Houston et al. (1979) remarked that beech scales planted on new trees exhibited a tendency to disperse upwards, and that natural populations of beech scale seemed to be most concentrated at a height somewhere above 1.5 meters. This suggests that the beech scale actively seeks out a particular height on a host tree, although not so strongly that it precludes infestation of other areas. This may be the result of increased nutritional content of moister bark further up the tree, combined with higher and more stable temperatures conducive to scale development at higher bole locations. Scales lower down the tree bole persist, but may produce fewer offspring due to the poorer microhabitat.

This alone may account for the lower colony densities on the areas of the tree bole below 1.5 meters, since nymphs typically do not migrate far from where the hatch. However, the beech scale may also exhibit active behavior that favors colony formation at higher sites, such as positive phototaxis during the more mobile nymph stage (Houston et al. 1979). This would allow the beech scale to take better advantage of the superior microhabitat further up the bole. Additionally, since the beech scale is dispersed by wind (Houston et al. 1979, Wainhouse 1980), colonizing areas further up the bole would allow for further dispersal (Wainhouse 1980). New colony formation on the lower areas of the tree bole are a consequence of nymphs not migrating over long distances, whether they hatched locally or are new recruits blown off another tree. Additionally, nymphs hatched higher up a tree bole may be transported downwards by stemflow, and may settle at lower locations as a result.

It was also noted that the trees with the largest densities of beech scale colonies showed a tendency to have bark moistures around 30% (Figure 8). It is possible that this represents an ideal level of moisture for the woolly beech scale, however the data collected in the present study is not sufficient to demonstrate this. The apparent peak may simply be due to an increased sample size near this value, increasing the chance that a given tree of high scale density occurs near this point.

7.16 Time

The effect of date of measurement was significant for all measured bark properties. This demonstrates that the particular conditions on any given day contribute to explaining the variation in bark characteristics on each tree.

In terms of bark temperature and moisture, this is best explained by differences in weather between sampling days. Sampling took place systematically and without regards to weather, and included days of heavy rain and cloud cover in addition to clear weather. However, since the effect of the interaction between location and sampling date is not significant, we can conclude that the observed differences in bark temperature and moisture existed independent of variations in overall temperature, incident moisture, and illumination due to sampling date.

Bark resistance was similarly affected by sampling date. After the tip of the bark

penetrometer required replacement in July, there was a significant decrease in mean bark resistance. Since the measurements were used to determine the differences between the bark resistance at different locations on each tree, and not an absolute value, this was not judged to impact the final results beyond obfuscating any seasonal patterns in bark resistance.

7.2 Stemflow Collar Effects

7.21 Overview

In the present study we have suggested that bark thickness and moisture content are factors that influence the within-tree distribution of the woolly beech scale. However, we have also raised the possibility that desiccation, especially during the non-feeding stages of the beech scale's lifecycle, is an important limiting influence on its distribution. To provide experimental evidence of the extent of this influence, we designed stemflow collars that reduce the amount of stemflow moisture to the north sides of beech trees, thereby exposing the non-feeding stages of the scale insect to drier conditions

7.22 Scale Colony Count at the End of the Season

At the end of the experimental season, we observed a higher number of beech scale colonies on the north side of control trees. On the north side of trees with stemflow collars, there were fewer colonies. Furthermore, when comparing the magnitude of the north-south population differences, the effect of aspect on the number of scale colonies was significantly greater on control trees compared to stemflow collar trees (Figure 2). This suggests that the experimental treatment changed the population distribution of the beech scale. The north aspect of trees with stemflow collars, exposed to less stemflow moisture, was unsuitable for the recruitment of new colonies in the area. The south aspects were less affected by the experimental treatment (the collar had holes on the south side to allow some stemflow to pass) and so were able to recruit more colonies, allowing the south aspects to become as populous as the north aspects. This suggests that desiccation is an important source of mortality to the early stages of beech scale development, and that stemflow moisture is an important factor in determining exposure to desiccation at these stages. However, there is an important limitation in comparing the

north and south colony counts on stemflow collar trees at the end of the experimental season; in our experimental design, the treatment was considered to be applied to the north sides of trees only. On the south side of the stemflow collars, there were holes that allowed stemflow to escape, preventing stemflow from overflowing out of the collar. The majority of this stemflow fell directly to the litter, however some came into contact with the bark on the south aspects of the trees. As a result, the exposure to stemflow moisture on the south sides of stemflow collar trees was more variable than on the north sides, which remained very dry. As a result, we cannot assume that the treatment was applied equally on both aspects of the stemflow collar trees, and neither does the south side of these trees represent a proper control group. In order to confirm the effect of the stemflow collars, we have considered the seasonal changes in beech scale populations on only the north sides of stemflow collar and control trees.

7.23 Seasonal Patterns in Scale Colony Counts

The effect of the stemflow collar on beech scale counts was evaluated using two different approaches. Overall, we found that the number of colonies approximately doubled between the colony count in June and the one in September.

The north side of trees with stemflow collars had recruited significantly fewer new colonies than the control trees, with no significant increase in the number of colonies on stemflow collar trees over the sampling period (Figure 3). This suggests that the stemflow collars effectively prevented the formation of new colonies, by reducing the viability of the beech scale's eggs or preventing access to the area by mobile nymphs. Since most scale colonies were found to contain one or more living scale insects at the end of the season, this means the actual population of beech scale insects should be accurately reflected by these patterns in colony formation.

As we discussed earlier, the non-feeding stages of the beech scale insect may be susceptible to desiccation. Precipitation represents the only situation where the non-living bark surface is rehydrated, and stemflow is the method by which most of this moisture comes into contact with the bark. However, during windy weather, a small number of rain droplets were able to bypass the stemflow collars. Nonetheless, by reducing exposure to incident moisture, we seem to have been able to prevent the

formation of new colonies.

It is important to realize that the experimental treatment did not significantly affect the bark moisture content at the time of measurement. This is likely because most measurements were taken in dry conditions. Moreover, stemflow collars only affect the very surface of the bark, and our moisture meter is measuring the moisture content of living bark tissue. Since beech bark is fairly impermeable (Billings and Drew 1938), there is no reason to expect stemflow moisture on the surface of the bark (or lack thereof) to affect the normal operation of the bark parenchymal tissue.

An alternate explanation for the recruitment-inhibiting effect of the stemflow collars is that the beech scale is positively phototaxic (Houston et al. 1979), or otherwise tends to migrate upwards. If it exhibits a tendency to migrate upwards towards an area somewhere above 1.5 meters, new colonies formed on the lower parts of the tree may be a result of mobile nymphs being washed partway down the bole of the tree by stemflow. The stemflow collars restrict stemflow on the north aspect of trees, effectively causing migrating nymphs displaced by stemflow to washed off the tree instead of potentially washed towards a lower location on the tree. Where wind is the primary factor affecting dispersal between trees (Wainhouse 1980), a balance between the migratory tendencies of the scale nymphs and the effects of stemflow as a dispersing agent may determine dispersal within trees. It seems likely that the beech scale nymphs, which are less sheltered from precipitation than the sessile adults, would be quite susceptible to being washed downwards by stemflow. This is supported by an observation by Houston (1994) that heavy autumn rains are negatively correlated with beech scale populations the following year.

7.24 Scales Per Colony

In the present study, we found that over 90% of scale colonies sampled contained at least one living beech scale. Furthermore, there were no fewer scales per colony on trees with higher densities of scale colonies. This suggests that for the range of colony densities observed in this experiment, the number of colonies was a reasonable indicator for beech scale populations. Additionally, we found no significant differences in the number of scales per colony in terms of aspect or height. Contrary to our original

hypothesis, this suggests that scale insects, once established, are able to survive equally well during the summer season at various locations on beech trees. The number of scale colonies varies more than the success of individual colonies across different locations on a tree. This suggests that a factor that inhibits scale colony establishment, but not survival, is at work.

One such factor may be bark thickness. Bark thickness has been shown to have a negative impact on other scale species (Wardhaugh et al. 2006). Areas that may have thinner bark, such as the (uncankered) north sides of trees (Lyubomir 1968) or areas further up the bole could be easier for scale colonies to establish themselves on. Areas with thicker bark would be more difficult to find an appropriate site to form a colony on, making the mobile beech scale nymphs less likely to form a colony at that location. While fewer good colony locations exist on certain areas of the tree, a beech scale established at one of these locations is able to extract sufficient nutrition for survival. Previous studies have established that aphids are generally nitrogen limited, and that available bark nitrogen is evenly distributed over the surface of beech trees (Wargo 1988). As a result we fully expect any particular beech scale colony on a susceptible beech tree to perform adequately once established, over a wide range of locations over the surface of a tree. This would explain why, in the present study, we found that scale colonies did not contain significantly fewer scales in areas where there were significantly fewer colonies, or more scales in areas that contained more colonies.

Additionally, there were no significant differences in the number of beech scales per colony between our stemflow collar and control groups. This is consistent with our previous assertion that the stemflow collars prevented the recruitment of new scale colonies. As we would expect, mortality among adult insects was not significant due to a reduction in stemflow moisture, as they may replenish moisture through feeding. Eggs and nymphs cannot, and so on stemflow collar trees there may exist higher mortality rates. This is particularly interesting as it suggests that any method that controls exposure to desiccation may be a potential management strategy for slowing the spread of the beech scale insect.

7.25 Bark Properties

We monitored the seasonal bark properties of both control and stemflow collar trees in order to identify any effect stemflow moisture would have on the properties of the beech scale's microhabitat. The only significant difference was that bark on the north sides of trees with stemflow collars became significantly less resistant to puncture than the bark on the north sides of control trees. We believe this is due to the outer bark, consisting of lignified dead cells, becoming more brittle as a result of being exposed to less moisture. While this has had no apparent effect on the success of established beech scale colonies, it may have an effect on the spread of Neonectria fungi. Chapela and Boddy (1988) demonstrated that several fungal species present in beech bark begin to develop only once bark tissue dries out. While we did not find any significant effect of the stemflow collars on bark moisture content, it's nonetheless possible that the drier conditions at the very surface of the bark may promote colonization by Neonectria fungi.

8. Conclusion

In the present study, we have described the colonization patterns the woolly beech scale forms on individual beech trees. Beech scale colonies were found to exist at higher densities on the north sides of trees. Scale colonies were also more numerous at a height of 2.5-2.8 meters than at a height of 1.0-1.3 meters. These patterns in colony formation were found to coincide with patterns in microhabitat quality; the higher locations along the tree boles were warmer and moister. The north sides of trees were found to be more resistant to puncture, probably as a consequence of the higher local populations of the beech scale. Additionally, the negative effect of the stemflow collars on new colony formation suggests that stemflow can be an important distributing agent for beech scale nymphs, and potentially an important source of moisture as well. An important limitation in the present study is that we did not measure bark thickness, which turned out to be potentially useful in describing the differences in scale colony densities between the north and south aspects.

We believe that this difference in scale densities between the north and south aspects is due to a difference in bark anatomy. On European Beech (*Fagus sylvatica*), it

has been demonstrated that the bark is thicker on the sunward side (south aspect in the present study) of the tree (Lyubomir 1968). European beech with thicker, more lignified bark exhibit consistently lower populations of the beech scale (Houston 1994). Our observation that there are quantitatively fewer beech scales on the south aspects of trees suggests that determining whether American beech are similar to their European counterparts in these respects may be a fruitful avenue of research.

Whether resistance to beech bark will ever become as widespread in North America, as it has in Europe, remains to be seen. Unlike the American Beech, the European Beech has had a long history of beech bark disease, and resistance occurs on the level of bark anatomy (Houston 1994) as well as metabolism (Gora et al. 1994). In the American beech, only inheritable resistance based on differences in nitrogen metabolism has been confirmed (Wargo 1988), although it remains possible that resistant phenotypes based on bark anatomy exist in North America as they do in Europe. For example, Latty et al. (2003) suggested that bark N content is a good predictor for scale infestation severity, and that both increase with tree DBH. However, we found that tree DBH and bark roughness did not clearly explain scale population density (Figure 8). This suggests that bark N content may be necessary, but not sufficient, to explain the increased incidence of beech bark disease on larger trees. A combination of bark chemistry and physical characteristics may prove to be more adequate.

While bark nitrogen content remains the best overall explanation for the distribution of beech bark disease within a population of trees, its major shortcoming is a failure to explain the distribution of beech scale colonies within individual trees. The present study quantitatively describes the distribution of scale colonies on individual trees and provides evidence that the physical characteristics of bark microhabitats are the process by which these patterns arise.

9. References

Bennett, K. D. 1985. The spread of Fagus grandifolia across eastern north America during the last 18 000 years. *Journal of Biogeography* 12: 147-164

Billings, W. D. & Drew, W. B. 1938. Bark factors affecting the distribution of corticolous bryophytic communities. *American Midland Naturalist* 20:302-330

Bishop, D. B., McCullough, D.G. & Ellis, T. 1994. Scale insects of christmas trees. Michigan State University Extension Bulletin E-2477.

Boddy, L. & Rayner, A. D. M. 1983. Origins of decay in living deciduous trees: The role of moisture content and a re-appraisal of the expanded concept of tree decay. *New Phytologist* 94: 623–641

Campbell, A., Frazer, B. D., Gilbert, N., Gutierrez, A. P. & Mackauer, M. 1974. Temperature requirements of some aphids and their parasites. *The Journal of Applied Ecology* 11:431-438.

Castlebury, L. A., Rossman, A. Y. & Hyten, A. S.. 2006. Phylogenetic relationships of *Neonectria/Cylindrocarpon* on *Fagus* in north America. *Canadian Journal of Botany* 84:1417-1433.

Chapela, I. H. & Boddy, L. 1988. Fungal colonization of attached beech branches. II. Spatial and temporal organization of communities arising from latent invaders in bark and functional sapwood, under different moisture regimes. *New Phytologist* 110:47-57.

Chapela, I. H. 1989. Fungi in healthy stems and branches of American beech and aspen: A comparative study. *New Phytologist* 113:65-75

Chilima, C. Z. & Leather, S. R. 2001. Within-tree and seasonal distribution of the pine woolly aphid *Pineus boerneri* on *Pinus kesiya* trees. *Agricultural and Forest Entomology* 3:139-145

Christy, H. R. 1952. Vertical temperature gradients in a beech forest in central Ohio. *The Ohio Journal of Science* 52:199-209.

Cogbill, C. 2005. Historical biogeography of the American beech. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 16-24.

Dadd, R. H. & Krieger, D. L. 1968. Dietary amino acid requirements of the aphid, *Myzus persicae*. *Insect Phytology* 14:741-764

Drake, J. A., Mooney, H. A., Di Castri, F., Groves, R. H., Krueger, F. J., Rejmanke, M. & Williamson, M. 1989. Biological invasions: A global perspective. John Wiley and Sons: Chichester, UK.

Eaton, J. S., Likens, G. E. & Bormann, F. H. 1973. Throughfall and stemflow chemistry in a northern hardwood forest. *The Journal of Ecology* 76:495-508

Ehrlich J. 1934. The beech bark disease, a *Nectria* disease of *Fagus*, following *Cryptococcus fagi* (Baer.). *Canadian Journal of Research* 10:593-692

Evans, C. A., Ayres, M. P., Twery, M. J., Houston, D. R. & Liebhold, A. 2004. Using models to identify forests at risk of major structure and compositional change due to beech bark disease. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 116-120.

Fernandez, M. R. & Boyer, M. G. 1988. Beech bark disease- a survey of the Toronto area. *Canadian Plant Disease Survey* 68:157-159

Field, C. 1983. Allocating leaf nitrogen for maximisation of carbon gain: leaf age as a control on the allocation program. *Oecologia* 56:341-347

Fyles, J. 1997. Appendix 2.10: Site information and maps for Morgan arboretum. Pages 79-82 In: Trofymow, J.A., CIDET Working Group. 1998. The Canadian Intersite Decomposition Experiment (CIDET): Project and Site Establishment Report. Nat. Resour. Can., Can. For. Serv., Pac For. Cent., Victoria, B.C. Inf. Rep. BC-X-378.

Glitzenstein, J. & Harcombe, P. A. 1979. Site specific changes in the bark texture of *Quercus falcata* michx. (Southern red oak). *American Journal of Botany* 66: 668-672

Gora V., Starke, R., Ziehe, M., König, J., Müller-Starck, G., & Lunderstädt, J. 1994. Influence of genetic structures and silvicultural treatments in a beech stand (*Fagus sylvatica*) on the population dynamics of beech scale (*Cryptococcus fagisuga*). *Forest Genetics* 1:157-164

Griffin, J. M., Lovett, G. M., Arthur, M. A. & Weathers C.K. 2003. The distribution and severity of beech bark disease in the Catskill mountains, NY. *Canadian Journal of Forest Research* 33:1754–1760

Gullan, P. J. & Kosztarab, M. 1997. Adaptations in scale insects. *Annual Review of Entomology* 42: 23-50

Hane, E. N. 2003. Indirect effects of beech bark disease on sugar maple seedling survival. *Canadian Journal of Forest Research* 33:807–813

Hartnett, D. C. & Bazazz, F. A. 1984. Leaf demography and plant-insect interactions: Goldenrods and phloem-feeding aphids. *The American Naturalist* 124:137-142

Heilman, W. E. & Zasada, J. 2000. Modeling the atmospheric dynamics within and above vegetation layers. Seventh Symposium on Systems Analysis in Forest Resources. p. 261-268.

Houston, D. R. 1975. Beech bark disease--the aftermath forests are structured for a new outbreak. *Journal of Forestry* 73:660-663

Houston, D. R., Parker, E.J., & Lonsdale, D. 1979. Beech bark disease: Patterns of spread and development of the initiating agent *Cryptococcus Fagisuga*. *Canadian Journal of Forest Research* 9:336-344

Houston, D. R. 1982. A technique to artificially infest beech bark with the beech scale, *Cryptococcus fagisuga* (Lindinger). USDA For. Serv. Res. Pap. NE-507. 8 p.1-8

Houston, D. R. 1994. Major new tree disease epidemics: beech bark disease. *Annual Review of Phytopathology* 32:75-87

Houston, D. R. 2004. Beech bark disease: 1934 to 2004: what's new since Erlich? Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 79-87.

Ip, D. W. 1992. Dutch elm disease. Nat. Resour. Can., Can. For. Serv., North. For. Cent., Edmonton, Alberta For. Leaflet 19.

Jakubas, W. J., McLaughlin, C. R., Jensen, P. J. & McNulty S. A. 2004. Alternate year beechnut production and its influence on bear and marten populations. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 79-87.

Kimoto, T. & Duthie-Holt, M. 2006. Exotic forest insect guidebook 2006. Canadian Food Inspection Agency, Catalogue number A104-23/2006E.

Koch, J. L. & Carey, D. W. 2004. The genetics of resistance of American beech to beech bark disease: Knowledge through 2004. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 79-87.

Kolb, Thomas E., Holmberg, Kristina M., Wagner, Michael R. & Stone, Jospeh E. 1998. Regulation of ponderosa pine foliar physiology and insect resistance mechanisms by basal area treatments. *Tree Physiology* 18:375-381

Krabel, D. & Petercord, R. 1998. Genetic diversity and bark physiology of the European beech *(Fagus sylvatica*): a coevolutionary relationship with the beech scale (Cryptococcus fagisuga). *Tree Physiology* 20:485-491

Lajoie, P. & Baril, R. 1954. Soil survey of Montreal, Jesus, and Bizard islands. (Agriculture and Agri-Food Canada document No. 3M-17939-12-53). Ottawa, ON.

Lajoie, P. & Stobbe, P. 1950. Soil surveys of Soulanges and Vaudreuil counties in the province of Quebec. (Agriculture and Agri-Food Canada document No. 15C-13613-1-50). Ottawa, ON.

Latty, E. F., Canham, C. D., & Marks, P. L. 2003. Beech bark disease in northern hardwood forests: the importance of nitrogen dynamics and forest history for disease severity. *Canadian Journal of Forest Research* 33: 257-268

Leaven, K. V. & Evans, C. A. 2004. A preliminary examination of beech bark disease and the influence of soil moisture on bark thickness and disease status in the northern Adirondack uplands. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 79-87.

Likens, G. E. & Eaton, J. E. 1970. A polyurethane stemflow collar for trees and shrubs. *Ecology* 51: 938-939

Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R. D. & Schabenberber, O. 2006. SAS for Mixed Models. 2nd ed. SAS Press.

Loach, K. 1970. Shade tolerance in tree seedlings. II. Growth analysis of plants raised under artificial shade. *New Phytologist* 69:273-286

Lyubomir, D. 1968. Untersuchungen über einige eigenschaften der buchenrinde. *European Journal of Wood and Wood Products* 26:28-33

MacKenzie, M. and Iskra, A. J. 2005. The first report of beech bark disease in Ohio comes nineteen years after the first report of the initiating scale. *Plant Disease* 89:203

Mantiri, F. R., Samuels, G. J., Rahe, J. E. & Honda, B. M. 2001. Phylogenetic relationships in Neonectria species having Cylindrocarpon anamorphs inferred from mitochondrial ribosomal DNA sequences. *Canadian Journal of Botany* 79:334-340

Mayer, M. & Allen, D. C. 1983. Chilocorus stigma (Coleoptera: Coccinellidae) and other predators of beech scale in central New York. Proc. IUFRO beech bark dis. working party conf., pp. 38-42.

Millette G. 1948. Soil map of the Morgan Arboretum. W.H. Brittain memorial forest science laboratory. Dept. Nat. Res. Sci., McGill University, Québec.

Papaik, M. J., Canham, C. D., Latty, E. F., & Woods, K. D. 2005. Effects of an introduced pathogen on resistance to natural disturbance: beech bark disease and windthrow. *Canadian Journal of Forest Research* 35:1832-1843

Perrin, R. 1983. Pectinase activities of *Nectria coccinea* (Pers. ex Fries) Fries in relation with beeehbark disease. Proc. IUFRO beech bark dis. work. party conf., pp. 54-58.

Perrin, R. 1984. Les activités pectinasiques de *Nectria ditissima* Tul. agent du chancre du hêtre *Forest Pathology* 14:219–225

Pest Management Regulatory Agency (PMRA Canada). 2005. Principles of spruce budworm integrated pest Management [Electronic version]. Catalogue number H114-11/2005E.

Peterson, P. M. 2002. Importance of site conditions and time since abandonment for coppice vegetation on Langeland, Denmark. *Nordic Journal of Botany* 22:463-481

Province of British Columbia Ministry of Forests and Range. 2006. *British Columbia's pine beetle action plan, 2006-2011*. Victoria:MOF. Retrieved August 18th, 2008, from: http://www.for.gov.bc.ca/hfp/mountain_pine_beetle/

Rowe, J. 1972. Forest regions of Canada. Can. J. For. Serv. Publ. No. 1300. Ministry of Fisheries and the Environment, Ottawa, Ontario.

Safranyik, L., Shrimpton, D.M., & Whitney, H. S. 1974. Management of lodgepole pine to reduce losses from the mountain pine beetle. Canadian Forestry Service, Pacific Forest Research Centre, Forestry Technical Report 1.

Shigo, A. 1972. The beech bark disease today in the northeastern U.S. *Journal of Forestry* 70:286-289

Spiller, N.J., Koenders, L., & Tjallinjii, W.F. 1990. Xylem ingestion by aphids – A strategy for maintaining water balance. *Entomologia Experimentalis et Applicata* 55: 101-104

Storer, A. J., Rosemier, J. N., Beachy, B. L., & Flaspohler, D. J. 2004. Potential effects of beech bark disease and decline in beech abundance on birds and small mammals. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 79-87.

Trofymow, J. A. & CIDET Working Group. 1998. The Canadian intersite decomposition experiment (CIDET): Project and Site Establishment Report. Nat. Resour. Can., Can. For. Serv., Pac For. Cent., Victoria, B.C. Inf. Rep. BC-X-378.

Twery, M.J. & Patterson III, W.A. 1983. Effects of species composition and site factors on the severity of beech bark disease in western Massachusetts and the White mountains of New Hampshire: A preliminary report. In: Proceedings, IUFRO beech bark disease working party conference; 1982 Sep 26-Oct 8; Hamden, CT.

USDA. 1998. Christmas tree pest manual. Michigan State University Extension Bulletin E-2676.

Wade, F. A. & Leather, S. R. 2002. Overwintering of the sycamore aphid, *Drepanosiphum platanoidis*. *Entomologia Experimentalis et Applicata* 104: 241–253

Wainhouse, D. 1980. Dispersal of first instar larvae of the felted beech scale, *Cryptococcus fagisuga*. *The Journal of Applied Ecology* 17: 523-532

Wardhaugh, C. W., Blakely, T. J., Greig, H., Morris, P. D., Barnden, A., Rickard, S., Atkinson, B., Fagan, L. L., Ewers, R. M. & Didham, R. K. 2006. Vertical stratification in the spatial distribution of the beech scale insect (*Ultracoelostoma assimile*) in *Nothofagus* tree canopies in New Zealand. *Ecological Entomology* 31:185-195

Wargo, P. M. 1988. Amino nitrogen and phenolic constituents of bark of American beech, *Fagus Grandifolia*, and infestation by beech scale, *Cryptococcus fagisuga*. *European Journal of Forest Pathology* 18: 279-290

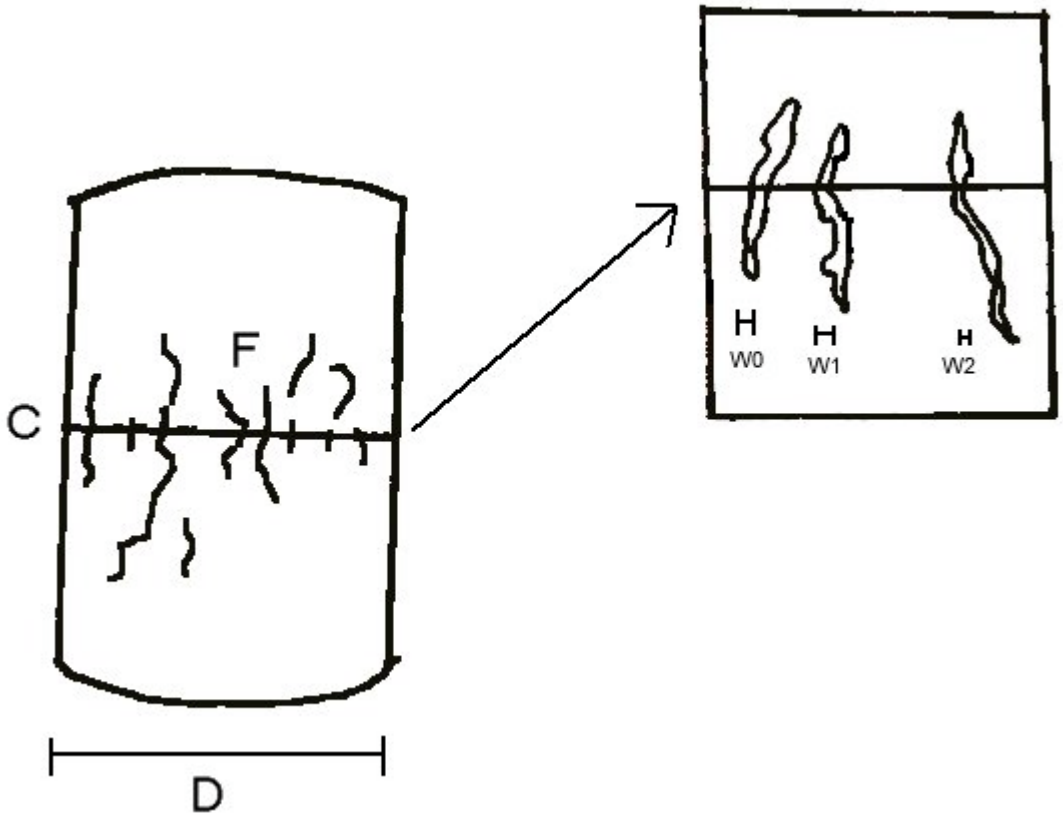
Wiggins, G. J., Grant, J. F. & Welbourn, W.C. 2001. *Allothrombium mitchelli* (Acari: Trombidiidae) in the Great Smoky Mountains National Park: incidence, seasonality, and predation on beech scale (Homoptera: Eriococcidae). *Annals of the Entomological Society of America* 94:896-901

Wiggins, G. J., Grant, J. F., Windham, M.T., Vance, R. A., Rutherford, B., Klein, R., Johnson, K. & Taylor, G. 2004. Associations between causal agents of the beech bark disease *complex [Cryptococcus fagisuga* (Homoptera: Cryptococcidae) and *Nectria* spp.] in the Great Smoky Mountains National Park. *Environmental Entomology* 33:1274-1281

Witter, J. A., Stoyenoff, J. L., Petrillo, H. A., Yocum, J. L. & Cohen, J. I. 2005. Effects of beech bark disease on trees and ecosystems. Pgs. 28-35. In: Evans, C. A., J. A. Lucas, and M. J. Twery. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium. Gen. Tech. Rep. NE-331. Newtown Square, PA, USDA Forest Service, Northern Research Station. 149 pgs.

Appendix

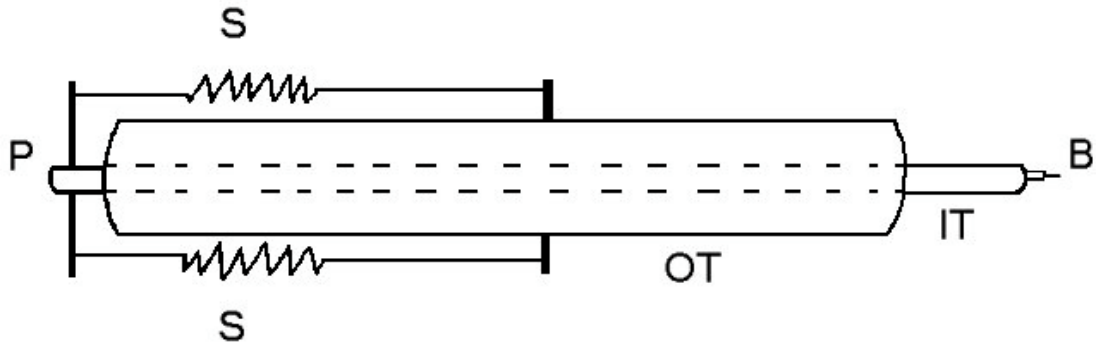
Appendix I: Determining Percent Bark Roughness



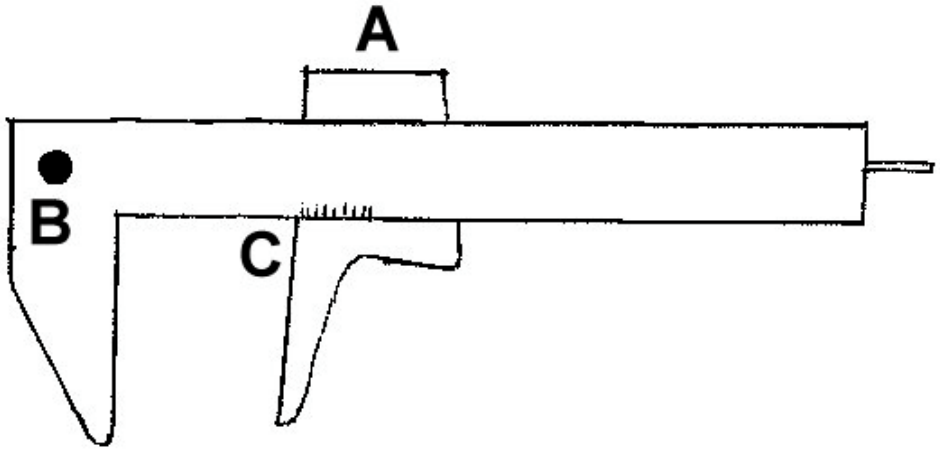
Bark Roughness was calculated by first measuring the width (W_n) of each bark fissure (F) on a tree bole of diameter D at cross section C. Then, we applied the formula:

$$\%\text{Bark Roughness} = (\Sigma(W_n)/\text{Circumference}) * 100$$

Appendix II: The Bark Penetrometer



When pressure is applied to the drill bit (B), the inner tube (IT) is pushed out of the outer tube (OT), extending the springs (S). The plug (P) is attached to a caliper (Shown below) to measure the extension of the springs



The caliper has a bolt driven through it at point B. This bolt is in contact with the penetrometer plug (Point "P") when the caliper is in the zero position. The plastic slider (A) is fixed to the penetrometer outer tube (Point "OT"). When force is applied to the plug, the central section (B) of the caliper extends. The plug extension can be measured to 0.1 mm accuracy using the Vernier scale at point C.

The distance the springs extend can be used to calculate the amount of applied force as measured in Newtons using Hooke's law, F=kx (F is force, k is the spring constant, and x is the distance). The spring constants of the springs used were 29.6 Newtons/meter (measured as 54.0 for the whole device). The minimum amount of applied force that was required to obtain an accurate reading was estimated to be 4 Newtons, since at a low

applied force the springs did not extend at all. There was an estimated 0.76 Newtons of frictional resistance. The nonfrictional internal resistance is by design: the springs hold themselves in place because their supports keep them stretched by 0.5 centimeters when the device is not being used. Any time the penetrometer is applied, it must overcome the force due to this initial extension. This distance must be considered when making any calculations of applied force. We chose not to express the penetrometer readings as amount of applied pressure, although this can be approximated knowing the diameter of the drill bit used and the force applied. In the present study, a drill bit 0.40 mm in diameter and about 2 mm long was installed and was field-replaceable.We tested the relationship between force applied to the device and the distance the springs extend, found it to be extremely linear ($R>0.95$), and judged that the extension of the springs was an adequate measure to compare bark resistance over different parts of a tree.

Appendix III: ANOVA Tables

A breakdown of the estimated differences in bark characteristics between locations on sampled trees:

Temperature

Estimates						
Label	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha
Nlo-Slo	-0.1562	0.2643	664	-0.59	0.5546	0.1
Nhi-Shi	-0.1543	0.2643	664	-0.58	0.5596	0.1
Nhi-Nlo	0.5151	0.2643	664	1.95	0.0517	0.1
Shi-Slo	0.5131	0.2643	664	1.94	0.0526	0.1

Moisture

Estimates						
Label	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha
Nlo-Slo	-0.39	1.21	260	-0.32	0.75	0.1
Nhi-Shi	-0.13	1.21	260	-0.1	0.92	0.1
Nhi-Nlo	2.64	1.21	260	2.18	0.03	0.1
Shi-Slo	2.37	1.21	260	1.97	0.05	0.1

Resistance to penetration

Estimates						
Label	Estimate	Error	DF	t Value	Pr > |t|	Alpha
Nlo-Slo	6.3202	3.114	398	2.03	0.0431	0.1
Nhi-Shi	7.4096	3.1258	398	2.37	0.0182	0.1
Nhi-Nlo	1.1919	3.1199	398	0.38	0.7026	0.1
Shi-Slo	0.1025	3.1199	398	0.03	0.9738	0.1

Effect of location and stemflow collars on the average number of live scales per colony.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	9.21	3.07	1.81	0.15
Error	44	74.5	1.69		
Total	47	83.7			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	1	4.77	4.77	2.82	0.10
aspect	1	4.33	4.33	2.56	0.12
height	1	0.097	0.097	0.06	0.81

ANOVA of the effect of the stemflow collars (treatment) on bark characteristics

Temperature

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	1	333	0.15	0.6957
date	10	333	103.91	<.0001
date*treatment	11	333	0.9	0.5429

Moisture

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treatment	1	130	2.67	0.105
date	4	130	2.66	0.0355
date*treatment	4	130	0.5	0.7363

Mean bark resistance before and after the replacement penetrometer tip

Source	DF	SS	Mean Square	F Value	Pr > F
Model	1	32019.3	32019.3	97.42	<.0001
Error	674	221528.0	328.7		
Corrected Total	675	253547.2			
Source	DF	Type I SS	Mean Square	F Value	Pr > F
tip	1	32019.3	32019.3	97.42	<.0001
Source		Type III SS	Mean Square	F Value	Pr > F
tip	1	32019.3	32019.3	97.42	<.0001

Appendix IV: Sphericity and Data Transformations

SAS PROC MIXED was used to conduct these analyses, as it allows a sphericity test to be performed on the data. Sphericity is assumed by repeated measures analysis. Additionally, PROC MIXED provides several ways to correct for violations of sphericity (such as the Huynh-Feldt correction) which were used in some instances. The covariance structure of these repeated measures analyses was selected by running the analysis several times with different covariance structures specified in SAS MIXED. The -2log likelihood (a measure of the goodness of fit of the model) of these covariance structures was compared to a model with an unstructured covariance specified (type=UN in SAS MIXED). The difference between the two measures of fitness is described by a chi-square distribution with degrees of freedom equal to the difference in the number of parameters between the two covariance models. In our experiment, an unstructured covariance consists of 24 parameters, and a compound symmetry structure of two parameters, producing a difference of 22 degrees of freedom between the two models (Littell et al. 2006). Based on the result of these analyses, the final covariance structure was chosen using the following simple algorithm:

1. If there was no significant difference between an unstructured covariance and compound symmetry (p=0.05), compound symmetry was used. This involves no correction for violations of sphericity.
2. If appropriate, an autoregressive covariance structure was tested against an unstructured covariance. This was only attempted for treatment effects, and never produced a viable covariance structure in the present study.
3. If compound symmetry was not an appropriate fit, the Huynh-Feldt covariance structure (type=HF in PROC MIXED) was tested against the unstructured one. If there was no significant difference (p=0.05), then this structure was used. This is a commonly used correction for violations of sphericity, and is slightly less conservative than the Greenhouse-Geiser method. It was the most common covariance structure used in our analyses.

4. If the Huynh-Feldt structure was not a good fit, then an unstructured covariance was used. This reduces power compared to the Huynh-Feldt correction but is appropriate if the data is too aspherical to correct with the Huynh-Feldt method.

When data failed a test for normality at p=0.05, we applied either a logarithmic, square-root, or cube-root transformation. Logarithmic transformations were applied as needed to continuous measurements such as length or height. For count data, a square root transformation was applied, unless the data represented a difference between two counts. In this case, a cube root transform was applied to accommodate for any negative integers. After transformation, all data passed a test for normality at p=0.05.

Appendix V: Beech Scale Migration

During the midsummer, as shortly after the first nymphs were observed to emerge from the beech scale eggs, a rectangular section of bark was removed from arbitrary donor trees with dense populations of the beech scale. These trees were not part of any sampling group, and were not required to meet the general sampling criteria. Six host trees were chosen according to the normal sampling criteria, and were randomly assigned (using dice) to have a piece of bark from the donor trees attached at either a low (1.3 meters) or high (2.5 meters) height. This was done by using thumbtacks and carpenter's glue to fix the donor bark to the east extremity of the bole. Since beech scales do not colonize dead bark, the purpose of this experiment was to determine the average vector of beech scale migration on a north-south/up-down axis. This would have been done by measuring the distance and direction (northwards, southwards and up or down) each insect migrated to, and calculating the average amplitude and direction of migration. Unfortunately, the majority of beech scale colonies and eggs were quickly washed off the dead donor bark by precipitation. As a result, no colonies had obviously formed as a result of this treatment, and no data were recorded.

Appendix VI: Additional Observations

Over the course of the present study, several observations were made that may be of use in further research. In the Morgan Arboretum, the highest observed density of trees infested by the wooly beech scale were in proximity to stands of hemlock. Many highly infested trees had boles in partial contact with hemlock branches, as has been observed before by Twery and Patterson (1983). Whether this is due to an environmental stress on the beech trees in this area, or because the close proximity of hemlock creates a microhabitat favourable to the wooly beech scale is unknown.

We had also noticed that in proximity to the stemflow collars, areas infected by Neonectria fungus appeared a faded red color rather than the usual vibrant red. Chapela (1989) demonstrated that *Neonectria* fungi spreads faster in wood with lower moisture content. This suggests that drier bark would be a better habitat for the Neonectria fungus. However, despite the fact that in the present study bark moisture did not significantly change in response to the use of stemflow collars, the sexual *Neonectria* fruiting bodies appeared less vigorous in the areas that did not receive stemflow. Perhaps the stemflow moisture is in some way necessary for *Neonectria* to produce healthy sexual fruiting bodies.