SUNY College of Environmental Science and Forestry

Digital Commons @ ESF

Dissertations and Theses

12-6-2019

NUTRITIONAL EFFECTS ON CAUSAL ORGANISMS OF BEECH BARK DISEASE IN AN AFTERMATH FOREST

Gretchen Dillon
gretchenadillon@gmail.com

Follow this and additional works at: https://digitalcommons.esf.edu/etds

 Part of the Forest Management Commons

Recommended Citation

Dillon, Gretchen, "NUTRITIONAL EFFECTS ON CAUSAL ORGANISMS OF BEECH BARK DISEASE IN AN AFTERMATH FOREST" (2019). *Dissertations and Theses*. 120.
https://digitalcommons.esf.edu/etds/120

This Open Access Thesis is brought to you for free and open access by Digital Commons @ ESF. It has been accepted for inclusion in Dissertations and Theses by an authorized administrator of Digital Commons @ ESF. For more information, please contact digitalcommons@esf.edu, cjkoons@esf.edu.

NUTRITIONAL EFFECTS ON CAUSAL ORGANISMS OF BEECH BARK DISEASE IN AN AFTERMATH FOREST

by

Gretchen A. Dillon

A thesis
submitted in partial fulfillment
of the requirements for the
Master of Science Degree
State University of New York
College of Environmental Science and Forestry
Syracuse, New York
December 2019

Department Forest and Natural Resources Management

Approved by:
Ruth Yanai, Major Professor
Jeffrey Garnas, Examining Committee
Martin Dovciak, Examining Committee Chair
Christopher Nowak, Department Chair
Scott S. Shannon, Dean, the Graduate School

© 2019
Copyright
G.A. Dillon
All rights reserved

Acknowledgements

I would especially like to thank the esteemed faculty at SUNY-ESF including Dr. Ruth Yanai, Dr. Mariann Johnston, and Dr. Thomas Horton for their patient assistance, and Dr. Greg McGee for securing microscopes and work space. Thank you to Christine Costello of the USFS; to my dedicated lab cohort, Yang Yang, Daniel Hong, Madison Morley, Alex Young, Alexandrea Rice, Jenna Zukswert, and Thomas Mann for countless Crayola crayon exercises and emotional support. Thank you to Mary Hagemann whose invaluable efforts help the B9 lab run more smoothly. This project could not have been completed without the help of the following summer, undergraduate, high school, and citizen scientist research technicians: Steve Abrams, Shaheemah Ashkar, Harshdeep Banga, Stephanie Chase, Kien Dao, Madeleine Desrochers, Imani Diggs, Bryn Giambona, Lia Ivanick, Abby Kambhampaty, Milda Kristupaitis, Vizma Leimanis, Grace Lockwood, Michael Mahoney, Charlie Mann, Allison Laplace McKenna, Julie Romano, Jason Stoodley, Emma Tucker, Trey Turnbalcer, and Sara Wasserman. Thank you to my family, biological and chosen, especially Lauren Martin, Sarah Dulany-Gring, my husband Michael, and Wilhelmina.

The Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE) project is funded by the National Science Foundation and the Long-Term Ecological Research Network.

Table of Contents

List of Tables

List of Figures

List of Appendices

Abstract

G.A. Dillon. Nutritional Effects on Causal Organisms of Beech Bark Disease in an Aftermath Forest, 50 pages, 3 tables, 5 figures, 2 appendices, 2019. APA style guide used.

Beech bark disease (BBD) invaded North America over a century ago but is still not completely understood. This disease occurs when an invasive scale insect, *Cryptococcus fagisuga*-Lind., feeds on the inner bark and cambium of American beech (*Fagus grandifolia*, Ehrh.) making trees susceptible to fatal infections by *Neonectria* fungi. These causal agents were examined in the context of experimental additions of N and P across six northern hardwood stands in New Hampshire. Scale cover varied significantly with tree diameter ($p = 0.02$) but was nearly identical (0.6%) at two heights on the bole (0.5 m and 1.5 m). Nearly all *Neonectria* samples collected were identified as *N. faginata*; 3% that were *N. ditissima*. New lesions developed on 58% of trees, with 96% developing at or below 0.5 m. Trees receiving P additions developed 2 times as many lesions as those not receiving P ($p = 0.04$). These results differ from previous research reporting higher BBD severity where P was low relative to N.

Key Words: Beech bark disease, BBD, *Neonectria*, *Cryptococcus fagisuga* Lind., *Fagus grandifolia* Ehrh., *Neonectria* fungi

G. A. Dillon
Candidate for the degree of Master of Science, December 2019
Ruth D. Yanai, Ph.D.
Department of Forest and Natural Resources Management
State University of New York College of Environmental Science and Forestry
Syracuse, New York

Chapter 1: Assessing Beech Bark Disease in Aftermath Forests:

A Review of Information, Identification, and Quantification Methods of Casual Agents

Beech bark disease history

Beech bark disease (BBD) is a pathogenic complex between a non-native scale insect and a fungus in the genus *Neonectria* that was introduced to Nova Scotia in the 1890's (Ehrlich, 1934; Shigo, 1964). Originally documented in southeastern Europe as early as 1838, BBD affects both American beech (*Fagus grandifolia* Ehrh.) and European beech (*F. sylvatica),* but its effect in Europe has been less severe than in North America (Gwiazdowski et al., 2006; Houston, 1994a; Houston, Parker, Perrin, & Lang, 1979). Beech bark disease continues to spread in the U.S. and is predicted to occupy most or all of the geographical range of beech by 2050 (Morin, Liebhold, Tobin, Gottschalk, & Luzader, 2007). As of 2013, almost all American beech in the northeastern United States were infected, with only 1 to 3% of trees unaffected (Houston, 1983; Stephanson & Coe, 2017). In some forests, such as the Adirondack region of New York, less than 1% of beech remain unaffected (Giencke, Dovčiak, Mountrakis, Cale, & Mitchell, 2014; Mason, Koch, Krasowski, & Loo, 2013).

In the northeastern United States, beech has experienced extensive mortality (Fernandez & Boyer, 1988; Jones & Raynal, 1987; Mize & Lea, 1979) which is altering forest composition and structure (Garnas, Ayres, Liebhold, & Evans, 201; Houston, 1975; Runkle, 2005; Shigo, 1972; Twery & Patterson, 1984). Increased BBD severity in stands is associated with increases in dead beech basal area and decreases in live beech basal area and density (Lovett, Arthur, Weathers, & Griffin, 2010) with beech increasingly present as standing dead and downed logs (Lovett et al.,

2010). Beech trees under stress can produce root sprouts, genetically identical individuals known as ramets, that can create dense thickets of monocultures (Houston, 1975; Ostrofsky & McCormack, 1986). The sprouts, which may develop with defects due to early initiation of cankers (Houston, 1975) compete for light and soil resources that other species need for growth and survival (Garnas et al., 2011a). Jones and Raynal (1986, 1987) found that sprout density was highest at 1 – 2 m from parent trees but can develop within 8 – 10 m, with density of sprouts correlating positively to parent tree diameter. Contrary to early beliefs, trees affected by BBD actually produce fewer root sprouts than unaffected trees (MacKenzie, 2005) and do not always contribute significantly to outward spread from a parent tree compared to saplings of seed origin (Giencke et al.,2014; Jones & Raynal, 1986). Geincke et al. (2014) found that saplings tended to cluster further away (~ 3-4 m) from severely diseased and dead trees; however, they did not determine whether the saplings in their study resulted from seeds or root sprouts. Beech saplings and other codominant, intermediate, and suppressed trees grow into the canopy space of dying beech trees (Giencke et al., 2014; Lovett et al., 2010).

An analysis of data from the United States Forest Inventory and Analysis program that evaluated the impacts of BBD on forest stand structure and composition at the landscape level found BBD-affected forests contained fewer large beech trees and more small diameter beech trees, increasing the density of small beech stems in North American forests (Garnas et al., 2011a). Garnas et al. (2011a) also found that tree species community composition did not change as a result of BBD. Studies conducted at regional scales show different patterns. In the Catskill Mountains of New York, sugar maple (*A. saccharum Marsh*) was shown to increase in basal area as beech declined in basal area, resulting in a prediction that species composition in northeastern forests will shift from mixed beech/maple stands to forests dominated by sugar maple (Griffin, 2005). As a result, forests in that area are predicted to experience decreases in beech litter input

and increases in sugar maple litter input (Lovett et al., 2010), and changes tree species compositions can lead to changes in nutrient cycles due to individual species characteristics. Lovett and others (2010) found that shifts away from beech towards maple dominated stands cause declines in forest floor C:N ratio, increases in the fraction of mineralized N that is nitrified, and increased foliar decomposition rates due to increases in sugar maple litter which has lower lignin content (Lovett, Weathers, Arthur, & Schultz, 2004) and is more decomposable than beech leaves (Melillo, Aber, & Muratore, 1982).

Causal organisms

Beech bark disease is initiated by the non-native beech scale *Cryptococcus fagisuga* (syn. *Cryptococcus fagi* Baer.), a sap-feeding insect that feeds on the inner bark and cork cambium (Shigo, 1964). Populations of first instar *C. fagisuga*, called nymphs or crawlers, are established when individuals are passively disseminated to host beech trees by wind or other organisms, such as humans or birds. Nymphs spread to other trees during late summer to late fall at rates around 3 to 15 km per year (Houston, Parker, & Lonsdale, 1979; Morin et al., 2007; Wainhouse 1980; Wieferich, McCullough, Hayes, & Schwalm, 2011). This stage of nymph is the only stage where the insect is mobile, once nymphs reach suitable trees, they insert their stylets into the cambium and remain immobile on the bark of the tree for subsequent instars. Once maturity is reached, beech scale reproduce parthenogenically. As of 2011, beech scale insects occupied ~50% of the geographical range of American beech in North America (Garnas et al., 2011a). Adult beech scale are small, only 0.5 to 1.0 mm long as adults (Brown, 1934), and populations occur along the length of the bole of the tree. Bark characteristics and abiotic factors can affect their longevity (Wainhouse 1980). Rougher bark at the base of the tree allows scale to cluster in protective

crevices. In colder climates, scale insects feeding towards the bottom of the tree will benefit from the insulating effect of snow, surviving through freezing temperatures (Burns & Houston, 1987; Latty, 2004; Teale, Letkowski, Matusick, Stehman, & Castello, 2009; Wainhouse, 1980).

A second scale insect, *Xylococculus betulae* (Pergande) (*Homoptera: Margarodidae*), native to North America, is also found on beech but doesn't play as large a role in the BBD complex (Houston et al., 1979a; Wiggins et al., 2004). This 4 mm orange-red insect enters bark to feed on phloem sap, causing erumpent spots that are 1 to 5 cm in diameter. These bark spots become rough and calloused as they dry with age (Shigo, 1962). Like *C. fagisuga*, this species provides suitable sites on host trees when their feeding activity causes the tree to create callused cracks that become colonized by *C. fagisuga*. This species is usually not detected in stands until after *C. fagisuga* has been observed and like *C. fagisuga* populations of *X. betulae* decline as tree health declines (Shigo, 1964; Wiggins et al., 2004).

Scale feeding activity predisposes beech to airborne fungal spores that germinate and create an infection in the tree (Ehrlich, 1934). In North America, the fungal component of BBD is caused by *N. ditissima* (previously *N. galligena*; anamorph *C. heteronema*) and *N. faginata* (previously *N. coccinea* var. *faginata*; anamorph *C. faginatum*) (Castlebury, Rossman, & Hyten, 2006; Chaverri, Salgado, Hirooka, Rossman, & Samuels, 2011; Hirooka, Rossman, Zhuang, & Salgado-Salazar, 2013). *Neonectria ditissima* is a generalist pathogen of North American and European hardwoods (Castlebury et al., 2006; Hirooka et al., 2013) while *N. faginata* is found exclusively on beech and is the most virulent and dominant fungus in the disease complex in North America (Houston, 1994a; Kasson & Livingston, 2009). Non-beech hardwood hosts show varying degrees of susceptibility to *N. ditissima*. Red maple (*Acer rubrum* L.), striped maple (*Acer pensylvanicum* L.), yellow birch (*Betula alleghaniensis* Brit.) and black birch (*Betula lenta* L.) are all highly susceptible, forming numerous stem and branch cankers that produce high inoculum

loads (Spaulding, Grant, & Ayers, 1936; Welch, 1934). Both species of *Neonectria* produce annual target cankers that decrease the merchantability of trees (Department of Agriculture, 2018). *Neonectria* infection on beech trails the arrival of scale insects by approximately 1-10 years (Ehrlich, 1934; Houston, 1994a; Houston, 2005), with habitat suitability peaking around 20-30 years after the insects begin colonizing trees (Garnas, Houston, Ayres, & Evans, 2011).

The two species of *Neonectria* can cooccur on individual trees (Kasson & Livingston, 2009), after entering the tree through wounds created by scale insects or otherwise (Ehrlich, 1934; Ostrofsky & Blanchard, 1983). The fungi create lesions of dead tissue that develop into cankers on the tree, with greatest canker development coinciding with spore release in the fall (Mason et al., 2013; Ostrofsky & Blanchard, 1983). *Neonectria* infects the cambial tissues of the tree causing necrosis and accumulations of lesions can merge and ultimately girdle the vascular cambium (Ehrlich, 1934; Houston, 1994). The vascular cambium is responsible for secondary growth in trees and produces vascular tissue cells that transport water and nutrients (xylem) and sugar and other large molecules (phloem) through the length of the tree (Nieminen, Blomster, Helariutta, & Pekka Mähönen, 2015). Girdling interrupts the functioning of this vasculature, causing transpiration disruption and dehydration which can lead to tissue death above the girdle, killing the crown and tree stem (Ehrlich, 1934; Houston, 1994). The girdling process also lowers the tree's overall ability to fight off infection and invaders which can also lead to death (Cale et al., 2015b).

Tree susceptibility to disease can be affected by abiotic factors including elevation, soil type, moisture, aspect, and temperature differences. Drought stress increases *Neonectria* lesion development, even in the absence of scale (Parker, 1977) and Ehrlich (1934) noted higher incidence of BBD on slopes compared to valleys. A wound-inoculation study by Perrin (1980) found that larger *Neonectria* lesions developed on scale-infested trees compared to those that were without scale. Other studies found that scale density was a poor predictor of *Neonectria* fruiting

structure density (Cale et al., 2015b; Garnas, 2009; Garnas et al., 2011b; Letkowski, 2009), though this does not necessarily preclude an important relationship between the two disease agents (Garnas 2009). Scale insect occurs equally around the circumference of the tree, (Ehrlich, 1934; Teale et al., 2009; Wiggins et al., 2004). Sites with high precipitation that occurred later in the summer season had higher carrying capacities for *Neonectria*, but climate was generally a weak predictor of variation in population growth rates (Garnas et al., 2011b).

Temporal stages of disease

Forests containing BBD can be characterized by the amount of disease agents present and the degree of beech mortality that has occurred (Shigo, 1972). Beech bark disease is described as having three stages: the advancing front, the killing front, and the aftermath zone.

Prior to BBD, beech mortality is low and beech scale and *Neonectria* are absent on trees (Shigo, 1972). The advancing front is characterized by the arrival and colonization of beech scale, low levels of *Neonectria* infection and normal beech mortality (low levels of disease mortality; Shigo, 1972). The killing front lag 3 to 6 years behind the arrival of the scale infection (Houston, 1975) and is characterized by a high beech tree mortality rate of over 50% along with heavy scale and/or severe lesions (Houston, 1975). In aftermath phase, beech mortality is low and ecological accommodations to the disease result in the changes to forest structure mentioned above (Shigo, 1972; McCaskill & Morin, 2012; Houston et al., 2005). Trees remain in a reduced state of health for years as populations of causal agents become established and wide-spread. There is a lower incidence of beech scale (< 40–70 scales/cm²; Cale et al., 2015a; Teale et al., 2009) and *Neonectria* lesions (0.005– 0.025 cm² canker/cm² bark; Cale et al., 2012; Giencke et al., 2014) in aftermath stands compared to earlier temporal stages. Over time *N. ditissima* is often replaced by *N. faginata*,

which has been more commonly associated with aftermath forests (Houston, 1994b); however, replacement or persistence of either species is likely driven by the amount of inoculum in the stand (Houston, 1994b; Kasson & Livingston, 2009). In this phase, scale and *Neonectria* persist in reduced numbers throughout beech stands (Garnas, Houston, Twery, Ayres, & Evans, 2013; Morin & Liebhold, 2015).

Nutritional implications of plant health

Both N and P play important roles in plant health and defense. Plants can acquire nitrogen from the soil and the atmosphere. Soil N is usually taken into the plant as nitrate (NO^{-}_{3}), an inorganic form of N, and is reduced to ammonia (NH_3) before assimilation into amino acids, proteins, nucleotides, hormones, chlorophyll, and other building blocks for growth, disease defense, and injury recovery (Lea, 1992). Phosphorous is acquired from soil as the orthophosphate ion (PO_4^{3-}) and is incorporated into nucleic acids, phosphoproteins, phospholipids, and energy molecules like adenosine triphosphate (ATP; Walters & Bingham, 2007). Additions of soil N and P positively impact beech tree and forest growth (Fisk et al., 2014; Goswami et al., 2018), are reflected in foliar nutrient status (Gonzales & Yanai, 2019) and is presumed to correlate with bark chemistry (Cale et al., 2015b).

Soil N and P can affect the development of plant diseases. A review article by Walters and Bingham (2007) details that for both biotrophic and necrotrophic fungal pathogens, elevated levels of soil N can lead to greater disease incidence and lesion area, however these results are not always consistent. Inconsistencies are most likely due to the unique relationships between plant hosts and pathogens, as the response of a pathogenic species to host tissue nutrient supply and the presence of host-defense compounds that are produced is complex and variable (Hoffland, Jeger, & van

Beusichem, 2000). Host-pathogen interactions are complicated; in response to pathogen infection, the host plant can evoke a protective defense reaction by producing secondary metabolites. In one study, *Neonectria* caused a defense response of increased phenolic concentrations in some beech trees to prevent further pathogen invasion into the vascular cambium (Ostrofsky et al., 1984). High levels of certain bark phenolic compounds (isorhamnetin and catechin) have been found to be negatively associated with BBD infection probabilities (Cale et al., 2015b) but are naturally occurring in uninfected beech trees rather than the result of a disease response.

Disease pathogens can also alter host plant chemistry and nutrient use. Pathogen growth on hosts can alter plant N uptake and partitioning (Walters & Bingham, 2007) and necrotized cambial cells can lead to girdling from coalesced lesions, which can limit radial growth and affect nutrient and water uptake and use (Dordas, 2008; Ehrlich, 1934; Houston, 1994). As different pathogens, *N. faginata* and *N. ditissima* may have different pathosystems that contribute differently to how BBD impacts trees (Cale et al., 2015b; Houston, 1994a; Kasson and Livingston, 2009). More information is needed on how soil nutrient conditions alter beech tree tissue nutrient status and how this status changes as a result of and in response to insect and fungi agents of BBD.

Nutritional investigations of beech bark disease

Beech tree nutritional status, as determined by soil nutrient additions, tree tissue stoichiometry and nutrient concentration in bark and leaves, have been associated with both increased and decreased BBD infection occurrence. Beech scale populations are influenced by tree bark nutrient concentration (Brown, 1975) and are greater on beech with higher bark amino nitrogen (Latty, Canham, & Marks, 2003; Wargo, 1988). In aftermath forests, lower concentrations of bark P and higher bark N:P were a predisposing factor in *N. faginata* infections (Castello &

Johnston, 2014; Cale et al., 2015b). Cale et al. (2015) found that bark N concentrations were significantly lower in infected trees than in control trees and that bark P concentrations were 33% lower in infected trees than in uninfected trees. Their data did not reveal any significant differences in P bark concentrations between *N. ditissima* and *N. faginata* afflicted trees. Other studies link high nutrient levels with elevated BBD. Elevated N:P ratios in beech foliage have been associated with greater occurrence of BBD (Ouimet, Duchesne, & Moore, 2015). Studies in Europe have found that trees fertilized with N or P develop more *Neonectria* lesions on *Fagus sylvatica* than trees in plots without N or P additions (Jönsson, 2000; Perrin & Garbaye, 1984). Adding further complexity, the pathogens of BBD may also change beech tree host tissues. It has been suggested that *C. fagisuga* scale infestations chemically alter bark tissue in favor of *Neonectria* infections (Houston, 1980). An inoculation study with *N. faginata* resulted in a decrease in bark phenols around the wound surface and increased phenols closer to the vascular cambium (Ostrofsky, Shortle, & Blanchard, 1984).

Identifying causal organisms of beech bark disease

In the field the two species of scale insects are easily distinguished from each other. The native scale, *X. betulae*, is buried within the bark but can be located by its distinctive, long, waxy excretory tube, that looks like a fine white hair protruding from tree bark. This is very different from *C. fagisuga*, which has no filament and is found on the exterior of the tree bark, covered by a white, waxy, felt-like secretion and occurring in groups (Kosztarab, 1996).

The fruiting bodies (ascomata) of *Neonectria* are visually identified on trees as tiny, red, round structures called perithecia that occur in clusters in cracks of bark. Perithecia are 200-300 µm diameter and 250–400 µm high and contain asci that bear sexual spores (ascospores)

measuring 10.5-12.5 x 5-6 μm in size. The anamorphic asexual state produces micro- and macroconidia from small, compact stroma (sporodochia; Castlebury et al., 2006). Lesions of mature *Neonectria* develop in oval-shaped groupings that can be visually distinct or diffuse, with individual lesions being sometimes difficult to discern.

Neonectria can be reliably identified to species via microscopy by measuring the length of mature sexual (ascospores) or asexual (macroconidia or microconidia) spores (Castlebury et al., 2006). Measuring the length of at least 25 ascospores from 2 to 3 perithecia per lesion is a reliable way to distinguish *N. ditissima* from *N. faginata* (Cale et al., 2015b; Castlebury et al., 2006; Cotter & Blanchard, 1981; Kasson & Livingston, 2009). Molecular tools, such as deoxyribonucleic acid (DNA) sequencing, are also favored as an accurate determination of species (Castlebury et al., 2006; Horton & Bruns, 2001; Ko, Stephenson, Bahkali, & Hyde, 2011). Results of direct sequencing can be confirmed by using a public sequence database such as GenBank, which exists as an open access annotated collection of nucleotide sequences and their protein translations that allow researchers to compare genetic sequences with more than 100,000 distinct organisms (Benson, Karsch-Mizrachi, Lipman, Ostell, & Wheeler, 2008).

Monitoring causal organisms

Monitoring scale and lesion development using digital photography has been effective in quantifying population density and occurrence (Gardner, 2005; Koch, Carey, Mason, & Nelson, 2010; Teale et al., 2009; Wainhouse, 1980; Wieferich, Hayes, & McCullough, 2013). Challenges include differentiating current year scales and lesions from those of previous years, differing densities of scale at different points on the bole, and the inclusion of dead scale, lichens, egg masses, and other organisms that are mistaken for living scale. In spite of these difficulties,

Wieferich, Hayes, and McCullough (2013) found that the area of wax visually assessed via photos explained ~80% of the variability in scale density. Individual scales are about 0.5 mm in size (Houston & O'Brien, 1983; Wainhouse & Gate, 1988) thus counting them in the field is difficult because of their size as well as the time it takes to point count populations.

In a study by Teale, Letkowski, Matusick, Stehman, and Castello (2009), digital analysis was found to be faster than visual processing by almost 30 minutes per sample. Digital analyses involve photos taken at different heights on the bole, using areas of bark usually 50 to 100 cm^2 in size. One method of quantifying the scale infestation is to randomly select one to three 1 cm by 1 cm squares within the photograph using ImageJ software to contrast the color to count or measure scale. *Neonectria* lesions are characterized by the area affected (Letkowski, 2009; Teale et al., 2009; Wieferich et al., 2013; Van Driesche & Japoshvili, 2012). Wieferich, Hayes, and McCullough (2013) suggested using at least three photographs per tree, with each photo showing 400 cm^2 of bark surface, to obtain accurate estimations of scale densities despite varying population numbers and locations.

Summary

Beech bark disease is changing the face of North America's northern hardwood forests by altering forest structure, reducing biodiversity, and decreasing timber value. This disease occurs when scale insects damage the bark and a canker-causing fungus infects and eventually kills the tree. Evidence suggests that nitrogen and phosphorus imbalance is a significant predisposing factor for BBD. Measuring the nutrient status of beech trees and quantifying BBD causal organisms and the complex effects that they have together on the tree may allow us to better evaluate factors affecting disease progression. Continued research on how nutrient manipulations affect the causal

agents of BBD is needed to better understand the effect this disease process has on northern hardwood forest ecosystem dynamics, structure and function.

Chapter 2:

Nutritional Effects on Causal Organisms of Beech Bark Disease in an Aftermath Forest

Introduction

Beech bark disease (BBD) is an invasive pathogenic complex (Ehrlich, 1934; Shigo, 1964) that has been described as the single greatest threat to American beech trees (*Fagus grandifolia* Ehrh; Houston, 1994a) that will lead to ecosystem changes greater than those caused by climate change and air pollution (Lovett et al., 2010). Within the next half century BBD is predicted to occupy most, if not all, of the geographical range of beech (Morin et al., 2007), and it will alter forest structure and species composition (Houston, 1975; Le Guerrier, Marceau, Bouchard, & Brisson, 2003; Runkle, 2005; Twery & Patterson, 1984), increase litter decomposition rates (Lovett et al., 2010) and alter long-term cycling of nutrients in forests, particularly of calcium (Ca; Arthur et al., 2017), carbon (C), and nitrogen (N; Lovett et al., 2010).

Beech bark disease causes high mortality as well as physical and species-related stand composition changes in northern hardwood ecosystems (Cale et al., 2013; Houston, 1994a; Mason et al., 2013) and involves insect and fungal components. An invasive felted beech scale, *Cryptococcus fagisuga* Lind. (Hemiptera, Eriococcidae), is a phloem-feeding insect that was introduced from Europe (Ehrlich, 1934) that feeds on the inner bark and cork cambium. This activity predisposes beech to canker-causing fungal infections by *Neonectria ditissima* [Tul. & C. Tul.] Samuels & Rossman) and/or *N. faginata* [Lohman, Watson & Ayres] Castl. & Rossman (Ehrlich, 1934; Houston, 1994b; Kasson & Livingston, 2009).

Beech scale infects trees > 1 cm in diameter (Cale et al., 2015b; Ehrlich, 1934; Morris, Small, & Cruzan, 2002). Subsequently, cankers of *Neonectria* species appear, typically on trees 12–37 years old and 2–11 cm in diameter (Houston & Valentine, 1988). The fungi create lesions of dead tissue that develop into annual cankers (Ehrlich, 1934; Houston, 1994a; Spaulding et al., 1936) eventually girdling the tree, causing growth reduction, crown dieback, and cambial and phloem death (Houston, 1994a). Beech bark disease also lowers the tree's overall ability to fight off infection and other pests and pathogens (Cale et al., 2015b; Ostrofsky & Blanchard, 1983).

Beech scale and *Neonectria* occur at various heights on trees. Some researchers found scale densities increasing with height on the bole up to 3 m (Wainhouse, 1980) while others found higher densities lower on the bole (Teale et al., 2009). The lowest 0.5 m of the bole has more bark fissures and is protected from sub-freezing temperatures beneath snowlines (Gove & Houston, 1996; Teale et al., 2009), as well as being closer to the leaf litter from which scale often emerges to attack trees (Stephanson & Coe, 2017). The position on the bole of lesion development has not previously been studied.

High availability of soil N has been associated with beech tree nutrient imbalances and stress (Moore, Mika, Schwandt, & Shaw, 1994; Wargo, 1980) and elevated N:P ratios in beech foliage have been associated with greater occurrence of BBD (Ouimet, Duchesne, & Moore, 2015). Similarly, Latty, Canham, and Marks (2003) found greater *C. fagisuga* infestations and more *Neonectria* lesions in trees with high bark N concentrations. In aftermath forests, higher bark N:P and lower concentrations of bark P were associated with BBD severity (Castello & Johnston, 2014; Cale et al. 2015b). These previous studies were correlational and not manipulative.

Our research studied the relationship between BBD causal agents and tree nutrient status in an aftermath forest setting. We used plots from a study on multiple element limitation in a northern hardwood ecosystem (MELNHE; Fisk, Ratliff, Goswami, & Yanai, 2014), a full-factorial N and P

manipulation in New Hampshire, USA. We investigated whether the severity of scale infestation varied with tree diameter, height on the bole, or N or P treatment. We assessed the occurrence of *N. faginata* and *N. ditissima*, which had yet to be reported for this study site. We investigated whether treatments of N or P impacted the date at which lesions first appeared in the fall and whether the number of lesions at first appearance varied with tree diameter, height on the bole, or N or P treatment. We hypothesized that both the scale and fungus would be more prevalent on bigger trees, lower on the bole, and on trees receiving N.

Methods

Site Description

This study examined 6 stands in the Bartlett Experimental Forest (BEF) that were part of an existing MELNHE project. Mean monthly temperatures ranged from -9 to 18ºC and mean annual precipitation was 1300 mm (Adams, Loughry, & Plaugher, 2003). The stands were similar in climate (humid continental), soils (Spodosols formed in granitoid glacial till; Vadeboncoeur, Hamburg, Blum, Pennino, Yanai, & Johnson, 2012; Vadeboncoeur, Hamburg, Yanai, Blum, 2014), and elevation (Table 1). Stands regenerated naturally after clearcutting and ranged in age from 31 - 136 years old in 2017 at the time of sampling. Four stands (C2, C3, C4, and C6) were secondary successional and dominated by pin cherry (*Prunus pensylvanica L.f.*), white birch (*Betula papyrifera Marsh*), yellow birch (*B. alleghaniensis Britton*), red maple (*Acer rubrum L.*), American beech and some sugar maple (*Acer saccharum Marsh*). Two stands (C7 and C8) were mature and were dominated by American beech, yellow birch, and sugar maple with some ash (*Fraxinus americana L.*), red maple, and white birch. Stands varied in beech and non-beech basal area (Table 1). In each stand there were four treatment plots, each 900 m² plus a 10 m buffer, with

treatments of N, P, N+P, and a control. Plots had annual applications of N and P at the rate of 30 kg N/ha/yr (as NH_4NO_3) and 10 kg P/ha/yr (as NaH_2PO_4) beginning in 2011.

Beech Tree Selection

In 2017, Five beech trees in each plot were selected for study using stem maps of the plots prepared in GIS. Trees were 3 m to 15 m apart, and trees < 5 m from each other were avoided to reduce the chance of sampling genetically identical individuals (Jones & Raynal, 1986). Additional trees were selected from the buffer area as needed based on diameter and disease status. Trees with conks of decay fungi were avoided as BBD causal agents may also interact with *Phellinus igniarius* and *Inonotus glomeratus* (Cale et al., 2015a). Sample trees ranged in diameter at breast height (1.4 m) from 9.5 – 38.1 cm, with a mean of 16.5 cm.

Invasive Beech Scale, *Cryptococcus fagisuga*, Imaging and Quantification

Eight 10 cm x 5 cm "L" shaped frames were painted on study trees in July 2017 at two heights (1.5 m and 0.5 m above the ground) and four cardinal directions (north, east, south, west; Figure 1). Adjustments to photo locations were made for limbs, knots, or bole irregularities. In July and August 2017, photos of tree bark were taken during dry weather. No flash was used, and the camera was centered directly in front of the area to be imaged.

Each image was cropped to 50 cm^2 using Image-J image-processing software (version 1.50i) developed at the National Institutes of Health (Bethesda, Maryland; Schneider, Rasband, & Eliceiri, 2012) and then superimposed with a grid containing 200 intersections using GIMP software (GIMP 2.8.10; Figure 1). Scale insect cover was quantified by tallying the number of intersections where a *C. fagisuga* wax mass was present. We did not attempt to differentiate new wax masses from those of previous years.

Neonectria Lesions

Plots were visited on September 22-23, October 7-8, October 21-22, and November 5-6, 2017. Five trees, pre-selected from stand inventory stem maps, were always visited first, but if those trees did not have lesions, neighboring trees were inspected until up to 5 trees with perithecia were located or until all the beech in the plot had been checked.

During each lesion collection trip, trees that had not previously developed lesions were visually inspected for lesion development, characterized by the appearance of small, bright red fruiting bodies (perithecia) usually grouped together in an ellipse. Lesions sometimes develop diffusely, with smaller numbers of perithecia appearing independent of the common elliptical shape. For our purpose, the term "lesion" refers to groupings of perithecia that were mostly to strongly elliptical. When a tree exhibited lesions, the following information was collected: tree DBH, approximate count of new lesions appearing on bole up to 2 m (recorded in classes of 5; 1-5, 5-10, etc.), and the location of the lesions on the bole (classes of 0.5 m; 0-0.5, 0.5-1.0, 1.0-1.5). Classes have overlapping numbers to allow for some subjectivity to classify the number of lesions depending on diffuseness, as some trees showed *Neonectria* growth that was not in the classic lesion formation. Upon recording lesion information at first sign of development, the tree was not revisited.

Neonectria samples from 65 trees were collected for morphological identification (n = 192). *Neonectria* ascospores (the mature sexual state) and sporodochia, if present, were collected from 3 to 4 lesions per tree during dry weather conditions. Sterilized blades were used to scrape up to 0.25 g of perithecia and sporodochia into sterile vials containing DI water. In addition, 77 samples were collected from a subset of 25 trees across the 6 stands into vials of 2% cetyltrimethyl ammonium bromide lysis extraction buffer for DNA analysis (CTAB, 100-mM Tris-HCL [pH 8.0],

1.4-M NaCl, 20-mM EDTA, 2% CTAB; Gardes & Bruns, 1993, as modified by Thomas Horton; Appendix 1). *Neonectria* lesion samples collected into CTAB were also collected into DI water so species identification by microscopy could be confirmed by DNA analysis. On October 21-22, heavy rain made lesions difficult to see on trees during the visit to C8, and that stand was not collected on that date. Samples were placed on ice in the field and transported to the lab where they were stored at 4°C until further processing.

Microscopy techniques used by Cotter and Blanchard (1981) along with ascospore photos and descriptions from Castlebury, Rossman, and Hyten (2006) were used to identify *Neonectria* samples to species. Slides were prepared using a squash mount and viewed at 1000X using oil immersion. The mean length of at least 25 ascospores from 2 to 3 perithecia per sample were measured (Cotter & Blanchard, 1981). Unsuccessful attempts at identification (n = 43) resulted from inadequate sampling material collected, an overgrowth of hyphae post collection, and contamination.

Of the 77 samples collected for DNA analysis, 20 lesions samples from 10 trees were selected from in N plots (8 lesions), P plots (9 lesions), an N+P plot (1 lesion) and a control plot (2 lesions) from 4 stands (C2, C3, C4, and C8). We used the translational elongation factor 1-alpha (EF1-α) region using *Neonectria*-specific primers, EF1-α-728 forward and EF1-α-1567 to distinguish the two *Neonectria* species (Castlebury et al., 2006). Genomic material was amplified using PCR with standard *Taq* polymerase and fragments were run on gel electrophoresis using 3% agarose gel and stained with ethidium bromide to determine if samples had successfully amplified. DNA from successfully amplified samples was digested with *DpnII* and *HinfI* restriction enzymes (New England Biolabs, Ipswich, MA, USA) and then run on 3% agarose gels, stained using ethidium bromide, and RFLP patterns were imaged using the Gel Doc EZ System. All 20 RFLP

patterns were identical and three were chosen for continued sequencing; RFLP patterns are unique to species and comparing sequences is a simple way to identify taxa to the species level (Horton & Bruns, 2001).

After reamplifying, PCR products were purified with a 24 Qiagen PCR Purification kit (Valencia, CA, USA) and DNA in samples was quantified using a NanoDrop ND-2000 (Thermo Fischer Scientific Inc., Waltham, MA, USA). The three RFLP replicate samples, each containing between 34 and 46 ng/uL DNA, were sent to Eurofins Genomics for sequencing following their protocols. The ITS1-F primer was used to amplify the fungal nuclear ribosomal internal transcribed spacer (ITS) region. Raw sequences were subjected to a basic local alignment search tool (BLAST) against the Genbank database (Altschul et al., 1997).

Data Analyses

Tree Diameter

Because scale cover and lesion numbers are known to vary with tree diameter, we tested whether tree diameters differed by stand and treatment (Control, N, P and N+P) using a linear model and ANOVA with the fixed effects of stand, treatment, and an interaction between stand and treatment. As expected, tree diameter differed by stand ($F_{5,94} = 14.89$; $p < 0.001$) with mature stands, C7 (t = 3.70, $p < 0.001$) and C8 (t = 2.45, $p = 0.02$) containing larger trees than successional stands (C3: t = 1.03, $p = 0.31$; C4: t = 0.15, $p = 0.88$; and C6: t = 0.73, $p = 0.47$); treatments ($F_{3,94} = 0.43$, $p = 0.73$) and the interaction of stand and treatment ($F_{15,94} = 0.63$, $p = 0.85$) were not significant.

All statistical tests were performed using R (Version 3.5.0).

Cryptococcus fagisuga Scale Cover

The effect of tree diameter and nutrient addition on scale cover of individual trees was tested using a linear mixed-effects model (nlme package in R; Pinheiro, Baes, DebRoy, Sarkar, & Rcore Team, 2019) and a nested, two-way analysis of variance (ANOVA) with tree DBH, treatment (N or P addition), and a treatment interaction (N*P) as fixed effects and forest stand and nutrient plot (nested within stand) as random effects in a split-plot design. Trees were sampling units (n = 118), nutrient addition plots were experimental units (n = 24), and stands were experimental blocks (n = 6). This full factorial approach compared N addition (N and N+P plots) to those without N addition (P and Control plots) and P addition to those without P addition (N and Control plots), along with N and P interactions. A square root transformation was applied to tree scale cover to meet the assumption of normality of the residuals. One outlier determined using Cook's distance was included in the analysis; conclusions were not changed by omitting this outlier.

A one-way analysis of variance showed that the aspect of the photo was not a significant predictor of scale cover ($F_{3,460} = 1.08$; $p = 0.36 = 0.009$; adjusted $R^2 = 0.0005$). Means and standard deviation of scale cover by aspect were: north (2.9% ± 3.8%), east (2.6 ± 3.5%), south (2.3 ± 3.0%), and west (2.3 ± 3.8%).

Neonectria Lesions

Lesion classes were converted to a continuous scale using the midpoint of each class (e.g. observations of 1-5 lesions recorded were converted to 3 lesions). Our experimental design treated trees as sampling units (n = 120), nutrient plots as experimental units (n = 24) and stands as

replicates (n = 6). One outliers were identified and included in the analysis; omitting it did not change statistical conclusions.

The effect of tree diameter and nutrient addition on tree lesion count was tested using a nested, two-way ANOVA with tree diameter with N and P addition and the interaction of N and P as fixed effects and with forest stand and plot (nested in stand) as random effects. A post hoc test of multiple comparisons of group means (multcomp package in R; Hothorn, Bretz, & Westfall, 2008) with a Holm p-value adjustment was used to evaluate treatment differences. We also used a linear model with number of lesions as the dependent variable and tree diameter as the independent variable to examine whether lesions numbers increased proportionally to tree diameter.

We tested whether lesion appearance date was predicted by treatment by using a linear model and a two-way ANOVA with tree diameter, plot treatment (Control, N, P and N+P), and a treatment interaction with tree diameter as fixed effects. We tested whether the number of lesions differed by stand characteristics using one-way ANOVAs with stand age, slope of the stand, and stand elevation as independent variables.

Results

Cryptococcus fagisuga Scale Cover

Invasive beech scale cover, assessed on 118 trees in six forest stands, ranged from 0 - 8.5%, averaged over 8 photos per tree. Tree diameters ranged from 9.5 - 38.1 cm DBH; larger trees had more scale cover per unit area than smaller trees ($F_{1,103} = 5.49$, $p = 0.02$). The effect of N addition ($F_{1,20} = 0.02$, $p = 0.90$) and P addition ($F_{1,20} = 0.03$, $p = 0.86$) were both positive but not significant (Figure 2). The NP treatment had lower scale cover than predicted by the main effects of N and P,

but this interaction was not significant ($F_{1,20}$ = 1.66, p = 0.21). Height on the bole was not a good predictor of scale cover; scale cover from photos taken at 1.5 m averaged 0.56%, which was indistinguishable from those taken at 0.5 m (0.57%; t = -0.16, p = 0.87 in a paired t-test).

Neonectria Lesions

We observed new lesions on 70 trees (58% of 120 trees sampled) based on observations made every two weeks from September 22 to November 6, 2017. Of the trees with lesions, 76% developed 5 or fewer lesions since the previous visit, two weeks earlier (Figure 3). Lesion counts ranged from 0 – 57.5 per tree (Figure 4). On most of the trees (n = 67), lesions appeared low on the bole, between the ground and 0.5 m. Only three trees showed initial lesion development higher than 0.5 m. Two of them showed lesion development to 1.0 m (both in N additions plots, with one in C3-N and one in C8-NP), and one tree developed lesions up to 1.5 m (in C7-P). Interestingly, the two trees with lesion development up to 1.0 m also had a high number of lesions observed, 27.5 and 57.5 lesions.

Trees in plots receiving P (P and NP) showed twice as many lesions upon initial observation than plots that did not receive P (N and Control; $F_{1,20}$ = 5.02, p = 0.04, for the main effect of P). The effect of N ($F_{1,20}$ = 0.04, p = 0.84) and the interaction of N and P ($F_{1,20}$ = 0.01, p = 0.93) were not statistically significant.

Larger diameter trees developed more lesions ($F_{1,76}$ = 13.0, p < 0.001). While lesions varied significantly by tree diameter ($F_{1,116}$ = 14.6, p < 0.001, R^2 = 0.10), the y-intercept of a linear regression of lesion numbers on tree diameter was not significantly different from zero (-2.7 cm ± 1.9 cm) indicating that the number of lesions was proportional to tree diameter and thus bark surface area (t = -1.37, p = 0.17; Figure 5).

Larger diameter trees developed lesions later in the season ($F_{1,110} = 30.00$, $p < 0.001$). Nutrient addition did not influence the date at which lesions were first observed ($F_{3,110} = 1.15$, $p = 0.33$) and the interaction between tree diameter and treatment was also not significant ($F_{3,110} = 0.39$, $p = 0.76$). Stand age was positive but not very significant ($F_{1,118} = 2.73$, $p = 0.10$, adjusted $R^2 = 0.01$), and slope ($F_{1,98} = 2.70$, $p = 0.10$, adjusted $R^2 = 0.02$), and elevation ($F_{1,118} = 1.14$, $p = 0.29$, adjusted $R^2 = 0.001$) did not impact lesion numbers.

The *Neonectria* samples collected for species identification by spore morphology were 97% *N. faginata*. Only 4 samples were identified as *N. ditissima*, all collected from trees receiving P additions (Table 2). Twenty of the lesions that were identified morphologically as *N. faginata* were confirmed via molecular genetic techniques using Genbank basic local alignment search tool (BLAST) search results (Table 3).

Discussion

The topic of nutritional effects on BBD in aftermath forests has gained attention as increased bark and foliar N and P have been found to be associated with BBD severity (Cale et al., 2015b; Castello & Johnston, 2014; Jönsson, 2000; Latty et al., 2003; Ouimet et al., 2015). These studies linked high N and high N:P in beech tissues with BBD severity. In our study, involving N and P fertilization, we found a different pattern: trees receiving P additions (70 kg P/ha applied over 7 years) had more lesions upon initial development than trees without P. Differences in whether N or P is limiting at different sites might explain why our results differed from previous studies. Most northeastern deciduous forests grow more in response to N than P and are considered N-limited (Vadeboncoeur, 2010) and studies on fungal diseases and plants suggest that N-limited

plants may be more susceptible to infection, although it can depend on the host-disease interaction (Walters & Bingham, 2007). Phosphorus fertilization in our study sites has increased tree diameter growth (Goswami et al., 2018). Other indications of P limitation include low foliar N:P ratios in control plots and N:P resorption ratios and green leaf N:P ratios more affected by the addition of P (Gonzales and Yanai, 2019). If previous studies conducted were in N-limited forests, then perhaps the *Neonectria* disease process was also N-limited and more responsive to N in host tree tissues. To our knowledge, this is the first study on BBD that has occurred in a P-limited forest, where *Neonectria* may be more responsive to P and thus grow more in response.

Elevated lesion development in response to P has been previously documented. A study on juvenile European beech found that saplings growing in soils with excessive N or P, as well as those in deficient soil N or P, had greater coverage of *N. ditissima* lesions compared to controls (Perrin & Garbaye, 1984); this study was different from ours both in the species and age of trees assessed. Studies on other plant diseases also show that soil additions of P can lead to either increased or decreased plant disease (Walters & Bingham, 2007). Plant hosts and pathogens have unique and complex relationships to nutrient availability due to individual variations in host tissue nutrient supplies and the presence of host-defense compounds that are produced (Hoffland, Jeger, & van Beusichem, 2000).

Soil N and P also impacts the morphological responses of fungal pathogens to host plants. In saprophytic fungi, like *Neonectria*, the nutrient levels of host plant tissue alter the pathogen's strategy of resource allocation; low nutrient levels encourage exploration strategies that cause fungal growth to grow radially in search of nutrients while higher nutrient levels result in an exploitation strategy where fungal colonies grow more densely on substrate (Dowson, Springham, Rayner, & Boddy, 1989). We counted lesions; methods that quantify perithecia per unit area of bark may better indicate *Neonectria* responses to nutrient additions.

We found that larger diameter trees had both more scale cover per unit area ($p = 0.02$) and more *Neonectria* lesions per tree ($p < 0.001$). In the case of lesions appearance, we found that lesion numbers were proportional to tree diameter and thus bole surface area. Previous studies on large trees and the causal agents of BBD present consistent information: larger trees are generally found have greater *C. fagisuga* incidence and severity (Cale et al., 2015b; Ehrlich, 1934; Garneau et al., 2012; Houston, 1994a; Latty et al., 2003; Morris et al., 2002), greater *C. fagisuga* carrying capacity (Garnas et al., 2011b), and more *Neonectria* lesions (Ehrlich, 1934; Garnas et al., 2011b; Houston & Valentine, 1988; Latty et al., 2003). One study in an aftermath forest in upstate New York, however, found that scale density and host tree diameter showed no significant relationship (Letkowski, 2009).

Our study failed to support the hypothesis that *C. fagisuga* populations occur closer to the bottom of the bole. This could be due to the range of diameter of our trees, as larger trees (> 25cm) have rougher bark towards the root collar that make suitable habitat for scale (Teale et al., 2009); only 10% of our trees fell into this category. We did observe that lesions started developing on the lowest portion of the bole, below 0.5 m. Because our study was limited to heights on the bole up to 1.5 m, we did not observe lesions developing higher on the bole or on branches.

We were not surprised to find both species of *Neonectria* and more *N. faginata* than *N. ditissima. Neonectria faginata* is known to replace *N. ditissima* as the dominant pathogen in aftermath stands (Houston, 1994b) and infected beech trees have been reported to have both *Neonectria* species (Houston, 1994b; Kasson & Livingston, 2009). Sizing ascospores via microscopy is reliable (Cale et al., 2015b; Castlebury et al., 2006; Cotter & Blanchard,1981; Kasson & Livingston, 2009) and identifying *Neonectria* to species is important for researchers investigating the nutritional impacts on causal agents of BBD, as well as how the pathosystems of *N. ditissima* and *N. faginata* differ (Cale, Garrison-Johnston, Teale, & Castello, 2017;

Houston,1994). We were able to confirm 20 *N. faginata* lesion samples with molecular genetic techniques. Confirming both species via sequencing would have been ideal but samples for DNA analysis were selected randomly and did not result in a sample of the less abundant species.

There are multiple methods for quantifying *C. fagisuga* insects. Early research used qualitative classification schemes that rated trees on an ordinal scale of severity, from none to heavy infestation (Griffin et al., 2003; Houston et al., 1979a; Twery & Patterson, 1984; Wiggins et al., 2004). Direct counts are a quantitative method of assessment but are time consuming, as these insects are only 0.5 to 1.0 mm long as adults (Gardner, 2005; Koch et al., 2010; Teale et al., 2009; Van Driesche & Japoshvili, 2012; Wainhouse, 1980). Recent methods quantify scale populations with digital photography, taking photos in the field and performing analyses in the lab, assessing the area of the wax masses in small, randomly selected areas of the photo (Cale et al., 2012; Teale et al., 2009; Wieferich et al., 2013). This approach to digital analysis samples a very small fraction of bark area, raising concerns about how representative the estimates are of the whole tree. We found this technique difficult to duplicate in a timely manner, taking twice as long as reported by other researchers (~10 minutes per photo to their 5 minutes). Our systematic point counting approach was adapted from methods commonly used for roots (Naples & Fisk, 2010; Newman, 1966; Tennant, 1975), woody debris (Fisk, Zak, & Crow, 2002; Van Wagner, 1968), and fungal hyphal length (Dempsey, Fisk, & Fahey, 2011). We suggest that this method offers a better compromise on accurately evaluating scale per area of bark sampled, the amount of time spent analyzing photos (2-5 minutes per photo), and method reproducibility. This improved method will make monitoring *C. fagisuga* easier for future research on the nutritional impacts on causal agents of BBD.

Chapter 3: Conclusions

This research provides an assessment of how experimental additions of N, P, and N+P influence the causal agents of beech bark disease in an aftermath forest in New Hampshire, USA. Previous research in this field has shown that nutrient imbalances in beech trees lead to increased severity of BBD; high N and high N:P ratios in beech tree bark and leaves have been linked to increased *C. fagisuga* and *Neonectria* populations on trees. This project adds even more complexity to previous research about the nutritional effects on BBD. We found that trees receiving P additions had more of lesions that appeared during four trips to study trees in the fall of 2017. *Neonectria* lesions than trees not receiving P. The study site on which these experiments were conducted has indications of being P-limited. Temperate forests have traditionally been considered N-limited, but recent ecosystem studies on nutrient limitation suggest that P or N limitation is possible. To our knowledge, this is the first study on BBD that has occurred in a P-limited forest. Quantitative studies on the causal organisms of BBD in aftermath forests are important as BBD continues to advance throughout the range of American beech in North America. Ecosystem-level changes in stand and species composition and nutrient cycling have been linked to BBD and are expected to shape our forests for the upcoming centuries. Continued analysis on how nutrient imbalances impact trees may provide key insights into the factors that worsen this disease and others like it. Definitive answers on this subject will have important management applications both in forest and agricultural settings.

References

Adams, M. B., Loughry, L. H., & Plaugher, L. L. (2003). *Experimental forests and ranges of the USDA Forest Service* (General Technical Report NE-321).

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein databse search programs. *Nucleic Acids Research, 25*, 3389-3402.

Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Wheeler, D. L. (2008). GenBank. *Nucleic Acids Research, 36*(1), D25–D30. Retrieved from https://doi.org/10.1093/nar/gkm929

Brown, K. S. (1975). The Chemistry of Aphids and Sucking Insects. *London Chem. Soc. Rev., 4*, 263-288.

Brown, R.C. (1934). Notes on the beech scale, Cryptococcus fagi (Baer.) Dougl., in New England. *J. Econ. Entomol., 27*, 327-334.

Burns, B. S., & Houston, D. R. (1987). Managing beech bark disease: evaluating defects and reducing losses. *Northern Journal of Applied Forestry 4*, 28-33.

Cale, J. A., Ashby, A.W., West, J. L, Teale, S. A., Johnston, M. T., & Castello, J. D. (2015a). Scale insects, decay and canker fungi in American beech, *For. Pathol., 45*, 71-75. https://doi-org.esf.idm.oclc.org/10.1111/efp.12127

Cale, J. A., Garrison-Johnston, M. T., Teale, S. A., and Castello, J. D. (2017). Beech bark disease in North America: Over a century of research revisited. *Forest Ecology and Management*, 394, 86-103. doi:10.1016/j.foreco.2017.03.031.

Cale, J. A., Letkowski, S. K., Teale, S. A., & Castello, J. D. (2012). Beech bark disease: an evaluation of the predisposition hypothesis in an aftermath forest. *For. Pathol., 42*, 52-56.

Cale, J. A., Teale, S. A., Johnston, M. T., Boyer, G. L., Perri, K. A., & Castello, J. D. (2015b). New ecological and physiological dimensions of beech bark disease development in aftermath forests. *For. Ecol. Manage., 336*, 99-108.

Castello, J. D., & Johnston, M. T. (2014). *An alternative model of beech bark disease*. Retrieved from: http://nsrcforest.org/sites/default/files/uploads/castellocale13full.pdf

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.

Chaverri, P., Salgado, C., Hirooka, Y., Rossman, A. Y., & Samuels, G. J. (2011). Delimitation of Neonectria and Cylindrocarpon (Nectriaceae, Hypocreales, Ascomycota) and related genera with Cylindrocarpon-like anamorphs. *Stud. Mycol., 68*, 57-78.

Cotter, H.V.T., & Blanchard, R.O. (1981). Identification of the two *Nectria* taxa causing bole cankers on American beech. *Plant Disease, 65,* 332–334.

Dempsey, M. A., Fisk, M. C., & Fahey, T. J. (2011). Earthworms increase the ratio of bacteria to fungi in northern hardwood forest soils, primarily by eliminating the organic horizon. *Soil Biol. Biochem., 43* (10), 2135-2141.

Department of Agriculture. (2018). *National Diagnostic Protocol for Detection of Neonectria ditissima (European canker).* Retrieved from https://www.plantbiosecuritydiagnostics.net.au/app/uploads/2018/11/NDP-21-European-canker-Neonectria-ditissima-V1.2.pdf

Dordas, C. (2008). *Agron. Sustain. Dev.*, *28,* 33. https://doi.org/10.1051/agro:2007051

Dowson, C. G., Springham, P., Rayner, A. D. M., Boddy, L. (1989). Resource relationships of foraging mycelial systems of *Phanerochaete velutina* and *Hypholoma fasiculare* in soil. *New Phytologist, 111,* 501 – 509.

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

Fernandez, M. R., & Boyer, M. G. (1988). Beech bark disease — a survey of the Toronto area. *Can. Plant Dis. Surv., 68,* 157–159.

Fisk, M. C., Ratliff, T. J., Goswami, S., & Yanai, R. D. (2014). Synergistic soil response to nitrogen plus phosphorus fertilization in hardwood forests. *Biogeochemistry, 118,* 195–204.

Fisk, M. C., Zak, D. R., & Crow, T. R. (2002). Nitrogen storage and cycling in old- and second-growth northern hardwood forests. *Ecology, 83,* 73–87.

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Mol. Ecol., 2,* 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Gardner, G. (2005). *Population and large-scale dispersal dynamics in the beech scale insect Cryptococcus fagisuga.* (Gen. Tech. Rep. NE-331). Retrieved from USDA Forest Service: https://www.fs.fed.us/ne/newtown_square/publications/technical_reports/pdfs/2005/ne_gtr331.pdf

Garnas, J. R. (2009). *Organism interactions and forest structural change in a nonnative disease complex: Beech bark disease in north america* (Order No. 3397908). Available from ProQuest Dissertations & Theses Global. (230880734). Retrieved from https://search.proquest.com/docview/230880734?accountid=14214

Garnas, J., Houston, D., Ayres, M., & Evans, C. (2009). *Population dynamics of the felted beech scale and associated Neonectria species, causal agents of beech bark disease* (Gen. Tech. Rep. NRS-P-51). Retrieved from https://www.nrs.fs.fed.us/pubs/gtr/gtr-nrs-p-51papers/44garnas-p-51.pdf

Garnas, J. R., Ayres, M. P., Liebhold, A. M., & Evans, C. (2011a). Subcontinental impacts of an invasive tree disease on forest structure and dynamics. *J. Ecol., 99,* 532-541.

Garnas, J. R., Houston, D. R., Ayres, M. P., & Evans, C. (2011b). Disease ontogeny overshadows effects of climate and species interactions on population dynamics in a nonnative forest disease complex. *Ecography, 34,*1-10.

Garnas, J. R., Houston, D. R., Twery, M. J., Ayres, M. P., & Evans C. (2013). Inferring controls on the epidemiology of beech bark disease from spatial patterning of disease organisms. *Agric. For. Entomol., 15,* 146-156. 10.1111/j.1461-9563.2012.00595.x

Garneau, D. E., Lawler, M. E., Rumpf, A. S., Weyburne, E. S., Cuppernull, T. M., & Boe, A. G. (2012). Potential effects of beech bark disease on small mammals and invertebrates in northeastern US forests. *Northeast. Nat., 19,* 391–410.

Giencke, L. M., Dovčiak, M., Mountrakis, G., Cale, J. A., & Mitchell, M. J. (2014). Beech bark disease: spatial patterns of thicket formation and disease spread in an aftermath forest in the northeastern United States. Canadian Journal of Forest Research, 44(9), 1042–1050. https://doi-org.esf.idm.oclc.org/10.1139/cjfr-2014-0038

Gonzales, K. E., & Yanai, R. D. (2019). Nitrogen-phosphorus interactions in young northern hardwoods indicate P limitation: foliar concentrations and resorption in a factorial N by P addition experiment. *Oecologia, 189*(3), 829-840. doi:10.1007/s00442-019-04350-y.

Goswami, S., Fisk, M. C., Vadeboncoeur, M. A., Garrison-Johnston, M., Yanai, R. D., & Fahey, T. J. (2018). Phosphorus limitation of aboveground production in northern hardwood forests. *Ecology, 99,* 438–449.

Gove, J. H., & Houston, D. R. (1996). Monitoring the growth of American beech affected by beech bark disease in Maine using the Kalman filter. *Environ. Ecol. Stat.,* 3, 167–187.

Gradowski, T., & Thomas, S. C. (2008). Responses of Acer saccharum canopy trees and saplings to P, K and lime additions under high N deposition. *Tree Physiol., 28,* 173–185.

Griffin, J. M. (2005). *The landscape pathology of beech bark disease in the Catskill Mountains, NY: the effects of land use history on disease progression, and the response of sugar maple to beech decline.* PhD Thesis, University at Albany, Albany, NY.

Griffin, J. M., Lovett, G. M., Arthur, M. A., & Weathers, K. C. (2003). The distribution and severity of beech bark disease in the Catskill Mountains, NY. *Can. J. For. Res., 33,* 1754–1760.

Gwiazdowski, R. A., Van Driesche, R. G., Desnoyers, A., Lyon, S., Wu, S., Kamata, N., & Normark, B. B. (2006). Possible geographic origin of beech scale, *Cryptococcus fagisuga* (Hemiptera: Eriococcidae), an invasive pest in North America. *Biological Control, 39,* 9-18.

Hirooka, Y., Rossman, A. Y., Zhuang, W. Y., & Salgado-Salazar, C. (2013). Species delimitation for Neonectria coccinea group including the causal agents of beech bark disease in Asia, Europe, and North America. *Mycosystema, 32*, 485-517.

Hoffland, E., Jeger, M. J., & van Beusichem, M. L. (2000). Effect of nitrogen supply rate on disease resistance in tomato depends on the pathogen. *Plant and Soil, 218*, 239 – 247.

Horton, T. R., & Bruns, T. D. (2001). The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology, 10*(8), 1855-1871.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous Inference in General Parametric Models. *Biometrical Journal, 50*(3), 346-363.

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. (1983). *American beech resistance to Cryptococcus fagisuga* (Gen. Tech. Rep. WO-37). Retrieved from https://www.fs.fed.us/nrs/pubs/gtr/gtr_wo37/11_houston_wo37.pdf

Houston, D. R. (1994a). Major new tree disease epidemics beech bark disease. *Annu. Rev. Phytopathol., 32*, 75-87.

Houston, D. R. (1994b). Temporal and spatial shift within the *Nectria* pathogen complex associated with beech bark disease of *Fagus grandifolia. Can. J. For. Res., 24*, 960-968.

Houston, D. R., & O'Brien, J. T. (1983). Beech bark disease. Retrieved from https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/fsbdev2_043310.pdf

Houston, D. R., Parker, E. J., & Lonsdale, D. (1979a). 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., Parker, E. J., Perrin, R., & Lang, K. J. (1979b). Beech bark disease: A comparison of the disease in North America, Great Britain, France and Germany. *European Journal of Forest Pathology, 9*, 199-211.

Houston, D. R., Rubin, B. D., Twery, M. J., & Steinman, J. R. (2005). *Spatial and Temporal Development of Beech Bark Disease in the Northeastern United States* (Gen. Tech. Rep. NE-331). Retrieved from: https://www.fs.fed.us/ne/newtown_square/publications/technical_reports/pdfs/2005/331papers/houston331-2.pdf

Houston, D. R., & Valentine, H. T. (1988). Beech bark disease: the temporal pattern of cankering in aftermath forests of Maine. *Can. J. For. Res., 18*, 38-42.

Jones, R. H., & Raynal, D. J. (1986). Spatial distribution and development of root sprouts in *Fagus grandifolia* (Fagaceae). *Am. J. Bot, 73*, 1723-1731. Doi: 10.2307/2444238

Jones, R. H., & Raynal, D. J. (1987). Root sprouting in American beech: production, survival, and the effect of parent vigor. *Can. J. For. Res.*, *17*, 539–544.

Jönsson, A. M. (2000). Soil treatment effects on bark lesions and frost sensitivity of beech (*Fagus sylvatica*) in southern Sweden. *Forest Ecology and Management*, *129* (1-3), 167-175.

Kasson, M., & Livingston, W. (2009). Spatial Distribution of Neonectria Species Associated with Beech Bark Disease in Northern Maine. *Mycologia, 101*(2), 190-195. doi: 10.3852/08-165

Ko, T. W. K., Stephenson, S. L., Bahkali, A. H., & Hyde, K. D. (2011). From morphology to molecular biology: can we use sequence data to identify fungal endophytes? *Fungal Diversity*, *50*(1), 113-120.

Koch, J. L., Carey, D. W., Mason, M. E., & Nelson, C. D. (2010). Assessment of beech scale resistance in full- and half-sibling American beech families. *Can. J. For. Res.*, *40*, 265-272.

Kosztarab, M. (1996). Scale insects of northeastern North America: identification, biology, and distribution. Martinsville: Virginia Museum of Natural History.

Lea, P. J. (1992). Ammonia assimilation in higher plants. In: Mengel K and Pilbeam DJ (Eds.), *Nitrogen Metabolism of Plants* (153 – 156). Oxford UK: Oxford university Press.

Le Guerrier, C., Marceau, D. J., Bouchard, A., & Brisson, J. (2003). A modelling approach to assess the long-term impact of beech bark disease in northern hardwood forest. *Canadian Journal of Forest Research*, *33*(12), 2416–2425. https://doi-org.esf.idm.oclc.org/10.1139/X03-170

Lonsdale, D. (1980). Nectria coccinea infection of beech bark: variations in disease in relation to predisposing factors. *Annls. For. Sci.*, *37*, 307-317.

Lovett, G. M., Arthur, M. A., Weathers, K. C., & Griffin, J. M. (2010). Long-term changes in forest carbon and nitrogen cycling caused by an introduced pest/pathogen complex. *Ecosystems, 13*, 1188–1200.

Lovett, G. M., Weathers, K. C., Arthur, M. A., & Schultz, J. C. (2004). Nitrogen cycling in a northern hardwood forest: do species matter? *Biogeochemistry*, *67*, 289-308.

Latty, E. F. (2004). *Stand-level patterns and ecosystem consequences of beech bark disease* (Gen. Tech. Rep. NE331). Retrieved from: https://www.fs.fed.us/ne/newtown_square/publications/technical_reports/pdfs/2005/331papers/latty331.pdf

Latty, E., & Canham, C., & Marks, P. (2003). Beech bark disease in northern hardwood forests: the importance of nitrogen dynamics and forest history for disease severity. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere. 33*. 257-268. doi: 10.1139/x02-183.

Letkowski, S. K. (2009). Beech bark disease: The relationship between scale and neonectria lesion densities in an aftermath forest (Order No. 1482177). Available from Dissertations & Theses @ SUNY College of Environmental Science and Forestry. (757376731). Retrieved from https://search-proquest-com.esf.idm.oclc.org/docview/757376731?accountid=26746

MacKenzie, M. (2005). Survival of the fittest: beech bark disease-resistant trees will leave more offspring. *In* Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium, Saranac Lake, New York, 16-18 June 2004. *Edited by* C.A. Evans, J.A. Lucas, and M.J. Twery. USDA Forest Service, Northeastern Research Station, Gen. Tech. Report. NE-331. 65-67.

Mason, M. E., Koch, J. L., Krasowski, M., & Loo, J. (2013). Comparisons of protein profiles of beech bark disease resistant and susceptible American beech (*Fagus grandifolia*). *Proteome Science*, *11*(2), 1-21. doi:10.1186/1477-5956-11-2

Melillo, J. M., Aber J. D., & Muratore, J. F. (1982). Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology*, *63*, 621

Mize, C. W., & Lea, R. V. (1979). The effect of beech bark disease on the growth and survival of beech in northern hardwoods. *Euro. J. For. Pathol*., *9*, 243–248.

Moore, J. A., Mika, P. G., Schwandt, J. W., & Shaw, T. M. (1994). *Nutrition and forest health, Interior Cedar-Hemlock-White Pine Forest: Ecology and Management*, Washington State Univ, Pullman, 173-176.

Morin, R. S, & Liebhold, A.M. (2015). Invasions by two non-native insects alter regional forest species composition and successional trajectories. *For. Ecol. Manage.*, *341*, 67-74. doi:10.1016/j.foreco.2014.12.018

Morin, R. S., Liebhold, A. M., Tobin, P. C., Gottschalk, K. W., & Luzader, E. (2007). Spread of beech bark disease in the eastern United States and its relationship to regional forest composition *Can. J. For. Res.*, *37*(4). 726-736. Retrieved from https://link-gale-com.esf.idm.oclc.org/apps/doc/A167696900/PPES?u=sunycesfsc&sid=PPES&xid=81287559

Morris, A. B., Small, R. L., & Cruzan, M. B. (2002). Investigating the relationship between Cryptococcus fagisuga and Fagus grandifolia in Great Smoky Mountains National Park. *Southeast. Nat.*, *1*, 415–424.

Naples, B. K., & Fisk, M. C. (2010). Belowground insights into nutrient limitation in northern hardwood forests. *Biogeochemistry*, *97*(2), 109-121. https://doi.org/10.1007/s10533-009-9354-4

Newman, E.I. (1966). A method of estimating the total length of root in a sample. *Journal of Applied* Ecology, 3(1), 139-145.

Nieminen, K., Blomster, T., Helariutta, Y., & Pekka Mähönen, A. (2015). Vascular Cambium Development. *The Arabidopsis Book,* (13). https://doi.org/10.1199/tab.0177

Ostrofsky, W. D., & Blanchard, R. O., (1983). *Characteristics and development of necrophylactic periderms in mature bark of American beech* (Gen. Tech. Rep. WO-37). Retrieved from https://www.fs.fed.us/nrs/pubs/gtr/gtr_wo37/16_ostrofsky_wo37.pdf

Ostrofsky, W. D., & McCormack Jr., M. L. (1986). Silvicultural management of beech and the beech bark disease. *Northern Journal of Applied Forestry, 3*, 89-91.

Ostrofsky, W. D., Shortle, W. G., & Blanchard, R. O. (1984). Bark phenolics of American beech (*Fagus grandifolia*) in relation to the beech bark disease. *European Journal of Forest Pathology, 14*(1):52-59.

Ouimet, R., Duchesne, L., & Moore, J.-D. (2015). Relation entre la composition foliaire et la présence de la maladie corticale du hêtre dans les stations du Réseau d'étude et de surveillance des écosystèmes forestiers du Québec, *Phytoprotection, 95*(1), 32-37. doi:10.7202/1031956ar

Parker, E. J. (1977). Some investigations with beech bark disease *Nectria* in southern England. *European Journal of Forest Pathology, 5*, 118-124.

Perrin, R. (1980). Contribution to the knowledge of beech bark disease etiology II. Experimental study of *Cryptococcus fagisuga* Lind-*Nectria coccinea* (Pers ex Fries) Fries association, respective part of each organism. *Ann. For. Sci*., *37*, 319-331.

Perrin, R., & Garbaye, J. (1984) Influence de la nutrition du hetre (Fagus sylvatica L.) sur la sensibilite au chancre provoque par Necria ditissima *Tul. Ann. Sci. For*., *41*, 449 – 460.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2019). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-142, https://CRAN.R-project.org/package=nlme.

R Core Team. (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Robinson, D., & Hayes, A. (2018). Broom: Convert Statistical Analysis Objects into Tidy Tibbles. R package version 0.5.0. https://CRAN.R-project.org/package=broom

Runkle, J. R. (2005). Twenty-four years of change in an old Tsuga canadensis woods affected by beech bark disease. *J. Torrey Bot. Soc.*, *132*, 483–491.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671-675.

Shigo, A. L. (1962). Another Scale Insect on Beech. Station Paper Number 168.

Shigo, A. L. (1964). Organism interactions in the beech bark disease. Phytopathology 54:263-269.

Shigo, A. L. (1972). The beech bark disease today in the northeastern U.S. *J. For.* 70, 286–289.

Spaulding, P., Grant, T. J., & Ayers, T. T. (1936). Investigations of Nectria diseases in hardwoods of New England. Journal of Forestry, 34, 169–179.

Stephanson, C. A., & Coe, N. R. (2017). Impacts of Beech Bark Disease and Climate Change on American Beech. *Forests*, *8*(5), 1-16. doi:10.3390/f8050155

Teale, S. A., Letkowski, S., Matusick, G., Stehman, S. V., & Castello, J. D. (2009). Quantitative, nondestructive assessment of beech scale (Hemiptera: Cryptococcidae) density using digital image analysis of wax masses. *Environ. Entomol., 38*, 1235–1240.

Tennant, D. (1975). A Test of a Modified Line Intersect Method of Estimating Root Length. *Journal of Ecology, 63*(3), 995-1001. doi:10.2307/2258617

The GIMP Development Team. (2019). *GIMP*. Retrieved from https://www.gimp.org

Twery, M. J., & Patterson, W. A. (1984). Variations in beech bark disease and its effects on species composition and structure of northern hardwood stands in central New England. *Can. J. For. Res., 14*, 565–574.

Vadeboncoeur, M.A. (2010). Meta-analysis of fertilization experiments indicates multiple limiting nutrients in northeastern deciduous forests. *Can. J. For. Res., 40*, 1766-1780. doi:10.1139/X10-127

Vadeboncoeur, M. A., Hamburg, S. P., Blum, J. D., Pennino, M. J., Yanai, R. D., & Johnson, C. E. (2012). The Quantitative Soil Pit Method for Measuring Belowground Carbon and Nitrogen Stocks. *Soil Sci. Soc. Am. J., 76*, 2241-2255. doi:10.2136/sssaj2012.0111

Vadeboncoeur, M. A., Hamburg, S. P., Yanai, R. D., & Blum, J. D. (2014). Rates of sustainable forest harvest depend on rotation length and weathering of soil minerals. *For Ecol Manag., 318*, 194–205.

Van Driesche, R. G., & Japoshvili, G. (2012). Comparative Densities of Beech Scale, *Cryptococcus fagisuga*, (Hemiptera: *Eriococcidae*) in the Country of Georgia and Massachusetts (USA), Parts of its Native and Invaded Ranges, on two Species of Beech. *Florida Entomologist, 2*: 422-427. doi:10.1653/024.095.0224

Van Wagner, C. E. (1968). The line intersect method in forest fuel sampling. *Forest Science, 14*, 20–26.

Wainhouse, D. (1980). Dispersal of first instar larvae of the felted beech scale, *Cryptococcus fagisuga.* J. Appl. Ecol. 17. 3:523-532.

Wainhouse, D., & Gate, I. M. (1988). The beech scale, pp. 76-85. In A. A. Berryman (ed.), *Dynamics of Forest Insect Populations*. Plenum, New York, NY.

Walters, D. and Bingham, I. (2007). Influence of nutrition on disease development caused by fungal pathogens: implications for plant disease control. *Annals of Applied Biology, 151*, 307-324. doi:10.1111/j.1744-7348.2007.00176.x

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.

Welch, D. S. (1934). The range and importance of *Nectria* canker on hardwoods in the northeast. *J. For., 32*, 997–1002.

Wickham, H. (2017). Tidyverse: Easily Install and Load the 'Tidyverse'. R package version 1.2.1. https://CRAN.R-project.org/package=tidyverse

Wieferich, D. J., Hayes, D. B., & McCullough, D. G. (2013). Evaluation of digital photography for quantifying Cryptococcus fagisuga (Hemiptera: Eriococcidae) density on American beech trees. *Journal of Economic Entomology*, *106*(3), 1324-1330. doi: 10.1603/EC12381

Wieferich, D., McCullough, D., Hayes, D., & Schwalm, N. (2011). Distribution of American Beech (Fagus grandifolia) and Beech Scale (Cryptococcus fagisuga Lind.) in Michigan from 2005 to 2009. *Northern Journal of Applied Forestry*, *4*, 173-179.

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. *Environ. Entomol*., *33*, 1274–1281.

Table 1. Characteristics of the six northern hardwood stands in this study

Stand	Year cut	Latitude	Longitude	Elevation	Slope	beech BA*	non-beech BA*
C2	1988	44º 04' N	71° 16' W	340	15-30%	0.4	1.2
C3	1980	44º 02' N	71° 18' W	590	8-20%	1.5	4.6
C4	1978	44º 03' N	71° 16' W	410	20-25%	0.4	8.2
C6	1975	44º 02' N	71° 16' W	460	13-20%	1.1	8.4
C7	~1890	44º 03' N	71° 18' W	440	5-10%	6.2	5.1
C8	1883	44º 03' N	71° 18' W	330	5-35%	4.6	7.0

* basal area (BA, $m2 \cdot ha–1$)

Table 2. ***Neonectria*** **lesion samples (n = 149) from trees (n = 65) collected in treatment plots and identified to species by microscopy as** ***N. faginata.*** **Samples identified as** ***N.ditissima*** **are in parenthesis.**

	C2		C3		C4		C6		C7		C8	
	Trees	Lesions	Trees	Lesions	Trees	Lesions	Trees	Lesions	Trees	Lesions	Trees	Lesions
C	0	0	5	14	2	6	1	3	4	9	2	4
N	2	3	4	9	1	2	0	0	4	9	5	11
P	2	5	5	12 (2)	4	11	1	3	3	6	5	7 (1)
NP	0	0	5	10 (1)	1	2	3	7	3	7	3	5

Table 3. DNA was extracted from 20 samples collected from tree lesions in an aftermath forest in Bartlett, NH. All samples exhibited the same patterns during restriction fragment length polymorphism (RFLP) analysis. BLAST results confirmed identification of the fungus as *Neonectria faginata*.

Final Designation	Accession	Alignment length	Total score	Gaps	Expected value	% Identity
N. faginata	DQ789685.1	132	183	0 %	5e-43	100%

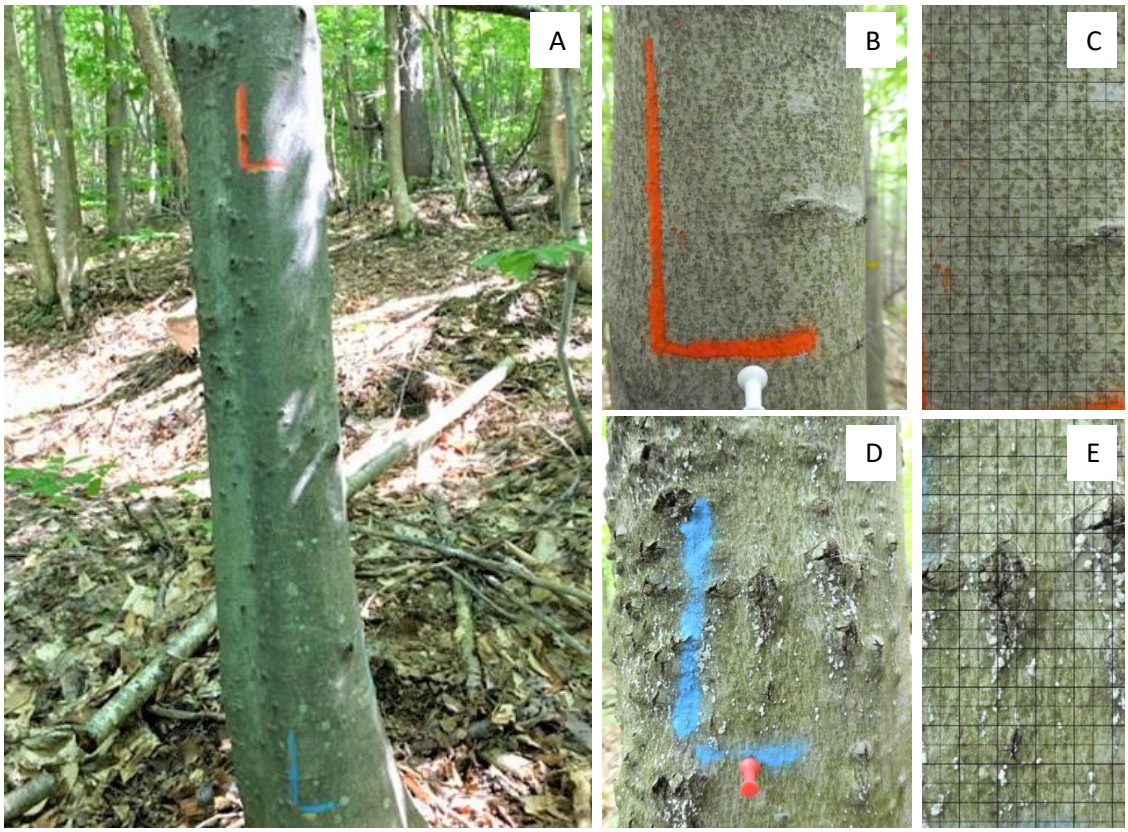

Figure 1. Trees were assessed for *Cryptococcus fagisuga* coverage. Photos taken at two heights on the bole (A, B, D) were analyzed using ImageJ and GIMP software. A 200-intersection grid was superimposed onto a 50 cm^2 area of the photo, guided by painted "L" frames, and the intersections with wax masses counted. Photo C shows 0% cover while photo E has 11% cover.

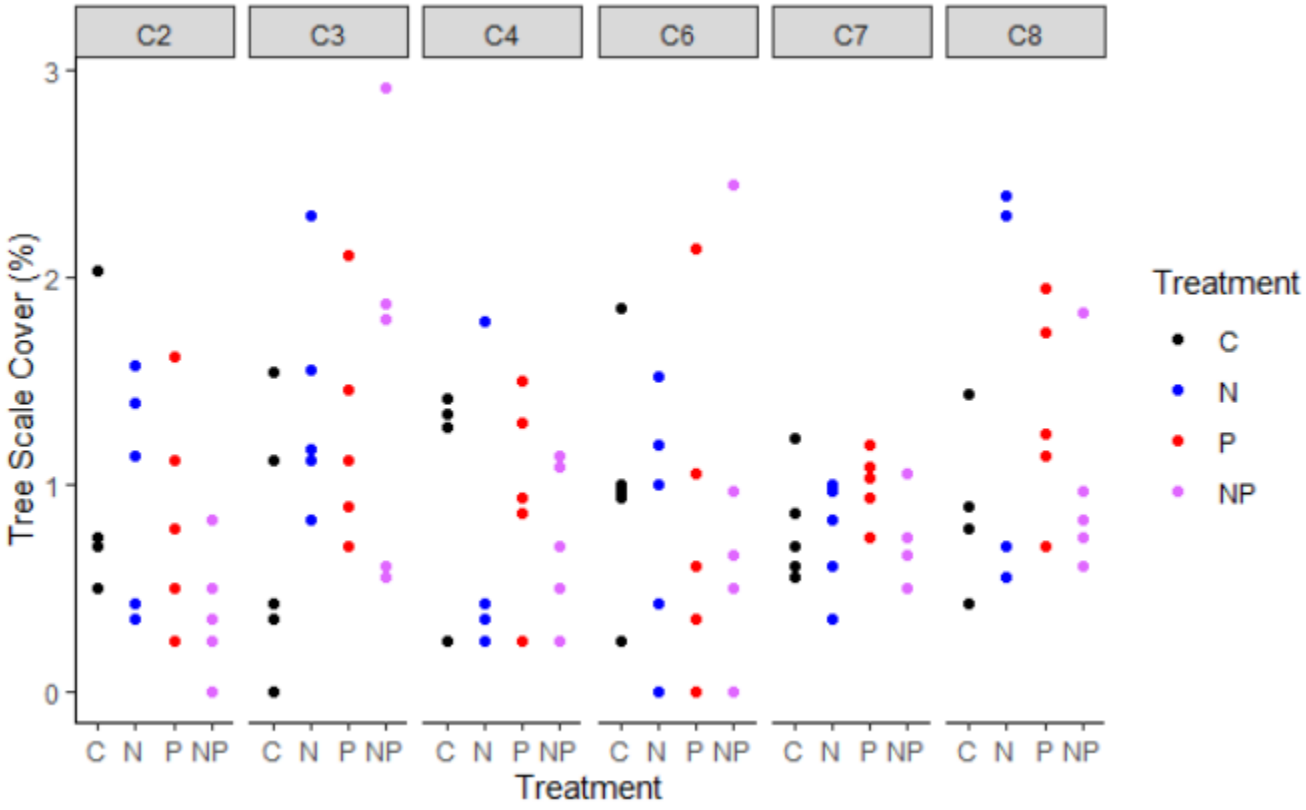

Figure 2. Scale cover observed on trees in six forested stands (C2 – C8), each containing treatment plots with added N, P, both, or neither (control).

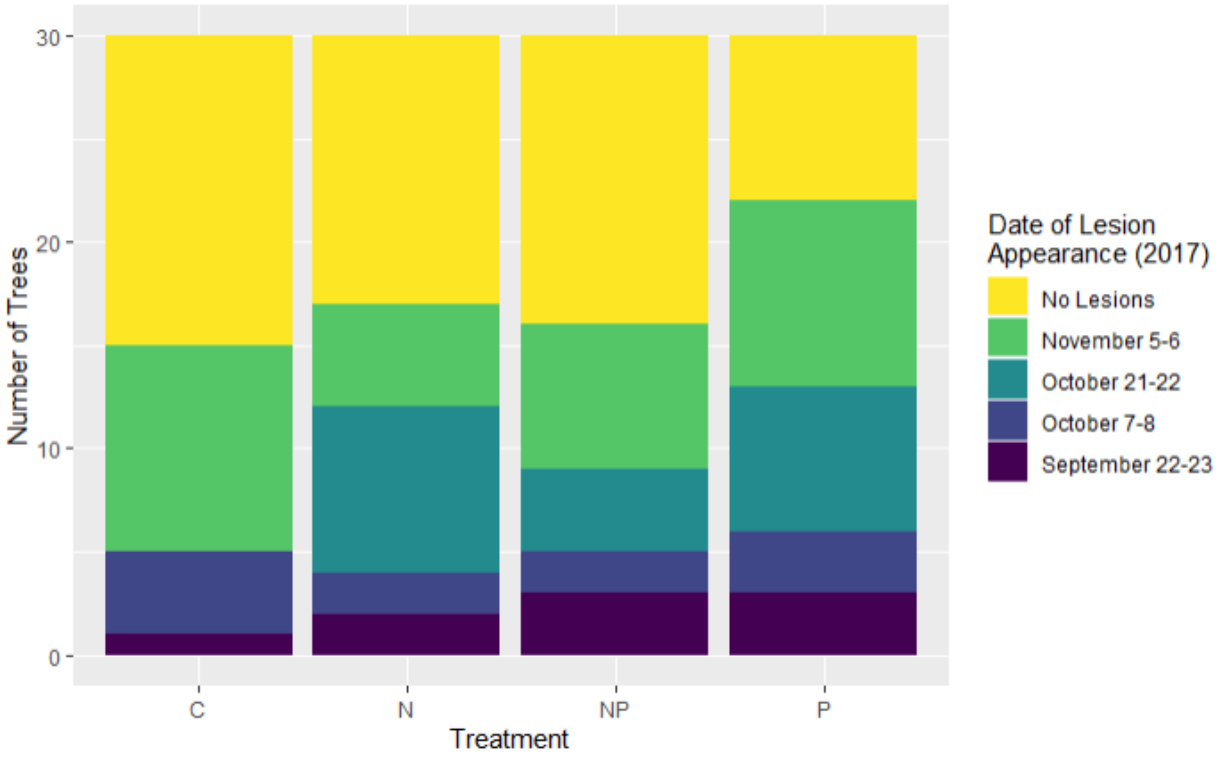

Figure 3. Number of trees exhibiting new lesion development over the 4 trips.

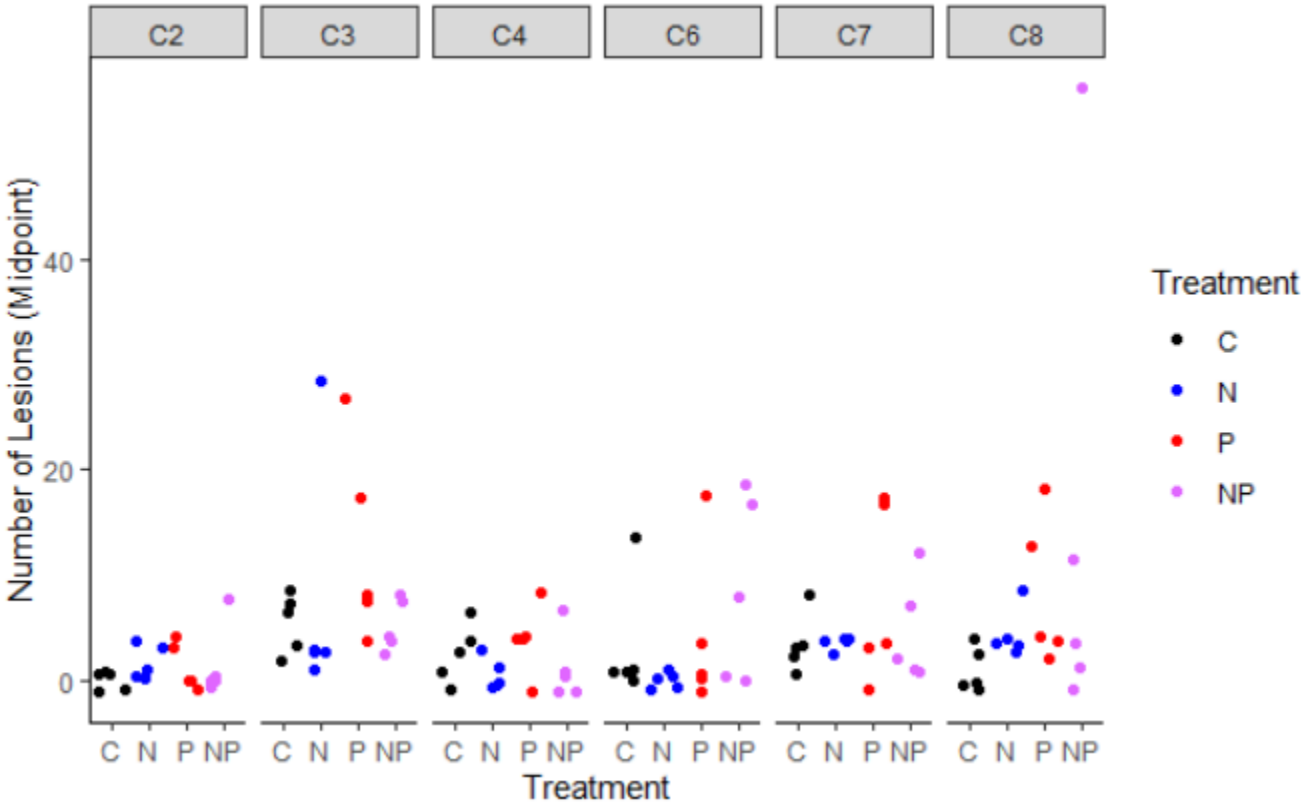

Figure 4. Numbers of lesions on each tree at first appearance.

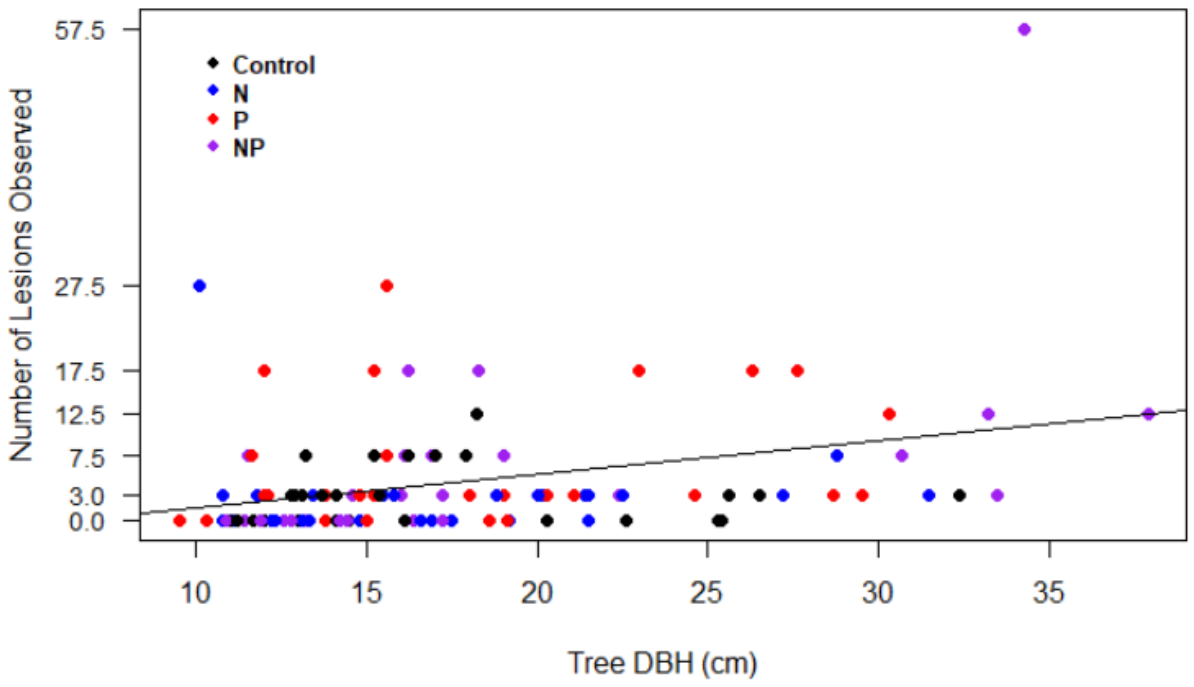

Figure 5. ***Neonectria*** **lesions were recorded in classes as observed in our research site in Bartlett Experimental Forest in New Hampshire, USA. Lesions increased with tree diameter and were proportional to bark surface area.**

Appendices

Appendix 1. Preparation for DNA extraction

The following protocol was used to extract DNA from samples containing up to 0.25g material. This method was provided by Thomas Horton as adapted from Gardes and Bruns (1993).

1) Add 300µl of 2% CTAB buffer to a labelled 1.5 ml Eppendorf tube containing mycorrhizal root tips or spore bearing tissue

2) Soften tissue by freezing (use -20° C freezer or dry ice or liquid nitrogen) and thawing (65° C) 2 times. Crush with a micropestle. Freeze thaw 1 more time.

3) Incubate 65° C for 45 minutes to 1 hour.

4) Add 300 µl of chloroform. Vortex for about 5-10 seconds.

5) Centrifuge at 13000 g (high speed) for 15 minutes at room temperature. Label new clean Eppendorf tubes for step 6.

6) Remove the upper phase and transfer it to a new, labeled, Eppendorf tube. **DO NOT TAKE UP ANY OF THE LOWER PHASE** If you do, centrifuge again for about 5 minutes and try again.

7) Precipitate DNA with 500µl cold isopropanol (-20°C). Put samples in freezer for at least 3 hours.

8) Centrifuge at 13000g for 10 minutes (in a cold room if available).

9) Carefully discard supernatant and wash pellet with 500 µl of cold 70% ethanol.

10) Centrifuge for 5 minutes.

11) Discard supernatant and let residual alcohol air dry or place in Speedvac.

12) Resuspend pellet in TE (50 to 100 µl). Then, for PCR dilute 1µl in 100 (10-3), trying other dilutions as needed (10-2, 10-4, etc.)

Appendix 2. Details of field research methods

The details of my field methods may prove useful to future researchers.

For imaging, colored push pins were pressed into the bark to indicate the cardinal direction (red for north, white for east, blue for south, and yellow for west); this served as an aid to easy photo sorting. Photo order on the camera was: an introductory picture to indicate a new day of photos, the tree tag, top level in north, east, south, west then lower level in north, east, south, west. Three or more pictures per location were taken to obtain a clear one. No photos were deleted in the field. Photos were sorted upon returning from the field so that one photo per frame, eight per tree, remained.

Quality control involving the collaborative efforts of more than a dozen volunteer technicians involved verbal and written instruction in the field, in-field and in-lab demonstrations, in-field and in-lab coached training, and spot checking of work once the technician was independent. Technicians were asked to demonstrate techniques and to explain techniques and theory to peers to assess their skill and knowledge. When in doubt, collections or identifications were repeated to ensure accuracy.

Curriculum Vita

Gretchen A. Dillon

310 Kensington Road, Syracuse, NY 13210 | (412) 849-8747 | GretchenADillon@gmail.com

EDUCATION

SUNY College of Environmental Science and Forestry, Syracuse, NY (Expected 12/2019)
Master of Science, Forest Ecology and Ecosystems in Natural Resource Management GPA: 3.7

The Maxwell School, Syracuse University, Syracuse, NY (Expected 12/2019)
Master of Public Administration with certificate in Conflict and Collaboration GPA: 3.8

The Pennsylvania State University, University Park, PA 12/2007
Bachelor of Science, Animal Science with Business/Management focus GPA: 3.5

RELEVANT COURSEWORK

- Public Budgeting
- International Management & Leadership
- Managing Interpersonal, Group, & Systemic Conflict
- Managing and Archiving Research
- Leadership and Public Policy
- Negotiation Theory and Practice

SKILLS

Interpersonal: public speaking, meeting facilitation, collaborative problem solving, sales
Computational: Word, PowerPoint
Statistical software: R, Stata, Excel
Information visualization: R, Excel, Adobe Illustrator, Tableau

HONORS & AWARDS

Albert & Barbara Cline Silviculture Scholarship Scholar ($1,300; 2018-2019 academic year)
Syracuse University Honor Society (2017/2018/2019)
Airbnb Super Host status (2017/2018/2019)
National Income Life Insurance Company Manager of the Month (2015)
Bank of America Quarterly Business Award (2010)
Bank of America Emerging Leader for Learning and Leadership Development Program (2010)
Sustainable Crop Protection in Agriculture (SUSPROT) Program, Belgium/France (2007)
American Society of Animal Science Undergraduate Scholar award (2006)

WORKSHOPS & TRAININGS

Conflict Management Training (16 hours); The Maxwell School, Syracuse University 4/2019
(1) Conflict Styles and Reflective Listening, (2) Interest-Based Problem Solving, (3) Group Facilitation, and (4) Cross-Cultural Communication; Administered by Syracuse University's Program for the Advancement of Research on Conflict and Collaboration (PARCC)

Alternative Dispute Resolution (ADR) Scrimmage (10 hours); Cornell University 4/2019
Mock pre-arbitration mediation case regarding unionized employee dismissal.

Professional Grant Development Workshop (16 hours); Cornell University 2/2019

Committee to Assess Building Space Usage (25 hours); SUNY- ESF 2018

Faculty-appointed position to assess and recommend improvements to reallocate graduate student space usage in Bray Hall, SUNY-ESF. Poster and report. 2019

ACADEMIC AND RELATED PROJECTS

Increasing Capital Project Planning and Delivery Efficiency for Public Libraries. Graduate Consultant, MPA Group Capstone Project. Contracted by New York City Department of Design and Construction to apply systems thinking to: identify causes of construction delay and cost overruns, identify improvements for agency management, citywide systems, local systems, state law improvements, and alternative delivery mechanisms. Developed cost benefit analysis, matrixed program management paradigm. 2019.

Committee to Assess Graduate Student Space Usage. Faculty-appointed position to assess and recommend improvements to reallocate graduate student space usage in Bray Hall, SUNY-ESF. Poster and report submitted to Dr. Christopher Nowak. 2019.

Conflict Analysis: Shaffer Equipment Company in Minden, WV. Paper for Fundamentals of Conflict Studies (Dr. Catherine Gerard, Syracuse University). Generation of conflict timeline, interdisciplinary analysis of conflict drivers, and proposed interventions; created logframe. 2018.

Enhancing Perinatal Care & Reducing Maternal/Infant Mortality in Niger. Paper for International Management and Leadership (Dr. J. E. Beagles, Syracuse University). Developed an international non-profit project and assessment plan. Created problem/solution trees, logframe, stakeholder Analysis, Gantt Chart, and budget. 2018.

FELLOWSHIPS & ASSISTANTSHIPS

Graduate Colloquium on Teaching and Learning, Teaching Fellow, Syracuse NY **Summer 2019**
Supervisor: Brandon Murphy

- Appointed to assist in planning, organizing, and conducting the annual graduate assistant colloquium that trains 75 incoming domestic and international graduate students
- Responsibilities included: facilitating and presenting learning sessions, providing administrative support, aiding faculty and administrative presenters, and conducting participant review and evaluations

Introduction to Accounting, Teaching Assistant, SUNY-ESF, Syracuse NY **Spring 2019, Spring 2018**
Supervisor: John McGraw

- Collaborated with instructor to grade weekly assignments for 60 students

Introduction to Soils Lab, Teaching Assistant**,** SUNY-ESF, Syracuse NY **Fall 2019**, **Fall 2018**
Supervisor: Russell Briggs

- Independently supervised indoor and outdoor lab demonstrations and sessions for 30 students
- Coordinated use of facility vehicles, tools, protective equipment, and chemicals with 3 other TAs

Forest Ecology and Silviculture, Teaching Assistant, SUNY-ESF, Syracuse NY **Fall 2017**
Supervisor: Christopher Nowak

- Graded, recorded attendance, and answered questions for class of 60 students
- Responsible for student safety and attendance during outdoor group labs for 30 students

SUNY-ESF, Field Crew Leader/ Research Assistant**,** Bartlett, NH **Summer 2017**
Supervisor: Ruth Yanai

- Supervised 12 research interns collecting data in nutrient manipulation plots in hardwood forest stands
- Coordinated daily work schedules, weekly housing management schedules, and trained members on forest safety and research techniques

POSTERS & PRESENTATIONS

Dillon, G.A., A.R. Young, J.L. Campbell, M.B. Green, and R.D. Yanai. Tree measurement error in the Forest Inventory and Analysis (FIA) plots in the northern region. Spotlight on Student Research, SUNY ESF, April 24, 2018. Poster.

Dillon, G.A., M. Mahoney, S. Chase, M.T. Johnston. Nutritional impacts on invasive beech scale quantification in beech bark disease aftermath forests. New York Society of American Foresters conference, Syracuse, NY, January 25, 2019. Poster.

Dillon, G.A. Will Gretchen find beech bark disease? North Woodstock, NH- Hubbard Brook Ecosystem Study 54th Annual Cooperators Meeting, July 9, 2017. Oral Presentation.

Lasser, G.A., D.S. Hong, Y. Yang, K.E. Phelps, G. Pu and R.D. Yanai. Effect of nutrients on foliar characteristics of pin cherry, American beech, yellow birch and white birch. Spotlight on Research, SUNY-ESF, April 25, 2017. Poster.

Lasser, G.A., D.S. Hong, Y. Yang, K.E. Phelps, R.D. Yanai. Effects of nitrogen, phosphorus, and calcium on foliar characteristics of Pin Cherry, American Beech, Yellow Birch, and White Birch. Rochester Academy of Sciences Fall Scientific Paper Session, Roberts Wesleyan College, November 12, 2016 and 2017 New York Society of American Foresters conference, Syracuse, NY. Poster.

Lasser, G.A., M.T. Johnston, M.J. Mahoney, V.A. Leimanis, and J.R. Stoodley. An investigation of nutritional effects on causal organisms of beech bark disease in aftermath forests. Rochester Academy of Sciences Fall Scientific Paper Session, St. John Fisher College, November 11, 2017 and 2017 Forest Ecosystem Monitoring Cooperative Conference, University of Vermont, December 15, 2017. Poster.

Mahoney M., V. Leimanis, M. Desrochers, B. Giambona, M.T. Johnston, R.D. Yanai, and G.A. Dillon. Impacts of fertilization on causal organisms of beech bark disease. Spotlight on Student Research, SUNY ESF, April 24, 2018. Poster.

Yanai, R.D., G.A. Dillon, J.E. Drake, T.R. McConnell, A.R. Young, J.L. Campbell, M.B. Green, B. S. Case, H.L. Buckley, and R.C. Woollons. Measurement error in forest inventory (FIA) and error propagation in biomass models. Ecological Society of America, New Orleans, L.A, August 9, 2018. Poster.

WORK EXPERIENCE

Airbnb, Super Host/Property Manager, Syracuse NY **1/2015- Present**

- Developed and managed part-time, in-home, bed and breakfast from residential home generating over $80K since start up with over 300 clients
- Experienced 18% and 11% increases during second and third years respectively due to self-driven marketing and customer satisfaction efforts

National Income Life Insurance Company, Supervising Agent, Syracuse, NY **7/2014 –3/2017**

- Sold life insurance policies by generating leads and conducting in-home appointments
- Managed team of 4, coordinated training/skill development for groups up to 12
- Generated $66.7K in new business during first year with an average 12-month retention rate of more than 79% and a 4-month retention rate of 87% against a company average of 75%.

Eastern Mountain Sports, Supervisor, Syracuse, NY **2/2013 – 7/2014**

- Supervised 15 staff and supported daily sales plan goals up to $25K in a $2M volume store
- Implemented processes resulting in improved store internal audit scores (from 72% to 90%)
- Achieved consistently higher customer satisfaction ratings than team members

Halfmoon Valley Animal Hospital, Office Assistant, Port Matilda, PA **1/2011 – 8/2012**

- Performed office support in a customer facing environment while managing office email, postal mail, fax, product ordering, and inventory/restocking
- Assisted veterinarians and veterinary technicians by monitoring animal respiration, performing restraint, and sanitizing surgical instruments
- Performed a full product review and created a pricing manual to help staff locate prices during a power outage or unexpected business interruptions

Rooo Valley Boarding Kennel, Coordinator, Port Matilda, PA **8/2008 - 4/2012**

- Developed formal safety and sanitation protocols for animal and waste management of 501C3 dog kennel that supports a greyhound rescue group
- Administered oral and topical medications, canine/human first aid, and dietary preparation
- Improved procedures for parasite reduction by ensuring grounds were properly mowed and maintained.

Bank of America, Specialty Fraud Analyst/Claims Analyst, Bank of America, Newark, DE **9/2005 – 1/2010**

- Investigated and researched customer claims and monetary disputes with merchants
- Developed solutions for daily service requests and inquiries of customers and merchants

Bank of America, Designated Team Floor Supervisor, State College, PA **7/2009 – 1/2010**

- Provided management coverage, associate and customer assistance in manager absence
- Used Excel to pull and distribute team/site statistics to track and improve team performance
- Rated and delivered listening feedback to associates to improve personal performance

Bank of America, Education Trainer/Staffing Support, State College, PA **3/2008 – 1/2010**

- Coached new associates on collection techniques to improve performance
- Coordinated efforts with management teams to meet call center recruiting goals during hiring
- Completed interviews and I-9 procedures in Recruiting Coordinator's absence