EVALUATING DIEBACK OF EASTERN WHITE PINE (*Pinus strobus* L.) IN THE SOUTHERN APPALACHIAN MOUNTAINS

by

ASHLEY NICOLE SCHULZ

(Under the Direction of Kamal J.K. Gandhi)

ABSTRACT

During the last decade, *Pinus strobus* L. trees in the Appalachian Mountain region of the United States have been displaying symptoms of dieback, including branch flagging, resinosis, and crown thinning. Many of these economically and ecologically important trees also have a scale insect, *Matsucoccus macrocicatrices* Richards, and various fungal pathogens associated with canker formation. For this study, we evaluated the health of *P. strobus* in 40 sites across the southern Appalachian Mountains, modeled the relationships between *P. strobus* health and abiotic and biotic conditions, and assessed correlations among *P. strobus* saplings, *M. macrocicatrices*, and cankers. Overall, we found that *M. macrocicatrices* and a canker-forming fungus, *Caliciopsis pinea* Peck, were present in 85% and 87.5% of the 40 sites, respectively. *Pinus strobus* health rating was associated with DBH, tree density, and latitude, where larger diameter *P. strobus* trees in less dense stands were healthier than smaller diameter trees in denser stands, and trees in Virginia were less healthy than trees in Georgia. Positive correlations were present within the tripartite, *P. strobus*-*M. macrocicatrices*-canker complex, suggesting that sapling dieback is associated with *M. macrocicatrices* and cankers. Further exploration of the relationships among *M. macrocicatrices*, cankers, and site conditions are encouraged to better understand the ecological drivers behind the *P. strobus* dieback that is occurring in the Appalachian Mountains.

INDEX WORDS: Appalachian Mountains, eastern white pine, *Caliciopsis pinea,* canker, *Matsucoccus macrocicatrices*, *Pinus strobus*

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DEDICATION

For my grandparents, Judy and Denny, who have stood by me since day one, and have been a prodigious source of encouragement and inspiration the past twenty-six years. Thank you for encouraging me to follow my dreams.

For my dear friend, Autumn, who introduced me to the world of entomology in the sixth grade. I am sorry I questioned your sanity when you said you wanted to be an entomologist. You were clearly on to something long before I finally caught the entomology “bug.” I hope Heaven is filled with insects, so you can keep pursuing your entomological dreams. Save me a net, my friend. I have faith that we’ll meet again one day.

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CHAPTER 1

THESIS INTRODUCTION AND LITERATURE REVIEW

1.1 Importance of Eastern White Pine

Eastern white pine (*Pinus strobus* L.) is a key conifer species in eastern North America that can be found within 31 states in the eastern and central United States (Little 1971). It is a major component of five Society of American Foresters forest cover types, including the eastern white pine-hemlock and eastern white pine-northern red oak-red maple forest cover types (Wendel 1980; Wendel and Smith 1990), prominent in the southern Appalachian region. Eastern white pine, as a dominant tree, is becoming more important in the southern Appalachian forest system, since eastern hemlock (*Tsuga canadensis* (L.) Carriére) trees have experienced mortality due to invasion by the exotic hemlock woolly adelgid (*Adelges tsugae* Annand) (Orwig and Foster 1998; Battles et al. 1999; Evans 2004). In addition to the five key forest types where eastern white pine is a dominant species, there are 23 other forest types where it plays a less dominant role. This suggests that eastern white pine is adaptable, and can grow in many forest ecosystems and site conditions. Other tree species found with eastern white pine in these other forest types include: red oak (*Quercus rubra* L.), white oak (*Q. alba* L.), hickory (*Carya* spp.), black gum (*Nyssa sylvatica* Marshall), American beech (*Fagus grandifolia* Ehrh.), Fraser magnolia (*Magnolia fraseri* Walt), maple (*Acer* spp.), and tulip poplar (*Liriodendron tulipifera* L.) (Wendel and Smith 1990). Eastern white pine is also commonly used for food and shelter by wildlife, such as white-tailed deer (*Odocoileus virginianus*

Zimmermann), snowshoe hares (*Lepus americanus* Erxleben), black bears (*Ursus americanus* Pallas), porcupine (*Erethison dorsatum* L.), bald eagles (*Haliaeetus leucocephalus* L.), and various songbirds (Martin et al. 1951; Wendel and Smith 1990). Since eastern white pine is one of the fastest growing northern conifers, it has been used for reforestation projects, landscaping, and the stabilization of areas that have been extensively strip-mined (Czapowskyj and McQuilkin 1966; Wendel and Smith 1990).

From an economic standpoint, eastern white pine has been grown for Christmas trees, furniture and match production, and ship masts (Betts 1954; Davenport and Walters 1967; Carter et al. 1988; Wendel and Smith 1990). In the southeastern U.S. (West Virginia to Georgia), the focal tree species covers 228,765 ha and has a combined volume of 54 million m^3 live trees on forestland, while the northeastern U.S. (New York to Maine) has a combined volume of 323 million m^3 (USDA Forest Service 2014). In addition to its wood production value, eastern white pine also has valuable chemical and medicinal qualities. The bark of the eastern white pine can be used as an astringent, and the wood is used to produce white pine tar which can be used as an antiseptic (Krochmal et al. 1969; Wendel and Smith 1990).

1.2 Dieback of Eastern White Pine

Over the past decade, forest resource managers and health specialists have noticed a lower branch dieback occurring in natural eastern white pine populations of the Appalachian Mountains, more specifically in Georgia and Virginia (Asaro 2011; Mech et al. 2013). Observed symptoms include branch flagging, crown thinning, canker development, and resinosis (excessive resin outflow) at the branch crotches of trees in all diameter classes. A closer inspection of the branches and main stems resulted in the discovery of small, immature stages of *Matsucoccus macrocicatrices* Richards (Hemiptera: Matsucoccidae) embedded in cankers, under lichen, and in branch crotches.

Collection data from 25 counties in the Southeast and a survey of 27 museums found that *M. macrocicatrices* was present in Georgia, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia in the southeast and Vermont in the northeast (Mech et al. 2013). Prior to this, *M. macrocicatrices* was not documented south of Massachusetts (Richards 1960; Martineau 1964; Kosztarab 1996).

Matsucoccus macrocicatrices is the only known species within the genus *Matsucoccus* to use eastern white pine as a host, but has never been documented to cause damage to eastern white pine trees (Richards 1960; Watson et al. 1960; Kosztarab 1996; Foldi 2004; Mech et al. 2013). Little is known about the biology of this cryptic insect. The most recent study conducted on the relationship between *M. macrocicatrices* and eastern white pine was conducted during 1957-1959 in eastern Canada. The study found that the female *M. macrocicatrices* would lay eggs under lichen or in crevices in the bark, and, when the eggs hatched, the immature *M. macrocicatrices* would crawl to the edges of *Septobasidium pinicola* Snell fungal mats, where they would become stationary, feed, and grow for two winters (Watson et al. 1960). The relationships among *M. macrocicatrices*, *S. pinicola*, and the host plant were not fully understood, but the researchers hypothesized that the fungus insulated the insect from cold temperatures and possibly from parasitism or predation (Watson et al. 1960). Prior to this study, von Hohnel (1907) observed the relationship between *M. macrocicatrices* and a *Septobasidium* fungus. It was noted that the fungus did not penetrate into the leaf tissue, determining that there must be a relationship between *M. macrocicatrices* and the fungus, because the fungus could not sustain itself without the scale insect (von Hohnel 1907). Couch (1930) reported conflicting data where *Septobasidium* species either lived saprophytically or epiphytically on the excretions of the insects (von Hohnel 1907), or parasitized *M. macrocicatrices*, and would overtake and eventually kill them (Burt 1916; Coker 1920; Petch 1921).

Similar symptoms to those seen in the eastern white pines of Georgia and Virginia have been reported in eastern white pine trees ranging from Maine to New Hampshire. The mortality of these trees has been largely attributed to cankers created by the ascomycetous fungus, *Caliciopsis pinea* Peck (Rose 2011; Rosenholm 2012). *Caliciopsis pinea* is native to the eastern U.S. and has been described as “reddish brown depressions in the bark that have small, globose, clustered, black pycnidia and stalked perithecia that arise from a stromatic cushion” (Funk 1963; Horst 2012). Past studies of *C. pinea* showed that the fungus had the ability to create sharply delimited cankers on the stems and branches of eastern white pine, but the cankers were not thought to create significant damage to eastern white pine trees (Ray 1936; Cram 2009).

1.3 Thesis Objectives

Since eastern white pine dieback has become more prevalent in the last decade, it has become imperative to assess the status of eastern white pine health throughout its distribution. The primary goal of this thesis is to analyze eastern white pine dieback dynamics in the southern Appalachian Mountains. In chapter two, we determine the range and severity of dieback of symptomatic eastern white pine trees in the southern Appalachians, and assess whether eastern white pine health varies with abiotic and biotic conditions such as tree density, basal area and topographic features. By assessing eastern white pine dieback, we can determine: 1) where the most dieback is occurring in the southern Appalachian Mountains; 2) if health varies across the different size classes (sapling, poletimber, and sawtimber); and 3) if any abiotic or biotic conditions, or set of conditions, is affecting eastern white pine health. We predict that eastern white pine dieback would be greater in: 1) higher latitudes (i.e., Virginia and West Virginia) versus lower latitudes (i.e., Georgia and South Carolina); 2) small versus larger diameter trees; and 3) stands with a greater density of eastern white pine than stands with less eastern white pine.

In chapter three, we assess potential correlations among *M. macrocicatrices*, cankers and health of eastern white pine in the southeastern and northeastern regions. By analyzing cankers and *M. macrocicatrices* on eastern white pine trees, we can gain a better understanding of the relationships that could be occurring between *M. macrocicatrices* and eastern white pine dieback, cankers and eastern white pine dieback, and *M. macrocicatrices* and cankers. We predict that correlations will occur among all of the factors involved in the eastern white pine-*M. macrocicatrices*-canker complex.

Since eastern white pine remains an economically important conifer species, and can be found in the already detrimentally impacted eastern hemlock-eastern white pine forest type, it is essential to determine what might be impacting its health. Few studies have assessed the associations between eastern white pine dieback and abiotic and biotic conditions, *M. macrocicatrices*, and cankers, so measurement and analysis of these conditions will be important to gain a better understanding of the mechanisms involved with eastern white pine dieback in the southern Appalachian Mountains.

1.4 Literature Cited

Asaro, C. 2011. Virginia Department of Forestry Forest Health Review. 11 p.

Battles, J.J., N. Cleavitt, T.J. Fahey, and R.A. Evans. 1999. Vegetation composition and structure in two hemlock stands threatened by hemlock woolly adelgid. Pages 55-61 *In* K.A. McManus, K.S. Shields, and D.R. Souto, editors. Proceedings of the Symposium on Sustainable Management of Hemlock Ecosystems in Eastern North America. USDA Forest Service GTR-NE-267. Durham, New Hampshire.

Betts, H.S. 1954. Eastern White Pine. USDA Forest Service publication. 8 p.

Burt, E.A. 1916. The Thelephoraceae of North America. Annals of the Missouri Botanical Garden 3: 319.

Carter, K.K., D.H. DeHayes, M.E. Demeritt, R.T. Eckert, P.W. Garrett, H.D. Gerhold, J.E. Kuser, and K.C. Steiner. 1988. Tree Improvement in the Northeast: Interim Summary and Recommendations for Selected Species. Maine Agricultural Experiment Station Technical Bulletin 131.

Coker, W.C. 1920. Notes on the lower Basidiomycetes of North Carolina. Journal of the Elisha Mitchell Scientific Society 35 (3-4): 113-182.

Couch. J.N. 1930. The Biological Relationship between *Septobasidium retiforme* and *Aspidiotus osborni*. In Proceedings, International Botanical Congress, 19 August 1930, Cambridge, England. University of North Carolina, Chapel Hill, NC.

Cram, M.M., C. Asaro and T. Edgerton. 2009. Field inoculations of *Caliciopsis pinea* and *Diplodia scrobiculata* on eastern white pine, p.13. *In* Proceedings, Southwide Forest Disease Workshop, 29 June-2 July 2009, Greenville, SC. USDA Forest Service, Forest Health and Protection, Asheville, NC.

Czapowskyj, M.M., and W.E. McQuilkin. 1966. Survival and early growth of planted forest trees on strip mine spoils in the Anthracite Region. USDA Forest Service, Research Paper NE-46. Northeastern Forest Experiment Station, Upper Darby, PA. 29 p.

Davenport, O.M., and R.S. Walters. 1967. Christmas Tree Culture in Kentucky. University of Kentucky Agricultural Experiment Station, Miscellaneous Publication 346. Lexington. 38 p.

Evans, R.A. 2004. Hemlock woolly adelgid and the disintegration of eastern hemlock ecosystems. Park Science 22 (2): 53-56.

Foldi, I. 2004. The Matsucoccidae in the Mediterranean basin with a world list of species (Hemiptera: Sternorrhyncha: Coccoidea). Annales de la Société entomologique de France (N.S.): International Journal of Entomology 40 (2): 145 – 168.

Funk. A. 1963. Studies in the genus *Caliciopsis*. Canadian Journal of Botany 41: 503-543.

Horst, R.K. 2012. Westcott's Plant Disease Handbook, 5th Ed. Springer Science and Business Media. 954 p.

Kosztarab, M. 1996. Scale insects of Northeastern North America. Identification, biology, and distribution. Virginia Museum of Natural History, Martinsburg, Virginia, 650 pp.

Krochmal, A., R.S. Walters, and R.M. Doughty. 1969. A guide to medicinal plants of Appalachia. USDA Forest Service, Research Paper NE-138. Northeastern Forest Experiment Station, Upper Darby, PA. 291 p.

Little, E.L. 1971. Atlas of U.S. trees, volume 1, conifers and important hardwoods: U.S. Department of Agriculture Miscellaneous Publication 1146, 9 p., 200 maps.

Martin, A.D., H.S. Zim and A.L. Nelson. 1951. American Wildlife and Plants. Dover, New York. 500p.

Martineau, R. 1964. Forest insect conditions. Annual Report of the Canada Department of Forestry, Forest Insect and Disease Survey. 42-46.

Mech, A.M., C. Asaro, M.M. Cram, D.R. Coyle, P.J. Gullan, L.G. Cook and K.J.K. Gandhi. 2013. *Matsucoccus macrocicatrices* (Hemiptera: Matsucoccidae): First Report, Distribution, and Association with Symptomatic Eastern White Pine (*Pinus strobus*) in the Southeastern U.S.. Journal of Economic Entomology. 106(6): 2391-2398.

Orwig, D.A. and D.R. Foster. 1998. Forest response to the introduced hemlock woolly adelgid in southern New England, U.S.A. Journal of the Torrey Botanical Society. 125: 60-73.

Petch, T. 1921. Fungi Parasitic on Scale Insects. Presidential Address. Trans. Brit. Mycol. Soc. 7:34.

Ray, W.W. 1936. Pathogenicity and Cultural Experiments with *Caliciopsis pinea*. Mycologia 28 (3): 201-208.

Richards, W.R. 1960. A new species of *Matsucoccus* Cockerell (Homoptera: Coccoidea). Canadian Entomologist 92: 179-181.

Rose, J.A. 2011. A Decline in State’s White Pines Found. West Virginia Department of Agriculture Market Bulletin. http://www.wvagriculture.org/market_bulletin/Past_Issues

Rosenholm, G. 2012. Pine canker a growing concern in the East. USDA Forest Service News Release No. DFO-08-12. http://www.na.fs.fed.us/nanews/nastories/News%20Release%20Canker%20FINAL%20062012%20w%20template.pdf.

USDA Forest Service. 2014. "Forest Inventory Data Online (FIDO)." Last modified 02/04/2014. http://apps.fs.fed.us/fia/fido/index.html.

Von Hohnel, F. 1907. Beitrage zur Kenntnis der Corticieen (II. Mitteilung). Sitzungsber. d. Kais. Akad. d. Wissensch. Wien, Math. Naturw. Kl. 116: 740.

Watson, W.Y., G.R. Underwood and J. Reid. 1960. Notes on *Matsucoccus macrocicatrices* Richards (Homoptera: Margarodidae) and its Association with *Septobasidium pinicola* Snell in Eastern Canada. The Canadian Entomologist. 92 (9): 662-667.

Wendel, G.W. 1980. Eastern white pine. *In* Forest cover types of the U.S. and Canada. p. 25-26. F. H. Eyre, ed. Society of American Foresters, Washington, DC.

Wendel, G.W. and H.C. Smith. 1990. *Pinus strobus* L. eastern white pine. In R.M. Burns and B.H. Honkala (eds.), Silvics of North America. Vol. 1. Conifers. USDA Forest Service, Washington, DC.

CHAPTER 2

IMPACTS OF ABIOTIC AND BIOTIC SITE CONDITIONS ON THE HEALTH OF EASTERN WHITE PINE IN THE SOUTHERN APPALACHIAN MOUNTAINS[1]

[1]Schulz, A.N., C. Asaro, D.R. Coyle, M.M. Cram, R.D. Lucardi, A.M. Mech, and K.J.K. Gandhi. To be submitted to *Forest Ecology and Management*.

Abstract

Eastern white pine (*Pinus strobus* L.) is an ecologically and economically important conifer species in the eastern region of North America. Since 2006, eastern white pines in the southern Appalachian region have been reported to show dieback with canker formations, as well as a scale insect, *Matsucoccus macrocicatrices* Richards (Hemiptera: Matsucoccidae) and fungal pathogens which are associated with the cankers. Our research objectives were to map the occurrence of *M. macrocicatrices* and *Caliciopsis pinea*, determine the range and severity of dieback of eastern white pine, and assess whether tree health varied based on abiotic and biotic conditions in the southern Appalachians. Forty sites were sampled in Georgia, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia. Overall, *M. macrocicatrices* and *C. pinea* were found in 85% and 87.5% of the 40 study sites, respectively. Eastern white pine health rating was higher (less healthy) in Virginia and West Virginia than Georgia and South Carolina. Further, larger pines in less dense stands were healthier than smaller pines in more dense stands. Similar trends were found among the categorized sapling (2.54-12.45 cm), poletimber (12.7-22.61 cm), and sawtimber (≥ 22.86 cm) trees, which showed that sawtimber-sized trees were healthier than poletimber- and sapling-sized trees. Further exploration of the mechanisms involved in eastern white pine dieback will be necessary to better predict dieback severity, and develop strategies that will assist with management of eastern white pine trees in the southern Appalachian region.

INDEX WORDS: Appalachian Mountains, canker, density, eastern white pine, latitude, *Matsucoccus macrocicatrices, Pinus strobus,* poletimber, sapling, sawtimber

2.1 Introduction

Eastern white pine (*Pinus strobus* L.) is an important component of many forest types in both deciduous and coniferous dominated forests in eastern North America (Barrett 1995). This species can grow in an array of environmental conditions; hence its broad distribution in the eastern United States and Canada. However, eastern white pine grows and competes best in a cool, humid environment, and on well-drained sandy soils of poor to moderate site quality where hardwood competitors cannot thrive (Wilson and McQuilkin 1965; Wendel and Smith 1990).

Within its native range, eastern white pine has many ecological applications, including its use for food and shelter by wildlife, such as white-tailed deer (*Odocoileus virginianus* Zimmermann), snowshoe hares (*Lepus americanus* Erxleben), black bears (*Ursus americanus* Pallas), porcupine (*Erethizon dorsatum* L.), bald eagles (*Haliaeetus leucocephalus* L.) and various songbirds (Martin et al. 1951; Wendel and Smith 1990). As one of the fastest-growing northern conifers, eastern white pine has also been used for landscaping and the stabilization of lands that have been extensively surface-mined (Czapowskyj and McQuilkin 1966; Wendel and Smith 1990).

In addition to its ecological importance, eastern white pine has historically been a key production species used for car construction, ship masts, agricultural equipment, caskets, matches, flooring, and crates, among other products (Betts 1954; Carter et al. 1988). Over time, extensive logging of eastern white pine forests nearly eliminated the mature eastern white pine resource. In the early 1930's, reports on eastern white pine's desirable characteristics and excellent growth rates drew the attention of many entities, prompting the Civilian Conservation Corps (CCC) to use eastern white pine for much of its reforestation program (Vimmerstedt 1962). Although eastern white pine was replanted through much of the eastern U.S., mass production of eastern white pine lumber dwindled, leaving trees to grow for biotic diversity, aesthetics, Christmas tree production, or other forest products (Ostry et al. 2010).

Over the past decade, forest resource managers and health specialists have been noticing a decline in health of populations of eastern white pines in the southern Appalachian region, more specifically in Georgia and Virginia (Asaro 2011; Mech et al. 2013). News releases have indicated that eastern white pine trees are expressing symptoms such as cankerous growths, significant sapping (resinosis), crown thinning, branch flagging, and decreases in crown density within eastern white pine trees of all age classes and over many site conditions (Asaro 2011; Rose 2011; Rosenholm 2012; Mech et al. 2013). A closer inspection of the branches and stems of eastern white pine revealed a common characteristic among the dying pines: a scale insect, *Matsucoccus macrocicatrices* Richards, embedded under lichen and in branch crotches, and cankers with various fungi, such as *Caliciopsis pinea* Peck (Mech et al. 2013).

The scale insect, *M. macrocicatrices*, belongs to the family Matsucoccidae, which contains one extant genus, *Matsucoccus* Cockerell (1909), and 39 known species throughout the world (Foldi 2004; Liu et al. 2014). Matsucoccidae are known, commonly, as the pine bast scales, which are some of the most serious pests of natural pine forests and plantations in China, Mediterranean Europe, and the U.S. (Foldi 2004; Liu et al. 2014). *Matsucoccus macrocicatrices* is the only documented *Matsucoccus* species on eastern white pine (Richards 1960; Watson et al. 1960; Kosztarab 1996; Foldi 2004; Mech et al. 2013). Until the mid-2000's, *M. macrocicatrices* was only known to exist on eastern white pine trees in Canada (New Brunswick, Nova Scotia, Ontario, and Quebec) and the northeastern U.S. (New Hampshire) (Richards 1960; Martineau 1964; Kosztarab 1996). However, recent studies have shown that *M. macrocicatrices* is present on eastern white pine in the southeastern region of the U.S., specifically in Georgia, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia (Mech et al. 2013). Until now, *M. macrocicatrices*' damage to eastern white pine has been considered negligible (Watson et al. 1960).

Another key factor that may be affecting the health of eastern white pine is *C. pinea*, a canker-forming ascomycetous fungus that is native to the eastern U.S. (Funk 1963; Horst 2012). *Caliciopsis pinea* is primarily found on eastern white pine, but it has been documented on other pine species in the eastern U.S., including Virginia pine *(Pinus virginiana* Mill.), table mountain pine (*P. pungens* Lamb.), and pitch pine (*P. rigida* Mill.) (Funk 1963). The cankers have been described as "reddish brown depressions in the bark that have small, globose, clustered, black pycnidia and stalked perithecia that arise from a stromatic cushion" (Horst 2012). Until recently, *C. pinea* was not known to create significant damage to mature eastern white pine trees. However, reports from the last few years have indicated that *C. pinea* cankers are damaging thousands of hectares of eastern white pine (Asaro 2011; Rose 2011; Rosenholm 2012). Although some sources indicate that the disease is most serious on suppressed saplings (Horst 2012), it has been found in mature trees, as well (Rose 2011).

Since eastern white pine is still an economically and ecologically important tree species in the eastern U.S., it is essential to evaluate whether any biotic or abiotic factors could be influencing its health in the southern Appalachian Mountains. By gaining a better understanding of the sites in which eastern white pine grow, we can determine whether a particular site condition, or set of site conditions, is correlated with eastern white pine dieback. The objectives of this research are to: 1) map the occurrence of *M. macrocicatrices* and *C. pinea* in the southern Appalachian region for this study, and provide an updated distribution with data from Mech et al. (2013); 2) determine the range and severity of dieback of symptomatic eastern white pine trees in the southern Appalachians; 3) assess if eastern white pine health varies based on abiotic or biotic factors in the plots; and 4) determine if eastern white pine health varies among the different size class categories (sapling, poletimber, and sawtimber). We hypothesize that: 1) eastern white pine dieback will be higher in northern sites (i.e., Virginia and West Virginia) versus southern sites (i.e., Georgia and South Carolina); 2) eastern white pine health will be associated with abiotic and biotic factors; and 3) sawtimber will have less dieback than the poletimber or sapling size classes.

2.2 Methods

2.2.1 Study Sites

Study sites were located in the major range of eastern white pine in the southern region of the Appalachian Mountains. We established 40 sites within six states (Georgia, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia) from January 2014-August 2014. Sites were chosen based on the USDA Forest Service, Forest Inventory Data Online (FIDO) that is available through the Forest Inventory and Analysis (FIA) National Program (USDA Forest Service FIDO 2014). Within the FIDO program, we searched for forest type area estimates for each category of land ownership (i.e., federal land, private land and state land) in each state from 2011-2012. Next, we found the area of forest land for the white/red/jack pine group in each ownership category, and focused on USDA Forest Service land instead of private land to ensure site accessibility in the future. Finally, we calculated the total number of sites that we would need per state for a total of 40 sites (Table 2.1).

Site location within each state was determined through Google Earth, Arc Geographic Information Systems (ESRI 2013), USDA Forest Service National Forest maps, and communication with forestry professionals within each state to determine where eastern white pine was most abundant in stands. As part of the site selection system for this project, sites had to: 1) be accessible; 2) present in federal USDA Forest Service land (wilderness and private land excluded); 3) be available for future sampling and monitoring; 4) have no anthropogenic disturbances (i.e.: silvicultural cutting, prescribed fire, etc.) planned for the next 5-10 years; and 5) be > 5 km from other sites within this project.

Sites were encompassed in the eastern temperate (Appalachian) forest of the U.S. (Commission for Environmental Cooperation 1997; Omernik and Griffith 2014). The general soil orders (and dominant suborders) found at these sites include Inceptisols (Udepts), Ultisols (Udults), and, to a lesser extent, Spodosols (Orthods) (Wendel and Smith 1990; USDA NRCS 1998).

Average annual precipitation and temperature fluctuated from Georgia to Virginia, where Georgia had higher average annual precipitation and temperature than Virginia and West Virginia. On average, Georgia acquires 1,267 mm of precipitation per year, North Carolina 1,249 mm/year, South Carolina 1,208 mm/year, Tennessee 1,316 mm/year, Virginia 1,095 mm/year, and West Virginia 1,138 mm/year (NOAA 2014).

Given these values, the average annual precipitation for the southern Appalachian region was around 1,212 mm/year. In terms of temperature, Georgia averaged 17.4°C, North Carolina 14.8°C, South Carolina 16.9°C, Tennessee 14.3°C, Virginia 12.8°C, and West Virginia 10.9°C (NOAA 2014).

All 40 sites fell within four of the five Society of American Foresters (SAF) forest cover types: eastern white pine-northern red oak-red maple (Type 20), eastern white pine (Type 21), eastern white pine-eastern hemlock (Type 22), or eastern white pine-chestnut oak (Type 51). Eastern hemlock [*Tsuga canadensis* (L.) Carriére], maple (*Acer* spp.), tulip poplar (*Liriodendron tulipifera* L.), red oak (*Quercus rubra* L.), white oak (*Q. alba* L.), hickories (*Carya* spp.), black gum (*Nyssa sylvatica* Marshall), American beech (*Fagus grandifolia* Ehrh.), Fraser magnolia (*Magnolia fraseri* Walt), birch (*Betula* spp.), Virginia pine, table mountain pine, pitch pine, and slash pine (*P. elliottii* Engelm.) are all overstory species that can be found with eastern white pine in the southern Appalachian region. Common understory species include: dogwood (*Cornus* spp.), mountain laurel (*Kalmia latifolia* L.), rhododendron (*Rhododendron maximum* L.), bracken fern (*Pteridium aquilinum* L. Kuhn), and buckberry (*Vaccinium stamineum* L.) (Wendel and Smith 1990).

2.2.2 Site Sampling

Three circular plots, at least 50 m apart, were established within each of the 40 sites, at least 5 km apart, for a total of 120 plots for the study. Each plot had a 10 m fixed radius and was permanently marked in the center by a tagged rebar stake and a tagged non-eastern white pine tree to ensure that the plots can be found for future monitoring. The coordinates for each plot center were determined using Global Positioning System

(GPS) technology, so site coordinates could be loaded into ArcGIS 10.2 (ESRI 2013) for mapping and future site monitoring.

Each plot was split into four quadrants based on the cardinal directions [Northwest (NW), Northeast (NE), Southeast (SE), and Southwest (SW)]. Latitude, slope, aspect, and elevation were documented as the topographic conditions for each plot. All live and dead standing trees that were > 2.54 cm diameter at breast height (DBH) within the plot were identified and measured for DBH. All eastern white pine and eastern hemlock trees (< 2.54cm DBH) within the plot were given a crown class rating of either "D" (dominant), "C" (codominant), "I" (intermediate), or "S" (suppressed). Each eastern white pine and eastern hemlock also received an overall health rating, which ranged from one, which was healthy, to five, which was completely dead (Figure 2.1). Health ratings were based on crown coverage, foliar transparency [based on the foliage transparency scale from Schomaker et al. (2007)], and tree size. We included tree size in the health rating assessment, since small trees naturally have a higher crown transparency and should not be equally compared to mature trees. All health ratings and other objective measurements were completed by the same person on all 120 plots to limit observer error.

We also counted the number of eastern white pine seedlings that were < 2.54 cm DBH, but > 61 cm tall within each plot (seedling A). Each seedling in the seedling A group was counted as dead if it had ≤ 25% crown coverage and alive if it had > 25% crown coverage. The number of eastern white pine seedlings that were < 2.54 cm DBH and < 61 cm tall were also documented within each plot (seedling B). Due to the abundance of seedlings in the seedling B group, subsampling was conducted where we counted the total number dead (≤ 25% crown coverage) and living (>25% crown coverage) seedlings that were present within a 1 m wide transect line along each cardinal direction.

Since marked plots will be monitored over the next few years, we selected four seedlings (from seedling group A) from each plot to tag and count the total number of dead and living nodes. Ideally, one tree was selected from each quadrant, but, if a seedling A-sized tree could not be found in each quadrant, all four trees were selected from a mix of the quadrants or a single quadrant. When the plots are resampled in the future, tagged seedlings may be reanalyzed to evaluate change in health over time.

After measurements were taken for each plot, we collected two eastern white pine seedlings (seedling group A) from outside of each plot (six seedlings per site) to assess for *M. macrocicatrices* and *C. pinea*. In the lab, we used a dissecting microscope to search for each life stage of *M. macrocicatrices* including: eggs, crawlers (first instar, mobile nymphs), cysts (heavily sclerotized, legless stage between legged crawler and adult stages), shells (shed skin from adult emergence), and adults. Due to the timing of sampling, we only found cysts and shells. We also assessed the size (using a mm^2 grid) and location (B1: first branch whorl and below, B2: second branch whorl to just above first branch whorl, etc.) of cankers, and presence/absence of *C. pinea*, which creates reddish brown depressions in the bark and has black, hair-like ascocarps which bear ascospores (sexual stage). *Caliciopsis pinea* also has an asexual stage, which appear as small, conical lobes. The lobes can grow into stalked ascocarps, or will remain small and bear spermatia (Overholts 1930; Ray 1936; Horst 2012). All seedlings were stored in a walk-in refrigerator at 4.4° C to prevent mold and temporarily preserve the cankers and

M. macrocicatrices. If at least one *M. macrocicatrices* insect was present on one of the six seedlings collected for each site, it was determined that *M. macrocicatrices* was present at the respective site. Similarly, if at least one *C. pinea* canker was present on one of the six seedlings collected for each site, or if *C. pinea* was detected and noted in the field, *C. pinea* was considered "present" at that respective site. Sites deemed "absent" may not have a true absence of *M. macrocicatrices* or *C. pinea* within the sites.

2.2.3 Mapping the Occurrence of *M. macrocicatrices* and *C. pinea*

Coordinates collected at each of the 40 sites in this study, and the coordinates from Mech et al. (2013) were compiled and entered into ArcGIS 10.2 (ESRI 2013) to create maps including the native range of eastern white pine (Little 1971; USGS 2013), and the presence and absence (i.e., no record) of *M. macrocicatrices* and *C. pinea* within Georgia, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia.

2.2.4 Statistical Analyses

All data were analyzed at the plot level using R v. 3.1.3 (R Development Core Team 2013). Eastern white pine health rating, the response variable used in all analyses, was calculated by averaging the health rating of all of the eastern white pine trees in each plot. Two plots had an eastern white pine health rating of 1, which were replaced with a health rating of 1.65 to close the gap between the smallest values and the second smallest values. DBH measurements were used to calculate basal area (m^2/ha) for eastern white pine and hardwood trees, as well as the average DBH of all eastern white pine trees (DBHA) within each plot. Further, we calculated eastern white pine and hardwood density (trees/ha), in addition to the square-root of the number of eastern white pine ($WPTree^{½}$) and hardwoods ($HWTree^{½}$) in a plot. Since many of the sampled plots did not have eastern hemlock, other pine, or rhododendron/mountain laurel, indicator variables (1 or 0) were used to indicate the presence or absence of these tree types in each of the 120 plots. *Caliciopsis pinea* and *M. macrocicatrices* presence were also included in the dataset as indicator variables based on the presence (1) or absence (0; no record) of *C. pinea* and *M. macrocicatrices*.

The latitude, slope, and elevation measurements that were taken at each plot were included as abiotic variables. Aspect data were also used, but converted using the Beers transformation (Beers et al. 1966):

$$\text{Transformed aspect} = \text{cosine } (45 - \text{azimuth}) + 1$$

This equation rescaled the 360° aspect to reflect site productivity by assigning a value of 2 to northeast facing slopes which receive less sunlight and are more mesic, and a value of 0 to southwest facing slopes, which receive more sunlight and are more xeric.

One-variable regressions were used as a preliminary analysis to assess the relationship between eastern white pine health rating and each of the abiotic, biotic, and indicator variables. Variables that were significant at the level of $p < 0.05$ were then included in fixed-effects models to determine which model was best able to predict eastern white pine health rating at the plot level. Akaike information criterion (AIC) and Bayesian information criterion (BIC) values from each model combination were used for model selection, where smaller values of AIC and BIC suggest a better model. Variables from the best model, as determined by AIC and BIC, were then included in a final mixed-effect model with site included as the random effect.

To evaluate eastern white pine health across the different size classes, we grouped each of the measured 2,061 eastern white pine trees into three categories: saplings (2.54 –

12.45 cm), poletimber (12.7 – 22.61 cm), and sawtimber ($\geq$ 22.86 cm) (USDA Forest Service FIA 2013). A Schapiro-Wilks normality test and residual plot was used to check the data for normality and homoscedasticity. Since data were not normal, we used a non-parametric, Kruskal-Wallis test, followed by post-hoc Mann-Whitney-Wilcoxon tests to determine if there were significant differences among the size class categories.

2.3 Results

2.3.1 Occurrence of *M. macrocicatrices* and *C. pinea*

Matsucoccus macrocicatrices was found in 85% of the 40 study sites sampled in 2014 (Table 2.2). In the other 15% of sites, *M. macrocicatrices* was not recorded. An updated map, which combined data collected from 2011-2014 (Mech et al. 2013), determined that around 77% of sites sampled during that time had *M. macrocicatrices* (Table 2.2; Figure 2.2). Some sites with no record of *M. macrocicatrices* were < 20 km from sites with a positive record of *M. macrocicatrices*. *Caliciopsis pinea* was found in 87.5% of the 40 sites sampled in 2014 (Table 2.3; Figure 2.2). Overall, 80% of sites had both *M. macrocicatrices* and *C. pinea*; 7.5% had no *M. macrocicatrices* or *C. pinea*; 7.5% had *C. pinea*, but no *M. macrocicatrices*; and 5% had *M. macrocicatrices*, but no *C. pinea*.

2.3.2 Abiotic and Biotic Site Conditions

On a scale of 1-5, where 1 is healthy and 5 is dead, eastern white pine health ratings ranged from 1.65-4.5 with a mean (± SE) of 3.36 ± 0.07 within the 120 plots. Mean (± SE) dieback of seedling group A (%) was 13.5 ± 2.48, while mean dieback of seedling group B was 2.87 ± 1.24 for all of the plots. The values for latitude, slope, aspect, elevation, eastern white pine density, $WPTree^{½}$, hardwood density, $HWTree^{½}$, DBHA, eastern white pine basal area, hardwood basal area, and the presence or absence of *M. macrocicatrices*, *C. pinea*, eastern hemlock, other pine, and rhododendron-mountain laurel were diverse in range (Table 2.4).

Five variables including latitude, eastern white pine density, DBHA, $DBHA^2$, $DBHA^3$, $WPTree^{½}$, and the presence/absence of *C. pinea* were significant and had the lowest AIC and BIC values (Table 2.5). The *C. pinea* presence/absence variable did not explain as much variance as the other variables, so it was dropped from the final model. The final, mixed-effects model with the random effect of site determined that eastern white pine health rating was significantly associated with the $WPTree^{½}$ ($P < 0.001$, $t = 3.75$), latitude ($P < 0.01$, $t = 2.89$), DBHA ($P < 0.01$, $t = 3.23$), $DBHA^2$ ($P < 0.01$, $t = -2.83$), and $DBHA^3$ ($P < 0.05$, $t = 2.12$) (Table 2.6). About 25% of the overall variability in the eastern white pine health rating prediction at the plot level was due to site randomness.

2.3.3 Eastern White Pine Size Class Assessment

Eastern white pine health varied among the size class categories ($\chi^2 = 304.83$; $P < 0.001$). Mann-Whitney-Wilcoxon tests revealed that there were differences in mean eastern white pine health rating between each of the size classes: sapling-poletimber ($P < 0.05$; W = 231,880.5), sapling-sawtimber ($P < 0.001$; W = 450,540.5), and poletimber-sawtimber ($P < 0.001$; W = 156,616). Mean (± SE) eastern white pine health rating ranged from 3.8 ± 0.03 for saplings, to 4.0 ± 0.05 for poletimber, and 2.9 ± 0.04 for sawtimber (Figure 2.3). Overall, the greatest difference occurred between poletimber and sawtimber trees, with a difference of 1.1 between the mean poletimber and sawtimber eastern white pine health ratings. The mean (± SE) eastern white pine health ratings for each crown class category (suppressed, intermediate, codominant, and dominant) showed similar results, where the mean (± SE) eastern white pine health rating was 3.41 ± 0.09 for suppressed trees, 3.93 ± 0.03 for intermediate trees, 3.75 ± 0.04 for codominant trees, and 2.73 ± 0.04 for dominant trees.

2.4 Discussion

This study on eastern white pine dieback in the southern Appalachian Mountains revealed the following major trends: 1) *M. macrocicatrices* and *C. pinea* were, respectively, found in 85% and 87.5% of sites sampled in 2014, while *M. macrocicatrices* has been found in 77% of sites sampled from 2011-2014; 2) eastern white pine health rating ranged from 1.65-4.5 with a mean (± SE) of 3.36 ± 0.07 for all 120 plots; 3) eastern white pine health rating increases with increasing latitude; 4) the healthiest trees, on average, were large trees in less dense stands, and the least healthy trees were small diameter trees in more dense stands; and 5) eastern white pine poletimber and saplings experienced greater dieback than sawtimber-sized trees.

The mean (± SE) health rating for eastern white pine trees was 3.36 ± 0.07 (1 = healthy and 5 = dead tree) for all 120 study plots across the southern Appalachian Mountains. This suggests that the trees may have already been in a moderate to advanced stage of dieback by the time we started sampling these sites. Houston and O'Brien (1983) classified the patterns of disease development over time and space, suggesting that there are three key classifications: the advancing front, killing front, and aftermath zone. This study may primarily be documenting the advancing front for the *M. macrocicatrices*-pathogen complex, though some areas may be transitioning into the killing front, especially in larger diameter trees (personal observations). Other studies of non-native insects have found similar trends where the emerald ash borer (*Agrilus plannipenis* Fairmaire) and beech bark scale (*Cryptococcus fagisuga* Lind.) had already caused much dieback before the symptoms appeared at the landscape-level (Houston 1994; Smith et al. 2015).

The mixed-effects model determined that eastern white pine health rating was associated with latitude, where lower latitude sites (Georgia and South Carolina) were healthier than higher latitude sites (Virginia and West Virginia). Perhaps *M. macrocicatrices* has been present in the northern areas for a longer time, thus causing more damage. Analyses of museum records have indicated that there are no previous records of the collection of *M. macrocicatrices* in the southeastern region of the U.S. (Mech et al. 2013), so further genetic work may be needed to determine the origin and dispersal pattern of the scale insect.

The model also found that eastern white pine health rating was associated with DBH and the density (square-root) of eastern white pine in the plots. This indicates that the healthiest trees tend to be larger diameter trees in less dense stands, while the least healthy trees may be small diameter trees in denser stands. In denser stands, we hypothesize that eastern white pine trees may have more competition for resources such as sunlight, water, nutrients, and root/stem space. These predisposing factors may cause trees to lose vigor or become stressed (Smith et al. 1997), allowing pathogens and pests to invade trees. Additionally, under the Resource Concentration Hypothesis (Root 1973), forest stands with densely packed eastern white pine may make it easier for insects, such as *M. macrocicatrices*, and pathogens, such as *C. pinea*, to find a suitable host. The mechanism for dispersal for adult stages of *M. macrocicatrices* is unknown, but they likely disperse by wind or animals, similar to hemlock woolly adelgid or red pine scale (*Matsucoccus resinosae* Bean and Godwin) (McClure 1989; McClure 1990).

Our models further determined that around 25% of the variance was accounted for by the random effect of site, while the other 75% may be due to pure randomness. This suggests that eastern white pine health rating may vary by about 0.5 units in plots with the same average eastern white pine DBH and/or the same eastern white pine density. Other factors that were unmeasured in this study may have an influence on eastern white pine health rating, and may help account for this randomness. We posit that these unmeasured factors may include precipitation, temperature, or soil type differences within and among the sites. A recent study conducted on some of the predisposing factors of beech bark disease further indicated that factors such as nitrogen and phosphorous levels in the soil, as well as bark chemistry could impact tree defenses and affect the aggressiveness of fungal pathogens (Cale et al. 2015).

Poletimber- and sapling-sized trees experienced the greatest dieback, while the sawtimber-sized trees had the least dieback. These results fit the trends found in the mixed-effects model, as well as observations by other researchers (Lombard 2003; Asaro 2011; Rose 2011; Rosenholm 2012). These trends may be explained by some physiological differences between small and large diameter trees. For instance, larger trees may be better defended than smaller trees. Generally, plants invest more in defense when they have more resources for defense allocation (Weiner 2004). Larger trees have deeper, more extensive root systems in addition to the roots they have at the soil surface, so they may be more capable of accessing water during drought than smaller trees with only shallow root systems (Dawson and Ehleringer 1991). Water access is an important component for plant defense, since water stress has the potential to concentrate nutrients in the sapwood, and reduce oleoresin exudation pressure which may benefit colonizing insects and fungi (Parker 1961; Vitè 1961; Mattson and Haack). Another physiological difference is that small diameter eastern white pine trees have thin, smooth bark, whereas larger trees have thicker, deeply grooved bark. As a result of having thinner bark, sapling- and poletimber-sized trees may be more susceptible to damage, and, hence, canker-forming fungi that exploit wounds. After the canker-forming fungal spores establish, the developing cankers may, over time, coalesce to girdle the stem, causing death to the stem, branches, and foliage beyond that point (Tainter and Baker 1996). To girdle the stem, the canker must be large enough (or many cankers must amalgamate) to affect the length of a tracheid, prevent xylem redistribution of water horizontally through the pits, and encompass a large portion of the stem diameter (MacKay and Weatherley 1973). Since sapling- and poletimber-sized trees are smaller in diameter, a smaller combined canker surface area may be efficient to girdle and kill them, whereas more coalescing cankers, larger cankers, or more time, overall, would be necessary to girdle and kill larger diameter trees. Although larger trees tend to remain healthier longer than the smaller trees, their rough bark texture and stress from scale insects and cankers may create conditions ideal for other biotic factors, such as bark beetles, to take over and kill the trees (Coulson and Witter 1984; Ferrenberg and Mitton 2014).

Overall, if regeneration of eastern white pine fails in these forest ecosystems, many of the common hardwood species such as tulip poplar, maples or oaks, may have the advantage, and take over as the dominant canopy species (Orwig et al. 2002; Ellison et al. 2005). These shifts in composition may alter soil nutrient cycling and stream health in riparian areas, especially in stands that have already experienced severe senescence of eastern hemlock (Stadler et al. 2005; Strohm 2014). Further exploration of the ecological drivers involved in eastern white pine dieback will be necessary to understand the impacts of dieback on forest ecosystems, and develop strategies that will assist with management of eastern white pine in the southern Appalachians.

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2.6 Literature Cited

Asaro, C. 2011. Virginia Department of Forestry Forest Health Review. 11 p.

Barrett, J.W. 1995. Regional Silviculture of the U.S. 3rd ed. John Wiley & Sons, Inc. 643 p.

Beers, T.W., Dress, P.E., Wensel, L.C. 1966. Aspect transformation in site productivity research. Journal of Forestry. 64: 691-692.

Betts, H.S. 1954. Eastern White Pine. USDA Forest Service publication. 8 p.

Cale, J.A., S.A. Teale, M.T. Johnston, G.L. Boyer, K.A. Perri, and J.D. Castello. 2015. New ecological and physiological dimensions of beech bark disease development in aftermath forests. Forest Ecology and Management 336: 99-108.

Carter, K.K., D.H. DeHayes, M.E. Demeritt, R.T. Eckert, P.W. Garrett, H.D. Gerhold, J.E. Kuser, and K.C. Steiner. 1988. Tree Improvement in the Northeast: Interim Summary and Recommendations for Selected Species. Maine Agricultural Experiment Station Technical Bulletin 131.

Commission for Environmental Cooperation. 1997. Ecological regions of North America: toward a common perspective. Commission for Environmental Cooperation, Montreal, Quebec, Canada. 71p. Map (scale 1:12,500,000). Revised 2006.

Coulson, R.N. and J.A. Witter. 1984. Forest Entomology: Ecology and Management. John Wiley and Sons, 669 p.

Czapowskyj, M.M., and W.E. McQuilkin. 1966. Survival and early growth of planted forest trees on strip mine spoils in the Anthracite Region. USDA Forest Service, Research Paper NE-46. Northeastern Forest Experiment Station, Upper Darby, PA. 29 p.

Dawson, T. E., and J. R. Ehleringer. 1991. Streamside trees that do not use stream water. Nature 350: 335-337.

Ellison, A.M., M.S. Bank, B.D. Clinton, E.A. Colburn, K. Elliot, C.R. Ford, D.R. Foster, B.D. Kloeppel, J.D. Knowepp, G. Lovett, J. Mohan, D.A. Orwig, N.L. Rodenhouse, W.V. Sobczak, K.A. Stinson, J.K. Stone, C.M. Swan, J. Thompson, B. Van Holle, and J.R. Webster. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology, 9: 479–486.

ESRI. 2013. ArcGIS Desktop: Release 10.2. Redlands, CA: Environmental Systems Research Institute.

Ferrenberg, S. and J.B. Mitton. 2014. Smooth bark surfaces can defend trees against insect attack: resurrecting a ‘slippery’ hypothesis. Functional Ecology 28: 837-845.

Foldi, I. 2004. The Matsucoccidae in the Mediterranean basin with a world list of species (Hemiptera: Sternorrhyncha: Coccoidea). Annales de la Société entomologique de France (N.S.): International Journal of Entomology 40 (2): 145-168.

Funk, A. 1963. Studies in the genus *Caliciopsis*. Canadian Journal of Botany 41: 503-543.

Horst, R.K. 2012. Westcott’s Plant Disease Handbook, 5th Ed. Springer Science and Business Media. 954 p.

Houston, D.R. and J.T. O’Brien. 1983. Beech Bark Disease. USDA Forest Service Forest Insect and Disease Leaflet 75.

Houston, D.R. 1994. Major New Tree Disease Epidemics: Beech Bark Disease. Annual

Review of Phytopathology 32: 75-87.

Kosztarab, M. 1996. Scale insects of Northeastern North America. Identification, biology, and distribution. Virginia Museum of Natural History, Martinsburg, Virginia, 650 pp.

Little, E.L., Jr. 1971. Atlas of United States trees, volume 1, conifers and important hardwoods: U.S. Department of Agriculture Miscellaneous Publication 1146. 9 p. 200 maps.

Liu, W., Y. Xie, Q. Yang, J. Xue, and F. Tian. 2014. New Research on *Matsucoccus matsumurae* (Kuwana) (Hemiptera: Matsucoccidae) in China. Acta Zoologica Bulgarica 6: 95-102.

Lombard, K. 2003. *Caliciopsis* Canker (pine canker): *Caliciopsis pinea*. New Hampshire Division of Forests & Lands and University of New Hampshire Extension Publication. 1p.

MacKay, J.F.G. and P.E. Weatherley. 1973. The effects of transverse cuts through the stems of transpiring woody plants on water transport and stress in the leaves. J. Exp. Bot. 24: 15-28.

Martin, A.D., H.S. Zim, and A.L. Nelson. 1951. American Wildlife and Plants. Dover, New York. 500 p.

Martineau, R. 1964. Forest insect conditions. Annual Report of the Canada Department of Forestry, Forest Insect and Disease Survey 42-46.

Mattson, W.J. and R.A. Haack. 1987. The Role of Drought in Outbreaks of Plant-eating Insects. Bioscience 37 (2): 110-118.

McClure, M.S. 1989. Importance of weather to the distribution and abundance of introduced adelgid and scale insects. Agricultural and Forest Meteorology 47 (2-4): 291-302.

McClure, M.S. 1990. Role of wind, birds, deer, and humans in the dispersal of hemlock woolly adelgid (Homoptera: Adelgidae). Environmental Entomology 19 (1): 36-43.

Mech, A.M., C. Asaro, M.M. Cram, D.R. Coyle, P.J. Gullan, L.G. Cook and K.J.K. Gandhi. 2013. *Matsucoccus macrocicatrices* (Hemiptera: Matsucoccidae): First Report, Distribution, and Association with Symptomatic Eastern White Pine (*Pinus strobus*) in the Southeastern U.S.. Journal of Economic Entomology. 106(6): 2391-2398.

NOAA National Climatic Data Center (NCDC). 2014. Climate at a Glance: Average Temperature and Precipitation from 1895 – 2014. U.S. Climate Divisional Database. http://www.ncdc.noaa.gov/cag/time-series.

Omernik, J.M. and G.E. Griffith. 2014. Ecoregions of the conterminous U.S.: evolution of a hierarchical spatial framework. Environmental Management 54(6):1249-1266.

Orwig, D.A., D.R.Foster, and D.L. Mausel, 2002. Landscape patterns of hemlock decline in New England due to the introduced hemlock woolly adelgid. Journal of Biogeography 29: 1475–87.

Ostry, M.E., G. Laflamme, and S.A. Katovich. 2010. Silvicultural approaches for management of eastern white pine to minimize impacts of damaging agents. Forest Pathology 40: 332 – 346.

Overholts, L.O. 1930. Mycological Notes for 1928-1929. Mycologia 22: 232-246.

Parker, A.F. 1961. Bark moisture relations in disease development: Present status and future needs. Recent Adv. Bot. 2: 1535-1537.

R Development Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Ray, W.W. 1936. Pathogenicity and Cultural Experiments with *Caliciopsis pinea*. Mycologia 28 (3): 201-208.

Richards, W.R. 1960. A new species of *Matsucoccus* Cockerell (Homoptera: Coccoidea). Canadian Entomologist 92: 179-181.

Root, R.B. 1973. Organization of a Plant-Arthropod Association in Simple and Diverse Habitats: The Fauna of Collards (*Brassica oleracea*). Ecological Monographs 43 (1): 95-124.

Rose, J.A. 2011. A Decline in State's White Pines Found. West Virginia Department of Agriculture Market Bulletin. http://www.wvagriculture.org/market_bulletin/Past_Issues

Rosenholm, G. 2012. Pine canker a growing concern in the East. USDA Forest Service News Release No. DFO-08-12. http://www.na.fs.fed.us/nanews/nastories/News%20Release%20Canker%20FINAL%20062012%20w%20template.pdf.

Schomaker, M.E., Zarnoch, S.J., Bechtold,W.A.. Latelle, D.J., Burkman,W.G., and Cox, S.M. 2007. Crown-condition classification: a guide to data collection and analysis. Gen. Tech. Rep. SRS-102. Asheville, NC: USDA Forest Service, Southern Research Station. 78 p.

Smith, D.M., B.C. Larson, M.J. Kelty and P.M.S. Ashton. 1997. The Practice of Silviculture: Applied Forest Ecology. 9th Ed. John Wiley and Sons Inc., 537 p.

Smith, A., D.A. Herms, R.P. Long, and K.J.K. Gandhi. 2015. Community composition and structure had no effect on forest susceptibility to invasion by emerald ash borer (*Agrilus planipennis*). The Canadian Entomologist 147: 318-328.

Stadler, B., T. Muller, D. Orwig, and R. Cobb. 2005. Hemlock woolly adelgid in New England forest: Canopy impacts transforming ecosystem processes and landscapes. *Ecosystems* 8: 233-247.

Strohm, C.J. 2014. Changing litter resources associated with hemlock woolly adelgid invasion affect benthic communities in headwater streams. University of Kentucky Theses and Dissertations-Entomology. Paper 8.

Tainter, F.H. and F.A. Baker. 1996. Principles of Forest Pathology. John Wiley and Sons, Inc. 805 p.

USDA Forest Service Northeastern FIA Program. 2013. Common Definitions Used By the Forest Inventory and Analysis Program. Last modified March 29, 2013. Retrieved July 14, 2015. http://www.fs.fed.us/ne/fia/methodology/def_ip.htm.

USDA Forest Service. 2014. "Forest Inventory Data Online (FIDO)." Last modified 02/04/2014. http://apps.fs.fed.us/fia/fido/index.html.

USDA Natural Resources Conservation Service (NRCS). 1998. Soil Orders Map of the U.S. National Soil Survey Center, Lincoln, NE USA. http://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/class/?cid=nrcs142p2_053589.

USGS Geosciences and Environmental Change Science Center. 2013.

Digital Representations of Tree Species Range Maps from "Atlas of United States Trees" by Elbert L. Little Jr. (and other publications). Accessed: 07/08/2015. http://esp.cr.usgs.gov/data/little/

Vimmerstedt, J.P. 1962. Southern Appalachian White Pine Plantations: Site, volume, and yield. USDA Forest Service Station Paper No. 149.

Vite, J.P. 1961. The influence of water supply on oleoresin exudation pressure and resistance to bark beetle attack in *Pinus ponderosa*. Contrib. Boyce Thompson Inst. 21: 37-66.

Watson, W.Y., G.R. Underwood, and J. Reid. 1960. Notes on *Matsucoccus macrocicatrices* Richards (Homoptera: Margarodidae) and its Association with *Septobasidium pinicola* Snell in Eastern Canada. The Canadian Entomologist 92 (9): 662 – 667.

Weiner, J. 2004. Allocation, plasticity and allometry in plants. Perspect Plant Ecol Evol Syst 6: 207-215.

Wendel, G.W. and H.C. Smith. 1990. *Pinus strobus* L. eastern white pine. In R.M. Burns and B.H. Honkala (eds.), Silvics of North America. Vol. 1. Conifers. USDA Forest Service, Washington, D.C.

Wilson, R.W., and W.F. McQuilkin. 1965. Silvics of Forest Trees of the U.S.. U.S. Department of Agriculture, Agriculture Handbook 271. Washington, DC. p. 329-337.

Table 2.1. Number of sites sampled per state based on the proportion of USDA Forest Service white/red/jack pine land (ha).

State	USDA Forest Service Land (ha)	Proportion	# Sites
Georgia	10,592.00	0.19	8
North Carolina	6,013.60	0.11	4
South Carolina	3,443.90	0.06	2
Virginia	17,605.00	0.31	13
West Virginia	8,580.20	0.15	6
Tennessee	9,977.50	0.18	7
Total	**56,212.20**	**1**	**40**

Table 2.2. Occurrence of *Matsucoccus macrocicatrices* in the southern Appalachian region in sites sampled from 2011-2014.

	2011 - 2013		2014		2011 - 2014	
State	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**
Georgia	8	1	8	0	16	1
Kentucky	0	3	NA*	NA*	0	3
North Carolina	6	1	3	1	9	2
South Carolina	2	3	2	0	4	3
Tennessee	1	2	5	2	6	4
Virginia	2	0	10	3	12	3
West Virginia	2	0	6	0	8	0
Total	21	10	34	6	55	16

*NA = not applicable. Kentucky was not sampled in 2014.

Table 2.3. Occurrence of *Caliciopsis pinea* in the southern Appalachian region in sites sampled in 2014.

State	Present	Absent
Georgia	8	0
North Carolina	3	1
South Carolina	2	0
Tennessee	4	3
Virginia	12	1
West Virginia	6	0
Total	35	5

Table 2.4. The range (low and high) and mean (± SE) of measured abiotic and biotic factors (n = 120).

Variable	Min	Max	Mean	SE
Eastern White Pine Health Rating[1]	1.65	4.5	3.35	0.07
Latitude	34.7	38.87	36.42	0.13
Slope (%)	1	50	15.89	1.02
Aspect	0	2	1.01	0.06
Elevation (m)	316	897	633.6	14.63
Eastern White Pine Density (trees/ha)	32	3,121	547.11	44.01
$WPTree^{½}$	1	8.06	3.78	0.15
Hardwood Density (trees/ha)	96	3,918	1,092.33	61.39
$HWTree^{½}$	1.73	11.09	5.59	0.16
DBHA (cm)	4.19	98.3	26.91	1.83
Eastern White Pine Basal Area (m^2/ha)	0.22	80.49	25.87	1.75
Hardwood Basal Area (m^2/ha)	0.35	46.1	19.15	0.90
M. macrocicatrices	0	1	0.15	0.03
C. pinea	0	1	0.13	0.03
Eastern Hemlock	0	1	0.43	0.05
Other Pine	0	1	0.36	0.04
Rhododendron/Mountain Laurel	0	1	0.32	0.04

[1] Health rating, where 1 = live tree, 2-4 = gradual thinning of the crown, and 5 = dead tree.

Table 2.5. Fixed-effects models to predict eastern white pine health rating at the plot level (n = 120).

Model #	Model	PAR	RMSE	RSQ	AIC	BIC
0	null	1	0.7207	0.0000	264.81	270.39
0x	SITE	40	0.6037	0.5279	252.75	367.04
1a	DBHA, $DBHA^2$, $DBHA^3$	4	0.6294	0.2557	240.38	249.31
1b	$WPTree^{½}$	2	0.6558	0.1781	243.27	251.64
1c	LAT	2	0.6807	0.1146	252.21	260.57
1d	ICP	2	0.6944	0.0786	257.00	265.36
2	DBHA, $DBHA^2$, $DBHA^3$, $WPTree^{½}$	5	0.5986	0.3326	224.28	241.01
3	**DBHA, $DBHA^2$, $DBHA^3$, $WPTree^{½}$, LAT**	**6**	**0.5690**	**0.4023**	**213.05**	**232.57**
4	DBHA, $DBHA^2$, $DBHA^3$, $WPTree^{½}$, LAT, ICP	7	0.5677	0.4102	213.46	235.76

Table 2.6. Mixed-effects model used to predict eastern white pine health rating at the plot level using the fixed variables and the random effect of site (n = 120).

Random Effect	**SD**			
Site	0.2874			
Residual	0.4955			
Fixed Effects	**Estimate**	**SE**	**t**	**P***
(Intercept)	-2.7244	1.7324	-1.5727	**0.12**
$WPTree^{½}$	0.1613	0.0430	3.7482	**0.0003**
DBHA	0.0633	0.0196	3.2323	**0.002**
$DBHA^2$	-0.0015	0.0005	-2.8298	**0.006**
$DBHA^3$	0.000008	<0.0001	2.1154	**0.038**
Latitude	0.1355	0.0469	2.8910	**0.005**

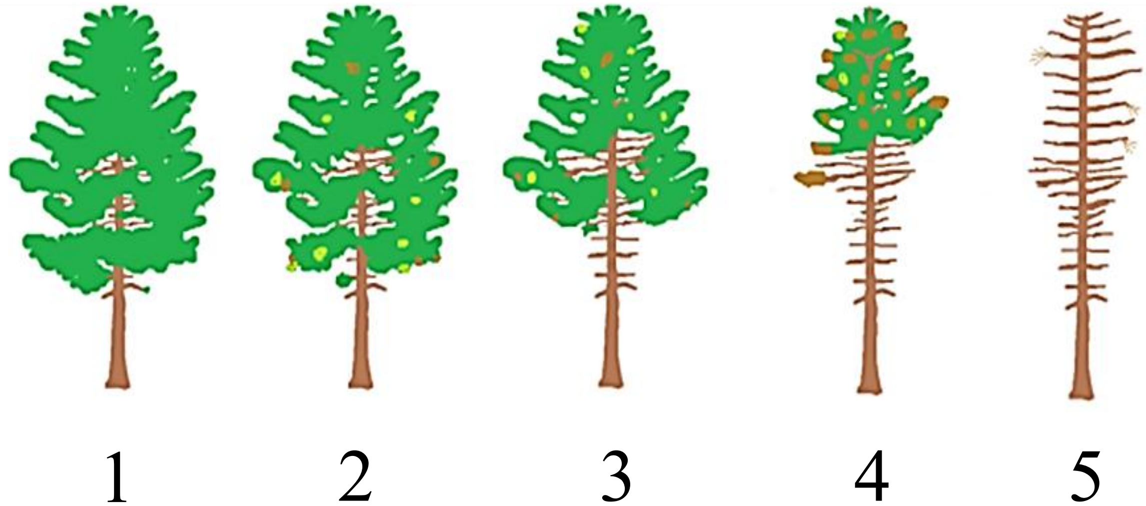
*Significant at $\alpha = 0.05$.

Figure Legend

Figure 2.1. Eastern white pine health rating scale, where 1 is healthy, 2-4 is a gradual thinning of the crown, and 5 is a dead tree.

Figure 2.2. Occurrence of *Matsucoccus macrocicatrices* from 2011-2014, and *Caliciopsis pinea* in 2014 in the southern Appalachian Mountains, U.S.

Figure 2.3. Average (± SE) eastern white pine health rating for different size classes (saplings, poletimber, and sawtimber).



Eastern White Pine Health Rating

Figure 2.1

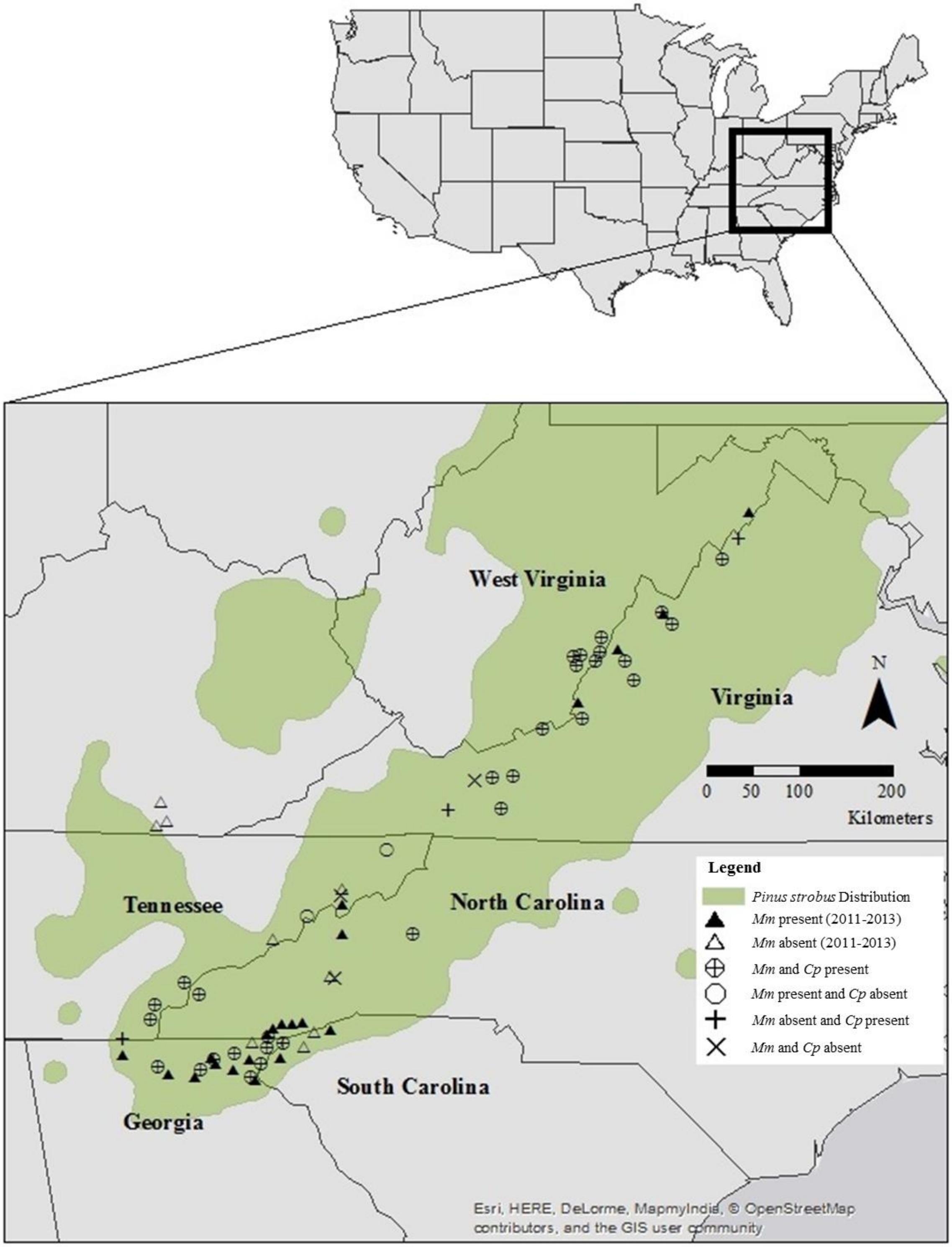

West Virginia
Virginia
N
0 50 100 200
Kilometers
Tennessee
North Carolina
South Carolina
Georgia
Legend
Pinus strobus Distribution
Mm present (2011-2013)
Mm absent (2011-2013)
Mm and Cp present
Mm present and Cp absent
Mm absent and Cp present
Mm and Cp absent
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community

Figure 2.2

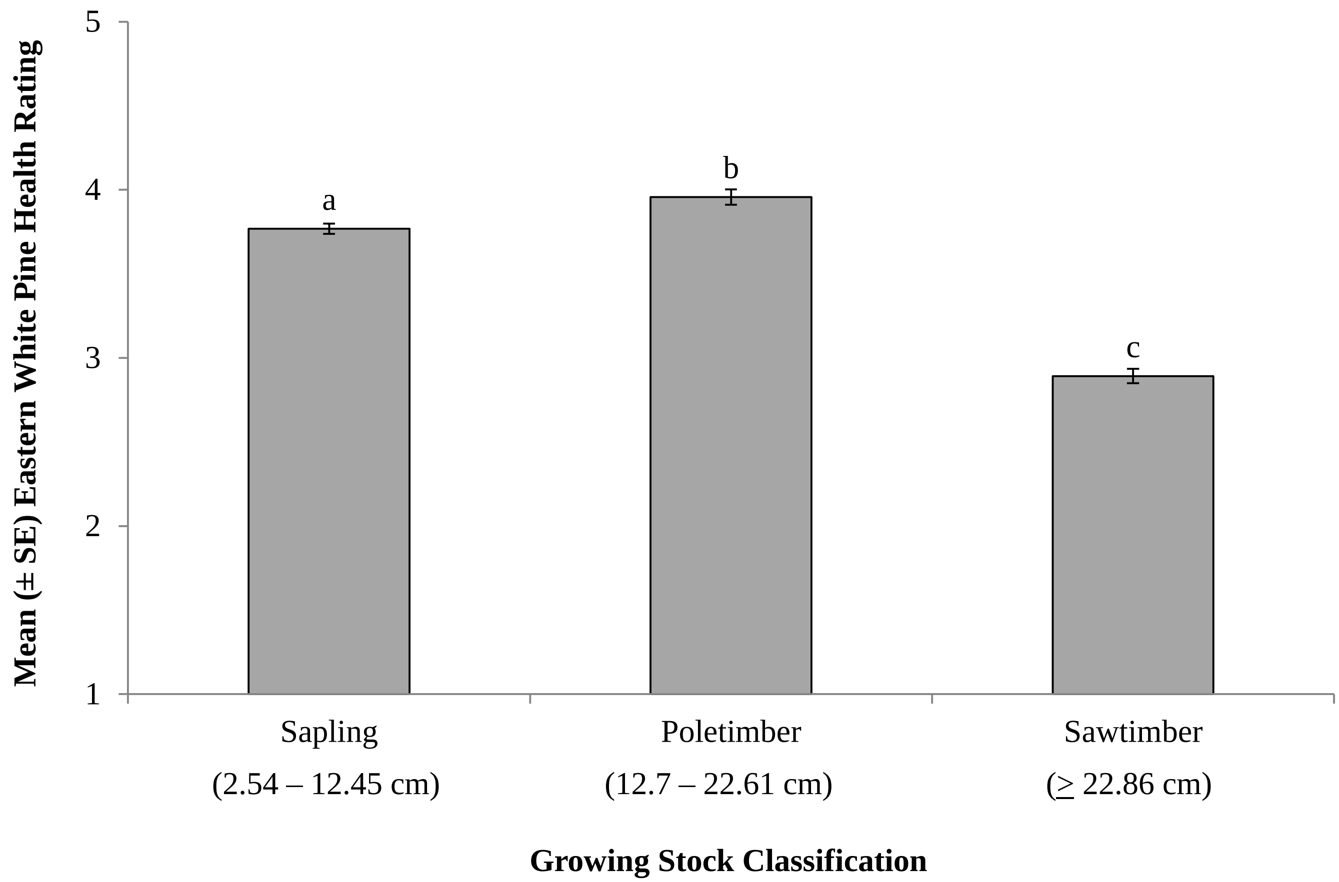

Mean (± SE) Eastern White Pine Health Rating
5
4
3
2
1
a
b
c
Sapling
(2.54 – 12.45 cm)
Poletimber
(12.7 – 22.61 cm)
Sawtimber
(≥ 22.86 cm)
Growing Stock Classification

Figure 2.3

CHAPTER 3

EFFECTS OF A SCALE INSECT-FUNGAL PATHOGEN COMPLEX ON EASTERN WHITE PINE HEALTH IN THE APPALACHIAN MOUNTAINS[2]

[2]Schulz, A.N., C. Asaro, D.R. Coyle, M.M. Cram, R.D. Lucardi, A.M. Mech, and K.J.K. Gandhi. To be submitted to *Forest Ecology and Management*.

Abstract

Eastern white pine (*Pinus strobus* L.) is an important conifer species across the eastern region of North America. In the last decade, eastern white pine have started displaying symptoms of branch flagging, resinosis, crown thinning, canker development with multiple fungal species, and *Matsucoccus macrocicatrices* Richards (Hemiptera: Matsucoccidae). For this study, we evaluated the eastern white pine-*M. macrocicatrices*-canker complex on 270 total symptomatic eastern white pine saplings from Georgia, Maine, Massachusetts, New Hampshire, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia. Results indicate that there were positive correlations between *M. macrocicatrices* and sapling dieback, cankers and sapling dieback, and *M. macrocicatrices* and cankers. Hence, there appears to be a mutualistic relationship between *M. macrocicatrices* and fungal pathogens that are associated with cankers. About 95% of *M. macrocicatrices* individuals sampled were associated with cankers, especially cankers formed by the fungus, *Caliciopsis pinea* Peck, which dominated the canker area. At least five other fungal genera were isolated from the cankers. We found a distinct latitudinal gradient where more cankers were present on saplings in northeastern than southeastern eastern white pine saplings. Overall, it appears that this unique and novel insect-pathogen complex is negatively affecting regeneration dynamics of eastern white pine in the United States.

INDEX WORDS: Appalachian Mountains, *Caliciopsis pinea*, canker, eastern white pine, *Matsucoccus macrocicatrices*, *Pinus strobus*, tripartite complex

3.1 Introduction

Eastern white pine (*Pinus strobus* L.) is one of the key conifer species in the Appalachian Mountains, and has been an economically important species since the beginning of logging in the United States (Harlow et al.1979). Betts (1954) indicated that the lumber industry in the U.S. was founded on eastern white pine. Production of eastern white pine lumber started around 1630 in New York, and soon spread to the surrounding New England states. By 1840, most of the original eastern white pine trees in the Northeast were cut, so production shifted to the Lake States, specifically Michigan, Minnesota, and Wisconsin (Betts 1954). The soft, straight-grained wood was used for car construction, ship masts, agricultural equipment, caskets, matches, flooring, crates, and more (Betts 1954; Carter et al. 1988). Extensive logging of eastern white pine forests nearly eliminated the mature eastern white pine resource (Ostry et al. 2010). In the early 1930’s, reports on eastern white pine’s desirable characteristics, notably its economic value, few insect and disease problems, and toleration of poor site conditions, and excellent growth rates drew the attention of many entities. Hence, the Civilian Conservation Corps (CCC) decided to use eastern white pine for much of its reforestation program (Vimmerstedt 1962). Post-1930’s, the use of eastern white pine lumber dwindled due to a diminished supply over the range, but it has remained a valuable species for biotic diversity, aesthetics, Christmas tree production, and other forest products (Ostry et al. 2010). Eastern white pine also has many ecological applications, including its use for food and shelter by wildlife, such as white-tailed deer (*Odocoileus virginianus* Zimmermann), and bald eagles (*Haliaeetus leucocephalus* L.), as well as stabilization of lands that have been extensively mined (Martin et al. 1951; Czapowskyj and McQuilkin 1966; Wendel and Smith 1990).

Eastern white pine has a plethora of insect pests and pathogens associated with it, including white pine sawfly (*Neodiprion pinetum* Norton), pine bark adelgid (*Pineus strobi* Hartig), white pine weevil (*Pissodes strobi* Peck), white pine blister rust (*Cronartium ribicola* A. Dietr.), Heterobasidion root disease (*Heterobasidion irregulare* Garbelotto and Otrosina), and white pine root decline (*Verticicladiella procera* Kendrick) (Baker and Craighead 1972; Livingston and Wingfield 1982; Ostry et al. 2010; Ostry et al. 2011). During the last few years, forest health specialists have noticed substantial dieback of eastern white pine across its native range. Recent news releases have indicated that eastern white pine trees of all age classes and over many site conditions have been expressing symptoms such as cankerous growths, significant sapping (resinosis), crown thinning, branch flagging, and decreases in crown density (Asaro 2011; Rose 2011; Rosenholm 2012). A closer inspection of the branches and stems of eastern white pine revealed a common characteristic among the dying pines: a scale insect, *Matsucoccus macrocicatrices* Richards (Figure 3.1), embedded under lichen and in branch crotches and cankers, such as *Caliciopsis pinea* Peck cankers (Mech et al. 2013) (Figure 3.2).

Matsucoccus macrocicatrices belongs to the family Matsucoccidae, which contains one extant genus, *Matsucoccus* Cockerell (1909), and 39 known species throughout the world (Foldi 2004; Liu et al. 2014). Matsucoccidae are known, commonly, as the pine bast scales, which are some of the most serious sap-sucking pests of natural pine forests and plantations in the U.S., China, and the Mediterranean basin (Foldi 2004; Liu et al. 2014). *Matsucoccus macrocicatrices* is the only documented

Matsucoccus species on eastern white pine (Richards 1960; Watson et al. 1960; Kosztarab 1996; Foldi 2004; and Mech et al. 2013). Until the mid-2000's, *M. macrocicatrices* was only known to exist on eastern white pine trees in Canada (New Brunswick, Nova Scotia, Ontario, and Quebec) and the northeastern U.S. (New Hampshire) (Richards 1960; Martineau 1964; and Kosztarab 1996). However, recent studies have shown that *M. macrocicatrices* is present on eastern white pine in the southeastern region of the U.S., specifically in Georgia, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia (Mech et al. 2013). Until now, *M. macrocicatrices*' damage to eastern white pine has been considered negligible (Watson et al. 1960).

Little research has been conducted on the life cycle, and reproductive and feeding strategies of *M. macrocicatrices*. Early evaluation of *M. macrocicatrices* revealed that it may have a two-year life cycle including egg, crawler (first stage larva), apodous intermediate, and adult stages (Richards 1960; Watson et al. 1960). It has been suggested that there are females and pre-adult males (resemble female morphology), which can pupate and emerge as adult males (Richards 1960). Although males have been described, it has been proposed that *M. macrocicatrices* may be parthenogenetic (Watson et al. 1960; Foldi 2004). Descriptions of *M. macrocicatrices* have indicated that the adults lack mouthparts (Richards 1960). The immature stages have a well-developed stylet (Richards 1960), but it is unknown whether they feed on the xylem or phloem of the eastern white pine trees.

Early descriptions of *M. macrocicatrices* have also suggested that there may be an insect-fungus-host relationship with the *Septobasidium pinicola* Snell epiphytic fungus

(Watson et al. 1960; Figure 3.1). A study exploring the relationship between *S. pinicola* and *M. macrocicatrices* determined that, although the relationship is not fully understood, the fungus may protect the insect from adverse weather conditions, and possibly from parasitism, while deriving nourishment from the insect (Watson et al. 1960). An earlier study suggested that *S. pinicola* lives as an epiphyte on eastern white pine, but it parasitizes and destroys *M. macrocicatrices* (Snell 1922). Other studies within the genus *Septobasidium* have come to similar conclusions that the fungus is either symbiotic (Couch 1931), or parasitic (Burt 1916; Coker 1920; Petch 1921) with *M. macrocicatrices*.

Another key factor that may be affecting the health of eastern white pine is *C. pinea*, a canker-forming ascomycetous fungus that is native to the eastern U.S. (Funk 1963; Horst 2012). *Caliciopsis pinea* is primarily found on eastern white pine, but it has been documented on other species in the eastern U.S., including pitch pine (*Pinus rigida* Mill.), table mountain pine (*Pinus pungens* Lamb.), shortleaf pine (*Pinus echinata* Mill.), and Virginia pine (*Pinus virginiana* Mill.) (Funk 1963). The cankers have been described as "reddish brown depressions in the bark that have small, globose, clustered, black pycnidia and stalked perithecia that arise from stromatic cushion" (Overholts 1930; Ray 1936; Horst 2012; Figure 3.2). Development of fruiting structures begins with the aggregation of fungal hyphae under the bark of a tree. This aggregation creates a flattened stroma, which continues to grow, and will eventually erupt from the bark of the tree. Once the stroma erupts, it provides a foundation for the production of ascocarps (hair-like structures), which enlarge and elongate, and go on to bear ascospores (main disseminating agents). Any conical lobes that do not elongate and turn into ascocarps are referred to as spermagonia. Spermagonia are capable of producing spermatia, which can also disperse, germinate and grow new colonies of *C. pinea*. Once established, the perennial *C. pinea* cankers are capable of producing annual crops of ascocarps with ascospores, and spermagonia with spermatia (McCormack 1936; Ray 1936; Funk 1963). It is questionable whether *C. pinea* spores need wounds to colonize the tissues of eastern white pine trees, though it has been noted that other species in the genus *Caliciopsis* take advantage of old lenticels and wounds from mechanical damage, insect feeding, ovipositing, or boring (Funk 1963).

Until recently, *C. pinea* was not thought to create significant damage to eastern white pine trees, although it was shown to create sharply delimited cankers on the trunks and branches of eastern white pine (Ray 1936; Cram 2009). Reports from the last few years, however, have indicated that *C. pinea* cankers have been damaging and potentially causing the mortality of thousands of acres of eastern white pine in the northeastern (Maine, Massachusetts, New Hampshire, New York, and Vermont) and southeastern (Georgia, Virginia, and West Virginia) regions of the U.S. (Asaro 2011; Rose 2011; Rosenholm 2012). Some sources indicate that the disease is most serious on suppressed saplings (Overholts 1930; Horst 2012), but it has also been found on mature trees (Rose 2011).

Since eastern white pine remains one of the most economically and ecologically important species in the eastern U.S., there is a need to better assess the relationships among the eastern white pine, *M. macrocicatrices*, and cankers that are present on the bole and branches of eastern white pine, to determine what could be associated with the dieback in the Appalachian Mountains. The objectives of this study are to: 1) determine the prevalence and distribution of *M. macrocicatrices* with *C. pinea*, other cankers, and no cankers, respectively; and 2) assess correlations among eastern white pine sapling dieback, canker surface area, and *M. macrocicatrices* in the southeastern and northeastern regions. We hypothesize that: 1) *M. macrocicatrices* will primarily be associated with *C. pinea* cankers; 2) eastern white pine sapling dieback will be correlated with cankers and *M. macrocicatrices*; and 3) cankers will be correlated with *M. macrocicatrices* in the southeastern and northeastern regions.

3.2 Methods

3.2.1 Study Sites

We established 40 sites in the major range of eastern white pine (Little 1971; USGS 2013) in the southern region of the Appalachian Mountains, specifically in Georgia, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia (Figure 3.3). Eight additional sites were established in the Northern Appalachian and Atlantic Maritime Highlands of New Hampshire, Massachusetts and Maine. These sites encompassed the eastern temperate (Appalachian) and northern (mixed wood plain and Atlantic highland) forests of the eastern U.S. (CEC 1997; Omernik and Griffith 2014). The general soil orders (and dominant suborders) found at these sites include Inceptisols (Udepts), Ultisols (Udults), Spodosols (Orthods), and, to a lesser extent, Entisols (Orthents) (Wendel and Smith 1990; USDA NRCS 1998).

Average annual precipitation fluctuated over the range of the sites, with southeastern states (Georgia: 1,267 mm, North Carolina: 1,249 mm, South Carolina: 1,208 mm, Tennessee: 1,316 mm, Virginia: 1,095 mm, and West Virginia: 1,138 mm) generally having higher annual precipitation than the northeastern states (Maine: 1,083 mm, Massachusetts: 1,152 mm, and New Hampshire: 1,124 mm) (NOAA 2014). Similar to annual precipitation, average annual temperature was higher in the southeastern states (Georgia: 17.4°C, North Carolina: 14.8°C, South Carolina: 16.9°C, Tennessee: 14.3°C, Virginia: 12.8°C, and West Virginia: 10.9°C) than the northeastern states (Maine: 4.6°C, Massachusetts: 8.4°C, and New Hampshire: 5.8°C) (NOAA 2014).

All sites were within one of the five Society of American Foresters (SAF) forest cover types: red pine (Type 15), eastern white pine-northern red oak-red maple (Type 20), eastern white pine (Type 21), eastern white pine-eastern hemlock (Type 22), or eastern white pine-chestnut oak (Type 51). In the southeastern region, eastern white pine occupied all crown classes (suppressed, intermediate, codominant, and dominant). In the northeastern region, eastern white pine was often a dominant or codominant tree. Other than eastern white pine, overstory vegetation within the southeastern and northeastern sites included hardwoods, such as American beech (*Fagus grandifolia* Ehrh.), birch (*Betula* spp.), maple (*Acer* spp.), red oak (*Quercus rubra* L.), tulip poplar (*Liriodendron tulipifera* L.), or white oak (*Q. alba* L.). Some sites also had eastern hemlock [*Tsuga canadensis* (L.) Carrière] or other pines, such as red pine (*Pinus resinosa* Aiton), Virginia pine (*P. virginiana* Mill.), table mountain pine (*P. pungens* Lamb.), pitch pine (*P. rigida* Mill.), or slash pine (*Pinus elliottii* Engelm). Common understory species included: dogwood (*Cornus* spp.), mountain laurel (*Kalmia latifolia* L.), rhododendron (*Rhododendron maximum* L.), bracken fern (*Pteridium aquilinum* (L.) Kuhn), and buckberry (*Vaccinium stamineum* L) (Wendel and Smith 1990).

3.2.2 Collection of Eastern White Pine

Within each of the southeastern sites, we established three plots at least 50 m apart. Slope, aspect, and elevation were noted for each plot. All trees that were $\geq$ 2.54 cm diameter at breast height (DBH) within the plot were measured. Six eastern white pine saplings were collected from each of the 40 sites (n = 240). Additional saplings were collected from another site in South Carolina (n = 6), and three saplings were collected from eight sites in total from New Hampshire, Massachusetts, and Maine (n = 24), for a total of 270 saplings. Each of the 270 saplings received an overall dieback rating based on the proportion of live to dead nodes on the tree, and were cut starting from the top first node and measured up to 100 cm to the base of the sapling. Any saplings that were ≥ 100 cm in length were cut at the 100 cm mark. Saplings that were < 100 cm were measured to determine their length. Small and large end diameters (mm) of the saplings were taken using calipers.

3.2.3 Sampling of *M. macrocicatrices*

On each eastern white pine sapling, we searched for, and, if found, counted the number of each life stage of *M. macrocicatrices* including: eggs, crawlers (first instar, mobile nymphs), cysts (heavily sclerotized, legless stage between legged crawler and adult stages), shells (shed skin from adult emergence), and adults. Due to the timing of sampling (January 2014-September 2014), we only found cysts and shells on the saplings. All samples were stored in a walk-in refrigerator at 4.4° C to prevent mold and temporarily preserve the *M. macrocicatrices*.

Eastern white pine specimens from each site were dry-pressed and deposited in the Herbarium Museum, University of Georgia, Athens according to the herbarium regulations. Collected cysts of *M. macrocicatrices* were preserved in ethanol and delivered to the Lucardi Genetics Laboratory, USDA Forest Service, Athens for a separate study. Extra cysts of *M. macrocicatrices* were deposited in the Museum of Natural History, University of Georgia, Athens.

3.2.3 Sampling of Fungal Species

For each eastern white pine sapling, we also assessed the size (using a mm^2 grid) and location (B1: first branch whorl and below, B2: second branch whorl to just above first branch whorl, etc.) of cankers, and the presence/absence of *S. pinicola*, which is identifiable by darker, brown-colored hyphae surrounded by lighter, brown- to cream-colored hyphae, and has an overall spongy appearance (Figure 3.1) (Snell 1922). Cankers were identified as having either *C. pinea* or other pathogens. *Caliciopsis pinea* cankers were identifiable by their asexual (shiny, black clusters of spermagonia) and sexual (black, hair-like ascocarps) structures (Figure 3.1). For the southeastern region saplings, any cankers that were not identified as *C. pinea* and were > 40 mm^2 were extracted, placed in sanitized plastic bags and labeled with the sapling and site information, size of the canker, as well as the number of *M. macrocicatrices* nymphs and shells that were associated with the canker. In addition to the cankers, any tissue that did not have visible canker, but had at least one settled *M. macrocicatrices* was extracted and packaged with the same information as the canker extractions. All samples were stored in a 4.4° C refrigerator until they were delivered to the Pathology Laboratory, USDA Forest Service, Athens for isolation.

Extractions were categorized as either: 1) *M. macrocicatrices* with no canker; 2) *M. macrocicatrices* absent but canker present; or 3) both canker and *M. macrocicatrices* present. To make isolates, extractions were surface sterilized for 10 seconds in 95% ethanol, then put in 1.05% NaOCl solution for four minutes (Blodgett and Stanosz 1997).

The surface sterilized tissue was then washed in sterile water for one minute and blotted dry with sterile paper towels. Each extraction was surface shaved, divided into three (Figure 3.4), and put on three types of media: modified Nash-Snyder media (Nelson et al. 1983), pine needle agar (PNA) media (1.5% water agar plus needles) (Blodgett et al. 2003), and potato dextrose agar with streptomycin and terigitol (PDA+S+T) media (Steiner and Watson 1965). Plated samples were incubated at 20 °C for over 4 weeks with weekly observation for identification or transfer of isolates to other media. Samples with unidentifiable mycelium isolates were transferred to carnation-leaf water agar (Nelson et al. 1983) or pine needle agar in an attempt to induce the isolate to produce spores for identification. Second transfers were observed weekly for another four weeks. Morphologically unidentifiable isolates will be genetically analyzed in summer 2015 to assist with identification to genus and species. Isolates that are selected for inoculation studies in summer 2015 will be stored on PDA slants and in 10% glycerin at -20 °C until ready for use.

3.2.4 Statistical Analyses

For each of the 270 eastern white pine saplings, we counted the total number of *M. macrocicatrices* (all stages) and assessed dieback (proportion of dead nodes). Since surface area of each sapling varied, we standardized both the *M. macrocicatrices* and canker data by dividing the total number of *M. macrocicatrices* and the total canker coverage (mm^2) by the respective surface area (mm^2) of each sapling, thus creating proportions of *M. macrocicatrices* and total canker, respectively. After standardization, all data were analyzed using R v. 3.1.3 (R Development Core Team 2013).

The data were first checked for normality using a Schapiro-Wilks normality test. Since data were not normal, and transformations were unable to normalize the data, we used a non-parametric Spearman's Rank Correlation Coefficient to analyze the relationships among *M. macrocicatrices*, cankers, and eastern white pine sapling dieback. Specifically, we assessed the correlations between: 1) the proportion of *M. macrocicatrices* and eastern white pine sapling dieback; 2) the proportion of total canker and dieback; and 3) the proportion of *M. macrocicatrices* and proportion of total canker, respectively.

Since there were many types of cankers present on the saplings, we calculated the percentage of *M. macrocicatrices* per sapling found either: 1) in cankers with fruiting bodies of *C. pinea*; 2) in other cankers (with no obvious fruiting bodies of *C. pinea*), and 3) without a canker (usually under lichen, moss or in node on tree without apparent cankerous tissue). Kruskal-Wallis Rank Sum tests were used to compare the groups.

To determine if the prevalence of *C. pinea* and other cankers (unknown fungal species) varied from southern latitudes to more northern latitudes of the eastern white pine range, we calculated the total canker surface area for *C. pinea* and other fungal species for each sapling, and standardized the values by sapling surface area. We then summed the standardized canker surface area (mm^2) for each canker type for each respective site and divided the summed values by the number of saplings analyzed from each site (n=6 for southeast sites; n=3 for northeast sites). A Spearman's Rank Correlation Coefficient test was used to evaluate whether there were correlations between *C. pinea* cankers and latitude, and other cankers and latitude.

To assess the distribution of the total canker surface area (cm^2) on the eastern white pine saplings, we summed the total canker area for each portion (B1, B2, B3, B4, etc.) of the 270 saplings. For instance, B1 includes all of the cankers from the first eastern white pine branch whorl to the base; B2 includes all of the cankers from the second branch whorl to just above the first branch whorl, and etcetera. Similarly, we assessed the distribution of *M. macrocicatrices* on the saplings, where we summed the total number of *M. macrocicatrices* for each portion of the saplings. Visual representations of the distribution of the cankers and *M. macrocicatrices* were made.

3.3 Results

3.3.1 Eastern white pine-*M. macrocicatrices*-canker relationship in the southeastern region

A total of 2,402 individual *M. macrocicatrices* were found on the 246 eastern white pine saplings that were collected from the southern Appalachian Mountains, with a mean (± SE) of 9.8 ± 1.9 and range of 0 to 265 *M. macrocicatrices* per sapling. In general, 52.9% of the saplings had no *M. macrocicatrices*, 37% had 1-20 *M. macrocicatrices*, and 10.1% had ≥ 21 *M. macrocicatrices* (Figure 3.5A). Mean (± SE) canker surface area (cm^2) on each sapling was 83.95 ± 9.57, and ranged from 0 cm^2 to 1,054.3 cm^2. The mean (± SE) number of *M. macrocicatrices* associated with *C. pinea* cankers was 38.4 ± 11.6, followed by 17.2 ± 5.6 associated with other cankers, and 3.0 ± 1.4 found outside a canker (Figure 3.6). Kruskal-Wallis tests revealed that there were differences in the mean number of *M. macrocicatrices* found in *C. pinea* cankers and no canker ($\chi^2 = 10.17$; $P < 0.01$), as well as other cankers and no canker ($\chi^2 = 13.19$; $P < 0.001$), but there were no differences between *C. pinea* and other cankers ($\chi^2 = 0.05$; $P >$

0.1) (Figure 3.6). Another way to view these values is that 66% of the collected *M. macrocicatrices* were associated with *C. pinea* cankers, 29% were associated with other cankers, and only 5% were found without a canker, so, overall, 95% of the collected *M. macrocicatrices* were found to be associated with cankers on the saplings. Mean (± SE) *C. pinea* canker size (mm^2) was 206.8 ± 16.1, while mean other canker size was 36.6 ± 2.1. *Septobasidium pinicola* was collected from saplings from three sites, including two sites in Virginia and one site in West Virginia.

We also found that there were positive correlations between the proportions of canker and dead nodes on the eastern white pine saplings ($P < 0.001$, $r_s = 0.62$, Figure 3.7A), the proportions of *M. macrocicatrices* and dead nodes on saplings ($P < 0.001$, $r_s = 0.46$, Figure 3.7B), and the proportions of *M. macrocicatrices* and total cankers ($P < 0.001$, $r_s = 0.55$, Figure 3.8).

Analyses of the other canker isolates revealed that, out of the 381 cankers isolated from the 246 saplings collected in the southern Appalachian Mountains, 20% were identified as *Phaeomoniella* spp., 5% were *Phomopsis* spp., 4% were *Chaetophoma* spp., 3% were *Pestalotiopsis* spp., and 2% were *Pezicula* spp. Other, currently unidentifiable genera (approximately 27 different genera) made up 31% of the isolates. A further 9% were classified as “unknown,” 4% did not have fungi, and 22% were contaminants such as *Penicillium* spp. (Table 3.1).

3.3.3 Eastern white pine-*M. macrocicatrices*-canker relationship in the northeastern region

A total of 929 individual *M. macrocicatrices* were found on the 24 eastern white pine saplings that were collected from the northeastern U.S., with a mean (± SE) of 38.7

± 7.1 and range of 1 to 110 *M. macrocicatrices* per sapling. Altogether, 0% of the saplings had no *M. macrocicatrices*, 45.8% had 1-20 *M. macrocicatrices*, and 54.2% had ≥ 21 *M. macrocicatrices* (Figure 3.5B). Mean (± SE) canker surface area (cm^2) on each sapling was 277.9 ± 43.9, and ranged from 25.6 cm^2 to 812.6 cm^2 on saplings.

The mean (± SE) number of *M. macrocicatrices* associated with *C. pinea* cankers was 56.1 ± 24.4, followed by 55.5 ± 15.8 that were associated with other cankers, and 4.5 ± 1.6 that were found outside a canker (Figure 3.6). Kruskal-Wallis tests revealed that, like the southeastern samples, there were differences in the mean number of *M. macrocicatrices* found in *C. pinea* cankers and no canker (χ^2 = 4.67; $P < 0.05$), as well as other cankers and no canker (χ^2 = 8.66; $P < 0.01$), but there were no differences between *C. pinea* and other cankers (χ^2 = 0.22; $P > 0.1$) (Figure 3.6). An equal percentage of the *M. macrocicatrices* collected were associated with *C. pinea* cankers and other cankers (48%), while the remaining 4% were found without a canker, so, largely, 96% of the *M. macrocicatrices* collected were found to be associated with cankers on the saplings. Mean (± SE) *C. pinea* canker size (mm^2) was 577.4 ± 93.8, while the mean size of other cankers was 49.9 ± 3.8. *Septobasidium pinicola* was collected from saplings from three sites, including two sites in Maine and one site in New Hampshire.

Overall, there were positive correlations between the proportions of canker and dead nodes on eastern white pine saplings (P < 0.05, r_s = 0.62, Figure 3.7A), the proportions of *M. macrocicatrices* and dead nodes on white pine saplings (P < 0.01, r_s = 0.44, Figure 3.7B), and the proportions of *M. macrocicatrices* and total cankers on eastern white pine saplings (P < 0.01, r_s = 0.53, Figure 3.8).

3.3.4 Trends over the latitudinal range

Results from a Spearman's Rank Correlation Coefficient test between each of the canker types (*C. pinea* and unknown species) and latitude indicate that there were positive correlations between *C. pinea* and latitude ($P < 0.01$, $r_s = 0.39$), and unknown spp. and latitude ($P < 0.01$, $r_s = 0.39$), indicating that there are more and larger cankers in the northeastern than southeastern sites (Figure 3.9). Overall, 65.1% and 64.9% of the total canker surface area was identified as *C. pinea* in the southeastern and northeastern sites, respectively.

3.3.5 *M. macrocicatrices* and canker distribution on saplings

Over 36% of the total canker area (cm^2) on the saplings was found below the first branch whorl. Similarly, 29% of *M. macrocicatrices* found on the saplings were found below the first branch whorl, often in cankers, or under moss or lichen that were growing at the base of the trees. Overall, the total canker area (cm^2) and number of *M. macrocicatrices* both decreased when going from the older, base tissue to the younger, meristem tissue (Figure 3.10).

3.4 Discussion

Our findings demonstrate that there were positive correlations among eastern white pine saplings, cankers, and *M. macrocicatrices* in the southeastern and northeastern regions of the United States. Similar to the beech scale insect (*Cryptococcus fagisuga* Lind.) on American beech, and Israeli pine bast scale (*Matsucoccus josephi* Bodenheimer and Harpaz) associated with *Sphaeropsis sapinea* (Fr.) on Aleppo pine (*Pinus halepensis* Mill.), *M. macrocicatrices* may be an important factor since the canker-forming fungi likely require entry wounds for infection (Houston 1994; Madar et al. 2005). We hypothesize that this tripartite interaction includes a healthy eastern white pine tree (Figure 3.11A) that is fed on by *M. macrocicatrices* (Figure 3.11B). The entry wound created by the stylet of the scale insect may aid the spores of canker-forming fungi with entry into the tree. After successful establishment in the feeding wounds of *M. macrocicatrices*, the canker-forming fungi can grow and penetrate into the sapwood (Figure 3.11C). The amalgamating cankers then cut off the flow of water and nutrients through the tracheids, reduce radial growth, create crown dieback, girdle, and eventually kill the trees (Coulson and Witter 1984; Houston 1994).

There may also be an inverse relationship where the callous tissue that develops around each canker may benefit *M. macrocicatrices* (Houston 1994). Cursory observations indicate that, often times, the living edge of the cankers will curl up to provide a crack or ridge in the tissue where the immature stages of *M. macrocicatrices* can settle (Figure 3.11 D). If *M. macrocicatrices* settles on the edge of the canker, it can feed by probing out into the living tissue surrounding the cankerous tissue with its long stylet, thus creating another wound for canker-forming fungal spores. This provides a positive feedback to the increased incidence of fungi, and, therefore, canker formations on the trees. It is important to note that the exact contributions of *M. macrocicatrices* and fungal pathogens to canker formation are still unclear.

About two-thirds of the total canker surface area was identified as *C. pinea* in both the southeastern and northeastern sites. Further, in the southeastern sites, 66% of *M. macrocicatrices* collected were found in cankers created by the *C. pinea* fungus. In the northeastern sites, 48% of *M. macrocicatrices* were found in *C. pinea* cankers. Studies have indicated that *C. pinea* can create sharply delimited cankers on trunks and branches, and cause dieback in small diameter eastern white pine (Ray 1936). Press releases have suggested *C. pinea* as a major factor affecting eastern white pine health (Rose 2011; Rosenholm 2012). Compared to other fungal pathogens found in the cankers, *C. pinea* may be more geographically widespread, able to easily colonize pine tissue, or more pathogenic to the tree.

There were at least five other fungal genera involved with non-*C. pinea* cankers, including *Chaetophoma* spp., *Pestalotiopsis* spp., *Pezicula* spp., *Phaeomoniella* spp., and *Phomopsis* spp.. Various species of *Phaeomoniella* have been shown to be pathogenic on host trees and shrubs in the genus *Prunus*, while species in *Pezicula* have been documented as endophytic, plant pathogenic fungi that aid with the decay and shedding of dead branches on trees (Kehr 1992; Ooki et al. 2003; Damm et al. 2010). *Phomopsis*, a hemibiotrophic fungus, may create stem cankers which are capable of killing small diameter douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] and other host plant species (Udayanga et al. 2011). Species in the genus *Pestalotiopsis* have been documented to create stem cankers on *Rhododendron*, though some species, such as *P. funerea* (Desm.), can be found on other host genera, including *Pinus*, *Picea*, and *Juniperus* (Farr et al. 1996). *Chaetophoma* has been documented as a saprophytic fungus that often exists in soils or leaf litter (Hurst et al. 1983). Overall, most of these fungal genera appear to be low to moderately pathogenic on other hosts.

We found a distinct latitudinal gradient where more and larger cankers were present in the central and northern Appalachian sites (Maine, Massachusetts, New Hampshire, Virginia and West Virginia) than the southern sites (Georgia, North Carolina, South Carolina, and Tennessee). Since *M. macrocicatrices* was only previously recorded in the northeastern U.S. and Canada, it may have been causing damage for a longer time. Also, eastern white pine is a more prevalent species in the northeastern than southeastern region where forest stands with eastern white pine often include a mix of hardwoods, hemlock or other pine species (Barrett 1995). With the presence of more concentrated host material, the buildup of insect populations may have been easier and faster due to an increased foraging efficiency (Root 1973; Kareiva 1983). Studies have suggested that specialist herbivorous insects that may not be highly mobile will often stay in more concentrated host plant patches or stands (Kareiva 1985; Jactel et al. 2004).

There was a distinct spatial pattern of cankers and *M. macrocicatrices* on the saplings, where most of the cankers and *M. macrocicatrices* were found at the base of the saplings. Often times, ovoid to elongate cankers appeared as large swellings centered at the base of the eastern white pine stems. These larger, older cankers at the base of the stem may have been present longer than the younger, smaller cankers on the younger tree tissue. Similar to bark beetles (Coulson 1979; Sullivan 2011), girdling of the stem at the base would be more damaging than girdling at the apical portion of the tree. If some of the cankers continue to grow with time, as we hypothesize, then there may be little chance for these saplings to recover.

Overall, *M. macrocicatrices*, cankers, and pathogenic fungi, such as *C. pinea*, appear to be drivers of the eastern white pine dieback in the mixed hardwood-conifer forests of the Appalachian Mountains. Although our results have shed some light on this novel tripartite complex, there is still much to be learned about the processes involved in the dieback of eastern white pine. Contemporary research of complexes such as beech bark disease have suggested that tripartite interactions often involve pest-induced changes in host physiology or antagonisms between plant defense hormones (Hatcher 1995; Thaler et al. 2002; Stout et al. 2006; Cale et al. 2014). Factors such as soil and bark chemistry, temperature, precipitation, host population genetics, and insect/pathogen dispersal ability can also be involved in the success or failure of the insects and pathogens in the tripartite complexes (McClure 1989; Cale et al. 2015). Future research may aim to determine the: 1) correlations among mature eastern white pine trees, *M. macrocicatrices*, and cankers throughout the range of eastern white pine; 2) fungal pathogens found in the non-*C. pinea* cankers and assess their pathogenicity; 3) feeding mechanisms, reproductive strategies, and phenology of *M. macrocicatrices*; 4) relationship between *S. pinicola* and *M. macrocicatrices*; and 5) strategies to best manage pathogenic fungi and *M. macrocicatrices*.

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3.6 Literature Cited

Asaro, C. 2011. Virginia Department of Forestry Forest Health Review. 11 p.

Baker, W.L. and Craighead, F. 1972. Eastern Forest Insects. Washington, D.C. USDA Forest Service. 608 p.

Barrett, J.W. 1995. Regional Silviculture of the U.S. 3rd Ed. John Wiley and Sons, Inc. 643 p.

Betts, H.S. 1954. Eastern White Pine. USDA Forest Service Publication. 8 p.

Blodgett, J.T., and Stanosz, G.R. 1997. *Sphaeropsis sapinea* morphotypes differ in aggressiveness, but both infect non-wounded red or jack pines. Plant Disease 81:143-147.

Blodgett, J.T., Bonello, P., and Stanosz, G.R. 2003. An effective medium for isolating *Sphaeropsis sapinea* from asymptomatic pines. Forest Pathology 33:395-404.

Burt, E. A. 1916. The Thelephoraceae of North America. Annals of the Missouri Botanical Garden 3: 319.

Cale, J.A., A.W. Ashby, J.L. West, S.A. Teale, M.T. Johnston, and J.D. Castello. 2014. Scale insects, decay and canker fungi in American beech. Forest Pathology 45(1): 71-75.

Cale, J.A., S.A. Teale, M.T. Johnston, G.L. Boyer, K.A. Perri, and J.D. Castello. 2015. New ecological and physiological dimensions of beech bark disease development in aftermath forests. Forest Ecology and Management 336: 99-108.

Carter, K.K., D.H. DeHayes, M.E. Demeritt, R.T. Eckert, P.W. Garrett, H.D. Gerhold, J.E. Kuser, and K.C. Steiner. 1988. Tree Improvement in the Northeast: Interim Summary and Recommendations for Selected Species. Maine Agricultural Experiment Station Technical Bulletin 131.

Coker, W.C. 1920. Notes on the Lower Basidiomycetes of North Carolina. Journal of the Elisha Mitchell Scientific Society 35 (3-4): 113-182.

Commission for Environmental Cooperation. 1997. Ecological regions of North America: toward a common perspective. Commission for Environmental Cooperation, Montreal, Quebec, Canada. 71p. Map (scale 1:12,500,000). Revised 2006.

Couch, J.N. 1931. The Biological Relationship between *Septobasidium retiforme* (B. & C.) Pat. and *Aspidiotus osborni* New. and Ckll. Quarterly Journal of Microscopial Science 2(74): 383-438.

Coulson, R.N. 1979. Population dynamics of bark beetles. Annual Review of Entomology 24:417–47.

Coulson, R.N. and J.A. Witter. 1984. Forest Entomology: Ecology and Management. John Wiley and Sons, 669 p.

Cram, M.M., C. Asaro, and T. Edgerton. 2009. Field Inoculations of *Caliciopsis pinea* and *Diplodia scrobiculata* on Eastern White Pine. Proceedings from the Southwide Forest Disease Workshop. June 29 – July 2, 2009.

Czapowskyj, M.M., and W.E. McQuilkin. 1966. Survival and early growth of planted forest trees on strip mine spoils in the Anthracite Region. USDA Forest Service, Research Paper NE-46. Northeastern Forest Experiment Station, Upper Darby, PA. 29 p.

Damm, U., P.H. Fourie, and P.W. Crous. 2010. *Coniochaeta* (Lecythophora), *Collophora* gen. nov. and *Phaeomoniella* species associated with wood necroses of *Prunus* trees. Persoonia 24: 60-80.

Farr, D.F., H.B. Esteban, and M.E. Palm. 1996. Fungi on *Rhododendron*: A World Reference. Parkway Publishers, Inc. 192 p.

Foldi, I. 2004. The Matsucoccidae in the Mediterranean basin with a world list of species (Hemiptera: Sternorrhyncha: Coccoidea). Annales de la Société entomologique de France (N.S.): International Journal of Entomology 40 (2): 145 – 168.

Funk. A. 1963. Studies in the genus *Caliciopsis*. Canadian Journal of Botany 41: 503-543.

Harlow, W.M., E.S. Harrar and F.M. White. 1979. Textbook of Dendrology, 6th Ed. New York, McGraw-Hill Book Co. 510 p.

Hatcher, P. E. 1995. Three-way interactions between plant pathogenic fungi, herbivorous insects and their host plants. Biological Reviews 70: 639-694.

Horst, R.K. 2012. Westcott's Plant Disease Handbook, 5th Ed. Springer Science and Business Media. 954 p.

Houston, D.R. 1994. Major New Tree Disease Epidemics: Beech Bark Disease. Annual Review of Phytopathology 32: 75-87.

Hurst, J.L., G.J.F. Pugh, and D.W.H. Walton. 1983. Fungal succession and substrate utilization on the leaves of three South Georgia phanerogams. British Antarctica Survey Bulletin 53: 89-100.

Jactel, H., E. Brockerhoff, and P. Duelli. 2004. A test of the biodiversity-stability theory:

Meta-analysis of tree species diversity effects on insect pest infestations, and re-examination of responsible factors. In M. Scherer-Lorenzen, C. Korner, and E.D. Schulze, (Eds.), Forest Diversity and Function. P. 235-262, Berlin: Springer.

Kareiva, P. 1983. Influence of vegetation texture on herbivore populations: Resource concentration hypothesis and herbivore movement. In Variable Plants and Herbivores in Natural and Managed Systems. (R.F. Denno and M.S. McClure, eds.): 259-289. Academic Press, New York.

Kareiva, P. 1985. Finding and losing host plants by *Phyllotreta*: Patch size and surrounding habitat. Ecology 66: 1809-1816.

Kehr, R.D. 1992. *Pezicula* canker of *Quercus rubra* L., caused by *Pezicula cinnamomea* (DC.) Sacc. European Journal of Forest Pathology 22: 29-40.

Kosztarab, M. 1996. Scale insects of Northeastern North America. Identification, biology, and distribution. Virginia Museum of Natural History, Martinsburg, Virginia, 650 pp.

Little, E.L., Jr. 1971. Atlas of United States trees, volume 1, conifers and important hardwoods: U.S. Department of Agriculture Miscellaneous Publication 1146. 9 p. 200 maps.

Liu, W., Y. Xie, Q. Yang, J. Xue, and F. Tian. 2014. New Research on *Matsucoccus matsumurae* (Kuwana) (Hemiptera: Matsucoccidae) in China. Acta Zoologica Bulgarica 6: 95-102.

Livingston, W.H. and M.J. Wingfield. 1982. First Report of *Verticicladiella procera* on Pines in Minnesota. Plant Disease 66: 260 – 261.

Madar, Z., Z. Solel, and M. Kimchi. 2005. Enhancement of Sphaeropsis Canker of

Aleppo Pine by the Israeli Pine Bast Scale. Phytoparasitica 33 (1): 28-32.

Martin, A.D., H.S. Zim, and A.L. Nelson. 1951. American Wildlife and Plants. Dover, New York. 500 p.

Martineau, R. 1964. Forest insect conditions. Annual Report of the Canada Department of Forestry, Forest Insect and Disease Survey 42-46.

McClure, M.S. 1989. Importance of weather to the distribution and abundance of introduced adelgid and scale insects. Agricultural and Forest Meteorology 47: 291-302.

McCormack, H.W. 1936. The Morphology and Development of *Caliciopsis pinea*. Mycologia 28 (2): 188-196.

Mech, A.M., C. Asaro, M.M. Cram, D.R. Coyle, P.J. Gullan, L.G. Cook and K.J.K. Gandhi. 2013. *Matsucoccus macrocicatrices* (Hemiptera: Matsucoccidae): First Report, Distribution, and Association with Symptomatic Eastern White Pine (*Pinus strobus*) in the Southeastern U.S.. Journal of Economic Entomology. 106(6): 2391-2398.

Nelson, P.E., T.A. Toussoun, and W.F.O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. Pennsylvania State University Press, University Park, PA.

NOAA National Climatic Data Center (NCDC). 2014. Climate at a Glance: Average Temperature and Precipitation from 1895 – 2014. U.S. Climate Divisional Database. http://www.ncdc.noaa.gov/cag/time-series.

Omernik, J.M. and G.E. Griffith. 2014. Ecoregions of the conterminous U.S.:

evolution of a hierarchical spatial framework. Environmental Management 54(6):1249-1266.

Ooki, Y., T. Fujita, and Y. Harada. *Pezicula cinnamomea* from cherry tree: pathogenicity tests and photomorphogenesis in culture. Mycoscience 44 (4): 319-326.

Ostry, M.E., G. Laflamme, and S.A. Katovich. 2010. Silvicultural approaches for management of eastern white pine to minimize impacts of damaging agents. Forest Pathology 40: 332 – 346.

Ostry, M.E., N.A. Anderson, and J.G. O'Brien. 2011. Field guide to common macrofungi in eastern forests and their ecosystem functions. General Technical Report NRS-79. Newtown Square, PA: USDA Forest Service-Northern Research Station. 82 p.

Overholts, L.O. 1930. Mycological Notes for 1928-1929. Mycologia 22: 232-246.

Petch, T. 1921. Fungi Parasitic on Scale Insects. Transactions of the British Mycological Society. 7(1-2): 18-40.

R Development Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Ray, W.W. 1936. Pathogenicity and Cultural Experiments with *Caliciopsis pinea*. Mycologia 28 (3): 201-208.

Richards, W.R. 1960. A new species of *Matsucoccus* Cockerell (Homoptera: Coccoidea). Canadian Entomologist 92: 179-181.

Root, R.B. 1973. Organization of a Plant-Arthropod Association in Simple and Diverse Habitats: The Fauna of Collards (*Brassica oleracea*). Ecological Monographs 43 (1): 95-124.

Rose, J.A. 2011. A Decline in State's White Pines Found. West Virginia Department of Agriculture Market Bulletin. http://www.wvagriculture.org/market_bulletin/Past_Issues.

Rosenholm, G. 2012. Pine canker a growing concern in the East. USDA Forest Service News Release No. DFO – 08 – 12. http://www.na.fs.fed.us/nanews/nastories/News%20Release%20Canker%20FINAL%20062012%20w%20template.pdf.

Snell, W.H. 1922. A New *Septobasidium* on *Pinus strobus*. Mycologia 14 (2): 55 – 60.

Steiner, G.W., and Watson, R.D. 1965. Use of surfactants in the soil dilution and plate count method. Phytopathology 55:728-730.

Stout, M. J., J.S. Thaler, and B.P.H.J. Thomma. 2006. Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. Annual Review of Entomology 51: 663-689.

Sullivan, B. T. 2011. Use of chemicals for prevention and control of southern pine beetle infestations. In: Coulson, R.N. and K. D. Klepzig. Southern Pine Beetle II. General Technical Report SRS-140. Asheville, North Carolina: United States Department of Agriculture Forest Service, Southern Research Station. 25-50.

Thaler, J.S., R. Karban, D.E. Ullman, K. Boege, and R.M. Bostock. 2002. Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. Oecologia 131: 227-235.

Udayanga, D., X. Liu, E.H.C. McKenzie, E. Chukeatirote, A.H.A. Bahkali, and K.D. Hyde. 2011. The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. Fungal Diversity 50: 189-225.

USDA Natural Resources Conservation Service (NRCS). 1998. Soil Orders Map of the U.S.. National Soil Survey Center, Lincoln, NE USA. http://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/class/?cid=nrcs142p2_053589.

USGS Geosciences and Environmental Change Science Center. 2013. Digital Representations of Tree Species Range Maps from “Atlas of United States Trees” by Elbert L. Little Jr. (and other publications). Accessed: 07/08/2015. http://esp.cr.usgs.gov/data/little/

Vimmerstedt, J.P. 1962. Southern Appalachian White Pine Plantations: Site, volume, and yield. USDA Forest Service Station Paper No. 149.

Watson, W.Y., G.R. Underwood, and J. Reid. 1960. Notes on *Matsucoccus macrocicatrices* Richards (Homoptera: Margarodidae) and its Association with *Septobasidium pinicola* Snell in Eastern Canada. The Canadian Entomologist 92 (9): 662 – 667.

Wendel, G.W. and H.C. Smith. 1990. *Pinus strobus* L. eastern white pine. In R.M. Burns and B.H. Honkala (eds.), Silvics of North America. Vol. 1. Conifers. USDA Forest Service, Washington, D.C.

Table 3.1. Genera-level identification of other cankers (n = 381) on the eastern white pine saplings collected in the southern Appalachian Mountains.

Fungal Genera	Number of Isolates	% of Total Isolates
Chaetophoma	17	4
Pestalotiopsis	11	3
Pezicula	7	2
Phaeomoniella	76	20
Phomopsis	20	5
Other genera	117	31
Unknown genera	34	9
Contamination (Mold)	83	22
No Fungal Growth	16	4
Total	381	100

Figure Legend

Figure 3.1. Immature cyst stage of *Matsucoccus macrocicatrices* A) in a leaf scar with apparent yellowing around their feeding site, B) under a lichen, C) wedged in a branch node, D) settled and feeding on the edge of a canker, E) in a canker, and F) in a *Septobasidium pinicola* mat.

Figure 3.2. Sexual (A and B) and asexual (C and D) structures of *Caliciopsis pinea* and cankers, and examples of other cankers (E and F) on the eastern white pine saplings.

Figure 3.3. Distribution of southeastern (six saplings/site) and northeastern (three saplings/site) collection sites for eastern white pine saplings.

Figure 3.4. Methodology for the identification of other cankers, where A) the surface layer of bark was removed, and B) each canker was cut into three sections and plated on three types of medium. Figure C) shows the cross section of a canker.

Figure 3.5. Frequency of observation of 0, 1-20, 21-40, 41-60, and ≥ 61 *Matsucoccus macrocicatrices* per sapling in the southeastern (A) and northeastern (B) regions.

Figure 3.6. Mean (± SE) number of *Matsucoccus macrocicatrices* associated with *Caliciopsis pinea* cankers, other cankers (non-*Caliciopsis pinea* cankers), and no cankers in the southeastern (black) and northeastern (striped) saplings.

Figure 3.7. Correlations between eastern white pine sapling dieback and proportion of total canker (A) and proportion of *Matsucoccus macrocicatrices* (B) in the southeastern (O) and northeastern (▲) regions.

Figure 3.8. Correlations between proportion of total canker and proportion of *Matsucoccus macrocicatrices* in the southeastern (O) and northeastern (▲) regions.

Figure 3.9. Standardized *Caliciopsis pinea* (A) and other canker (B) surface area (mm^2) for each sampled site (n = 49) along the latitudinal range of eastern white pine.

Figure 3.10. Distribution of the total canker surface area (cm^2) (A) and *Matsucoccus macrocicatrices* (B) from the base to the meristem of the eastern white pine saplings (n = 270).

Figure 3.11. Proposed complex, including (A) eastern white pine; (B) *Matsucoccus macrocicatrices*; and (C) cankers, which may (D) provide a crevice for more scale insects to colonize.

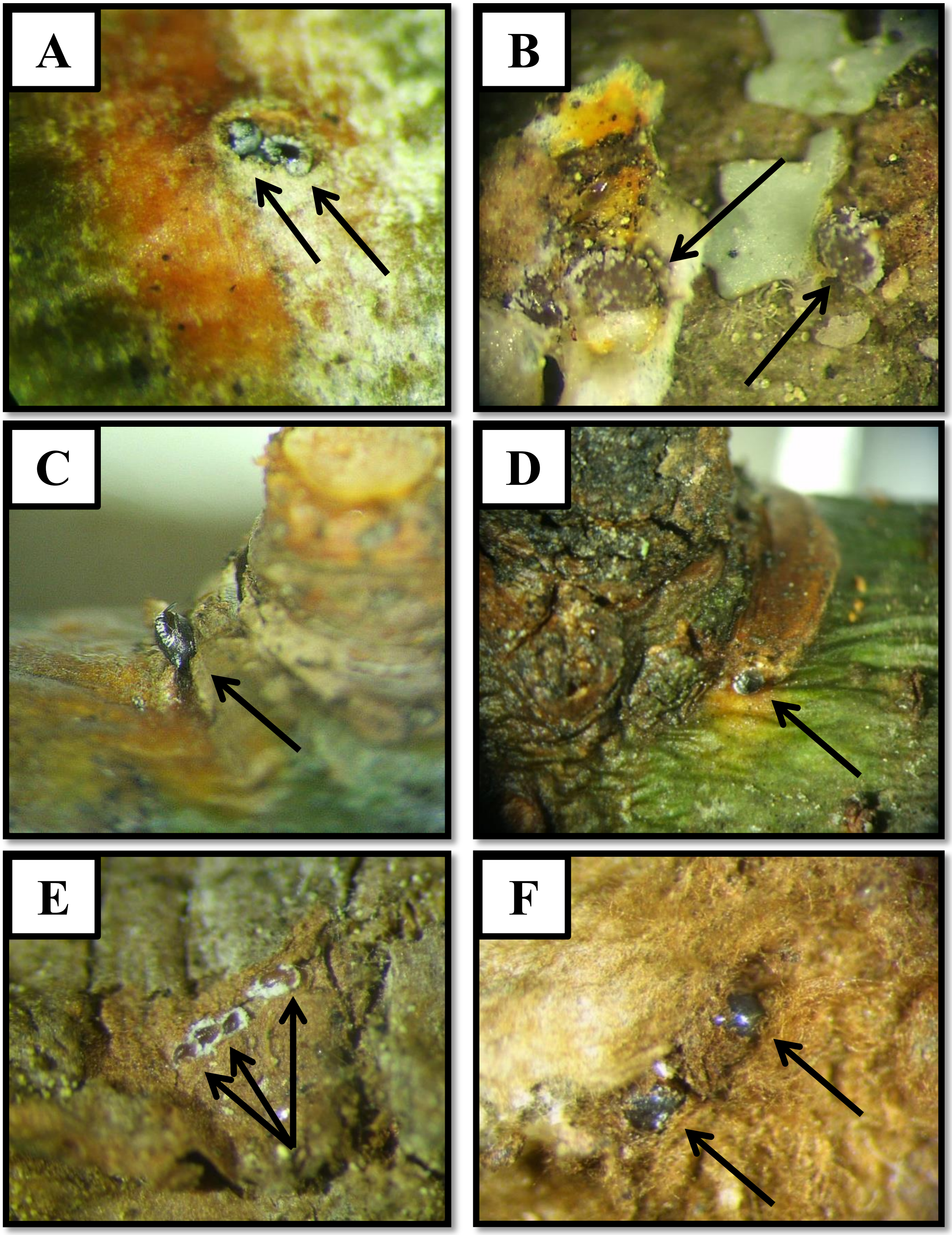


Figure 3.1

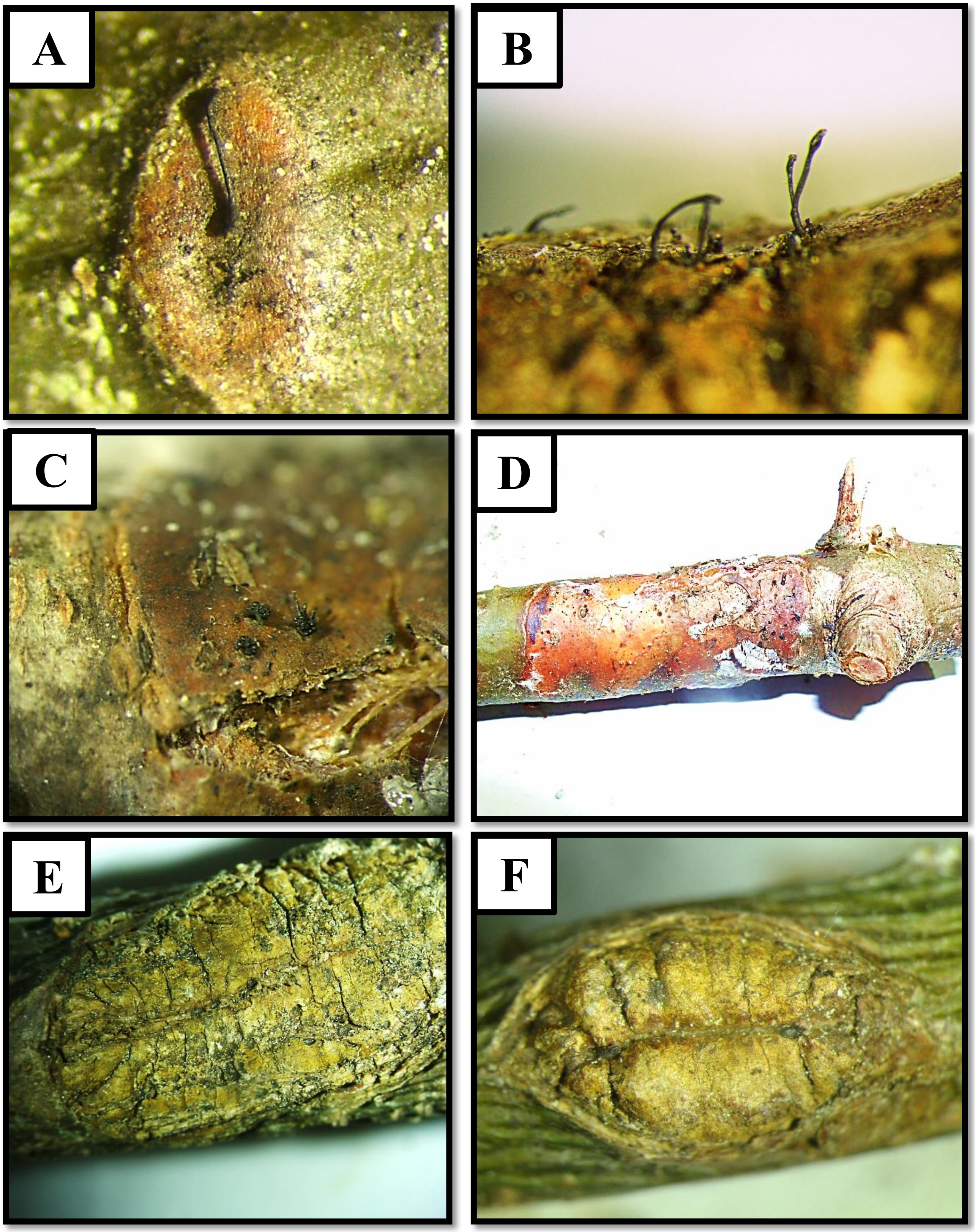


Figure 3.2

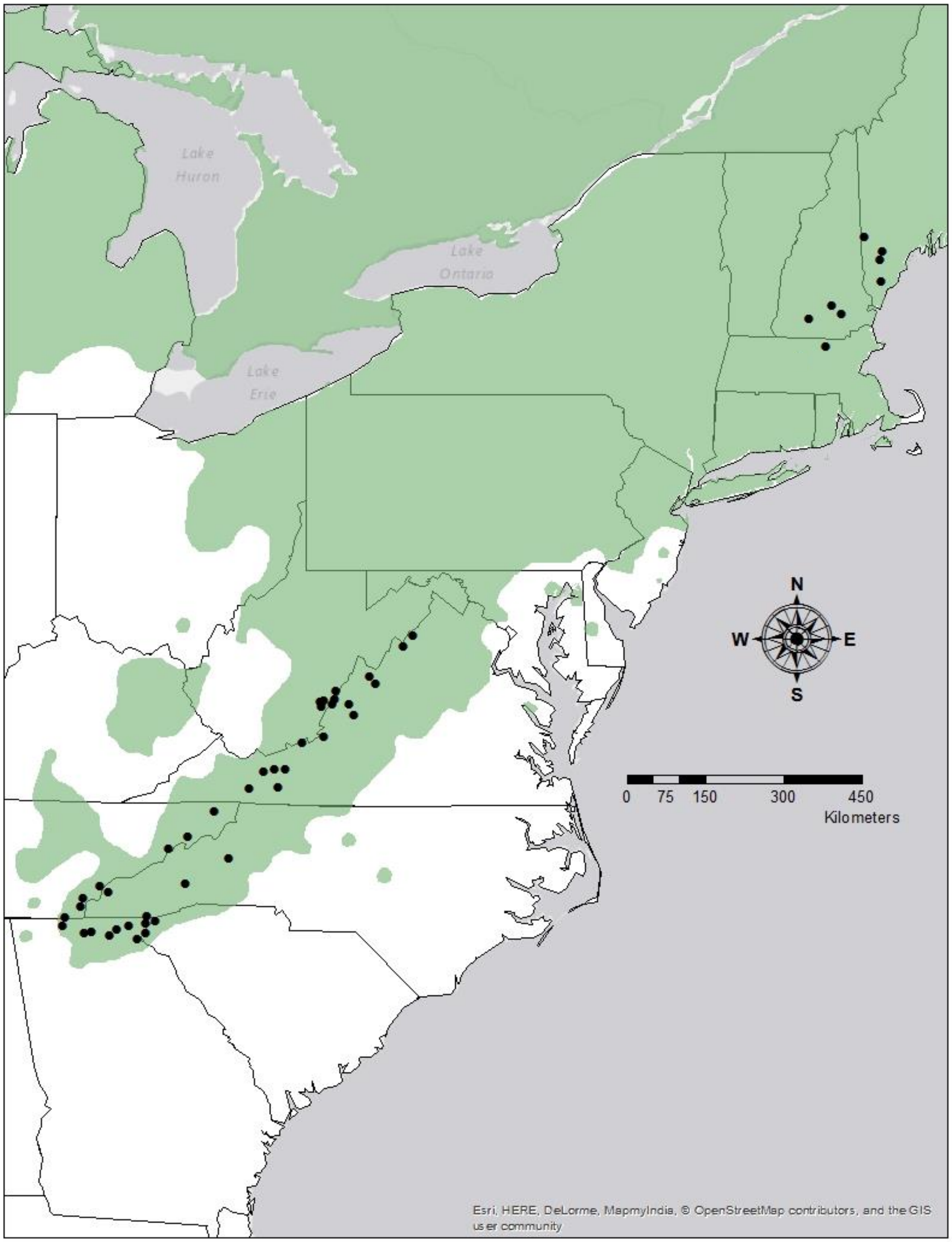

Lake Huron
Lake Ontario
Lake Erie
N
W
E
S
0 75 150 300 450
Kilometers
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community

Figure 3.3

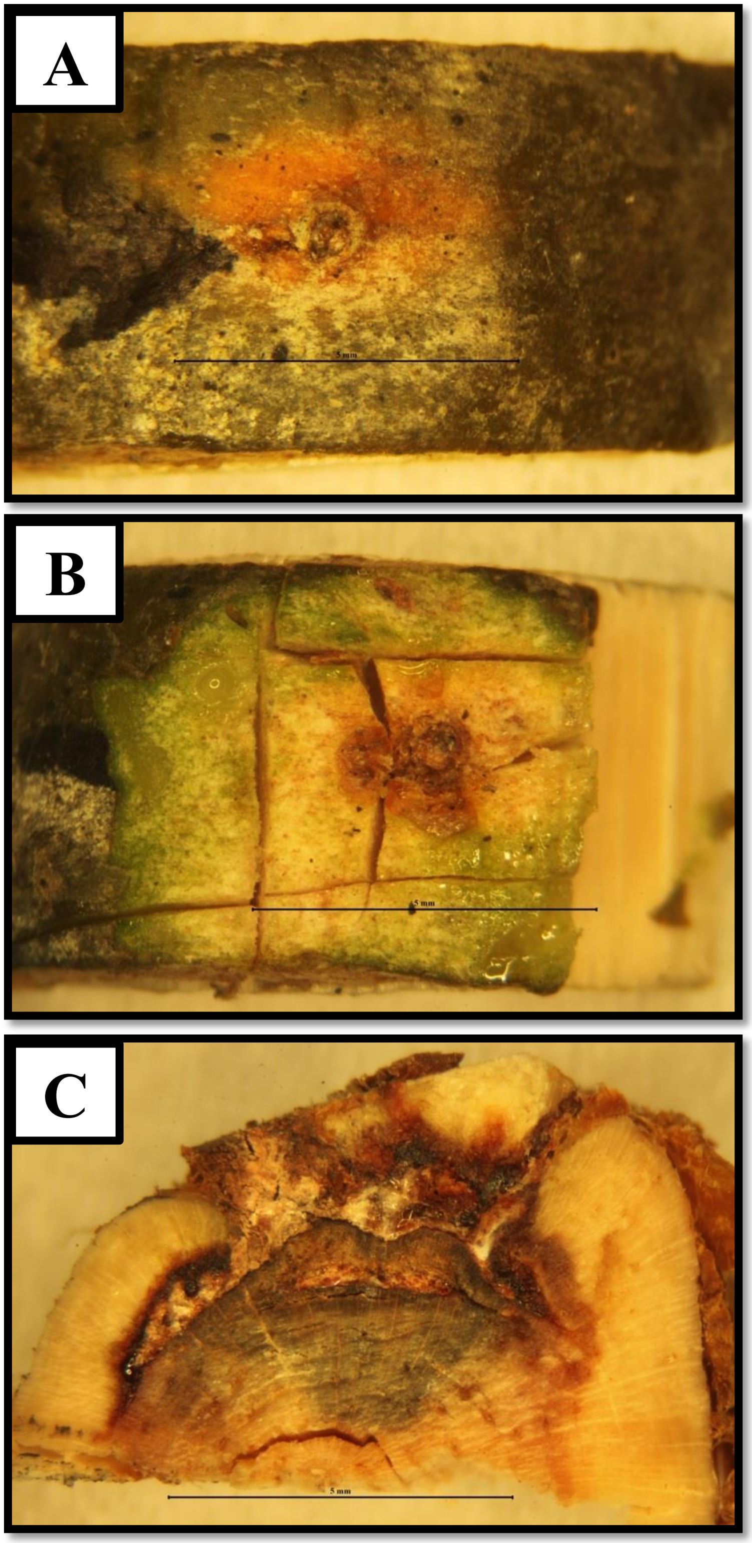


Figure 3.4

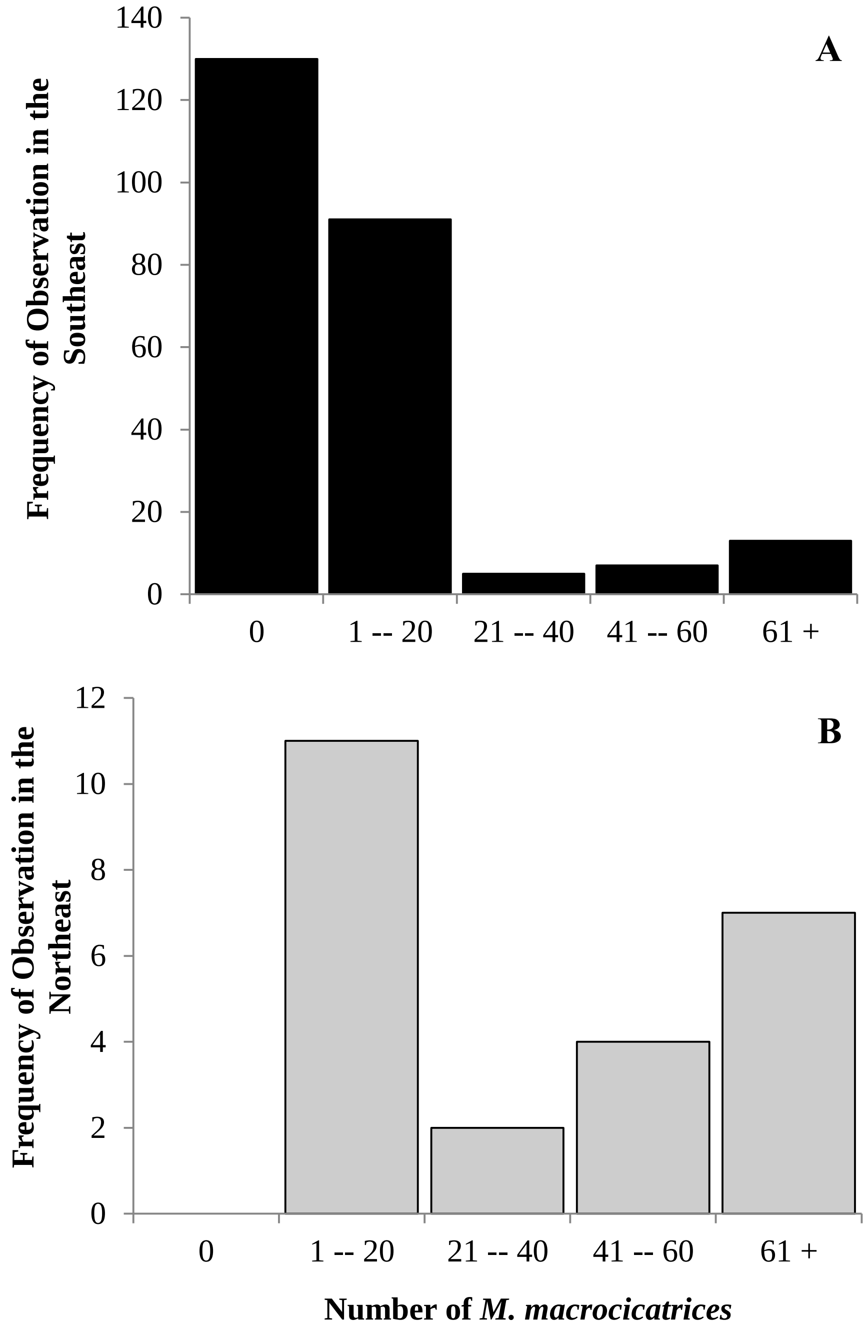

A
Frequency of Observation in the Southeast
140
120
100
80
60
40
20
0
0
1 -- 20
21 -- 40
41 -- 60
61 +
B
Frequency of Observation in the Northeast
12
10
8
6
4
2
0
0
1 -- 20
21 -- 40
41 -- 60
61 +
Number of *M. macrocicatrices*

Figure 3.5

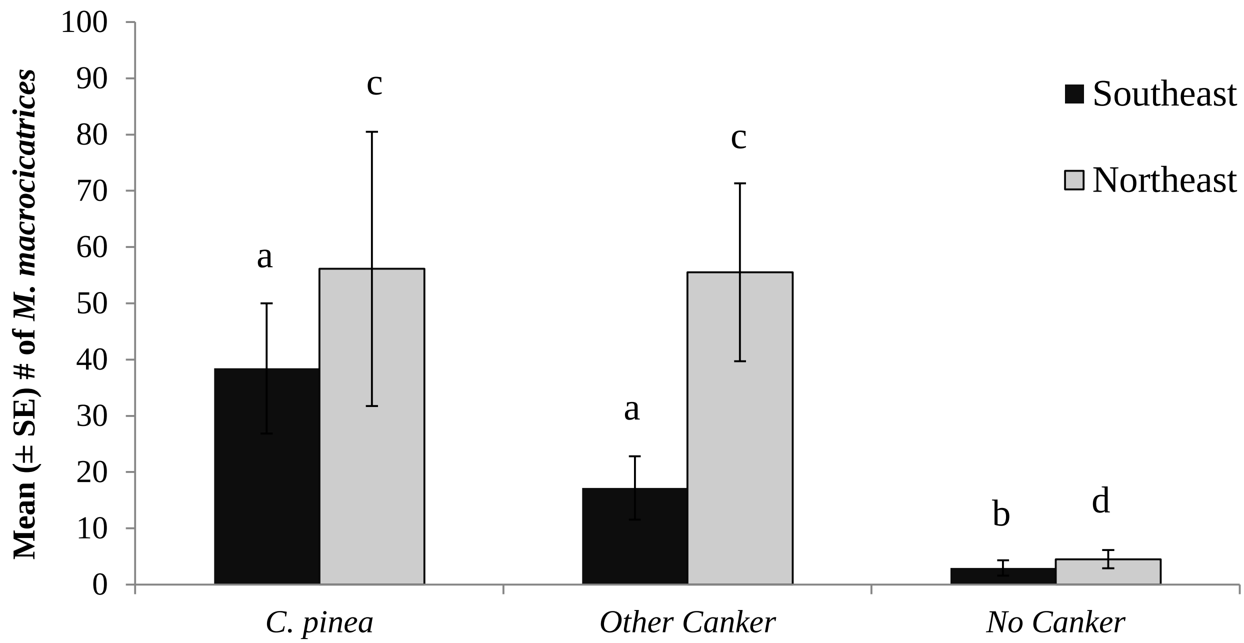

Mean (± SE) # of M. macrocicatrices
100
90
80
70
60
50
40
30
20
10
0
Southeast
Northeast
a
c
a
c
b
d
C. pinea
Other Canker
No Canker

Figure 3.6

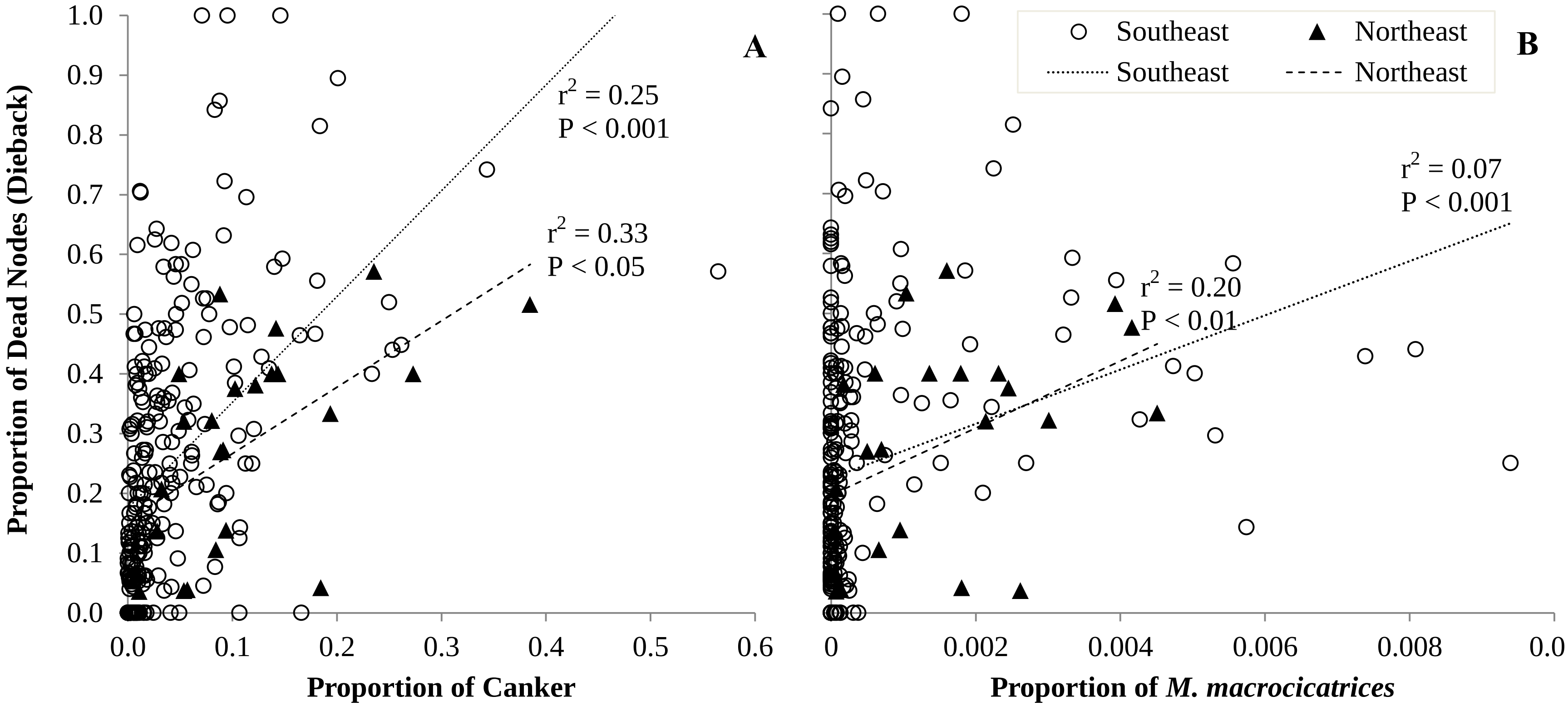

Proportion of Dead Nodes (Dieback)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
A
r2 = 0.25
P < 0.001
r2 = 0.33
P < 0.05
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Proportion of Canker
B
Southeast
Northeast
Southeast
Northeast
r2 = 0.07
P < 0.001
r2 = 0.20
P < 0.01
0
0.002
0.004
0.006
0.008
0.01
Proportion of M. macrocicatrices

Figure 3.7

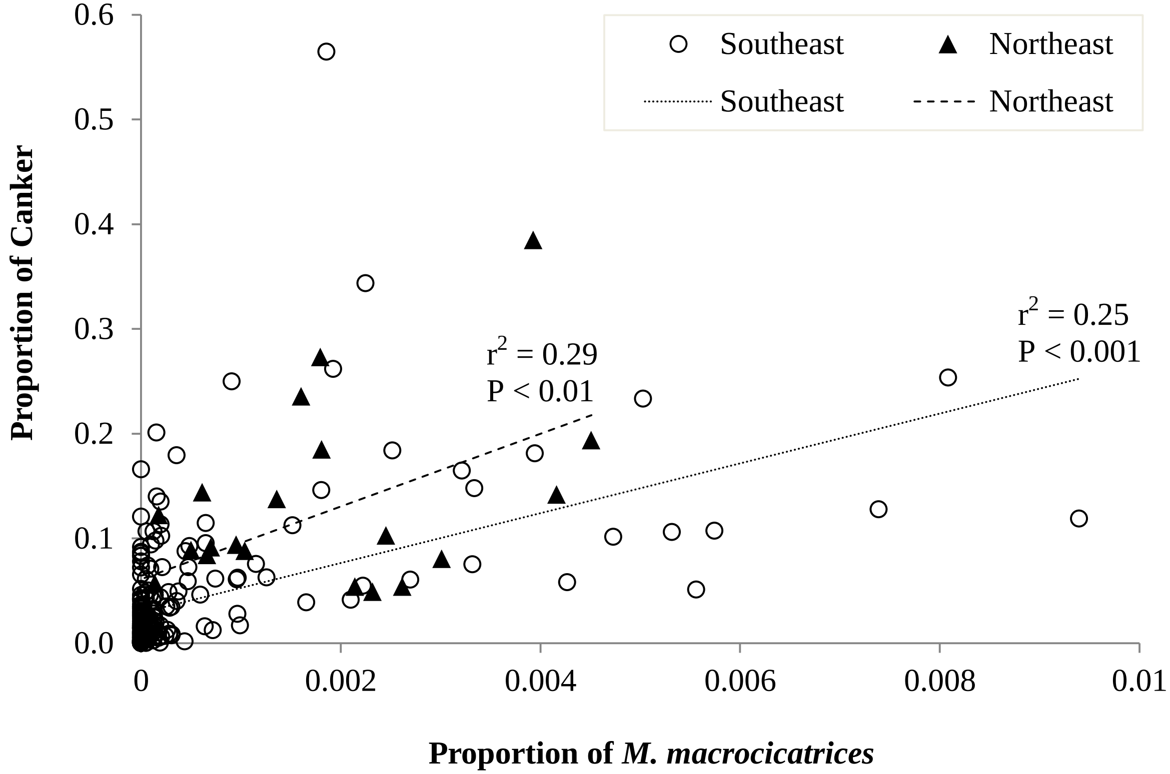

0.6
0.5
0.4
0.3
0.2
0.1
0.0
Proportion of Canker
Southeast
Northeast
Southeast
Northeast
$r^2 = 0.29$
$P < 0.01$
$r^2 = 0.25$
$P < 0.001$
0
0.002
0.004
0.006
0.008
0.01
Proportion of *M. macrocicatrices*

Figure 3.8

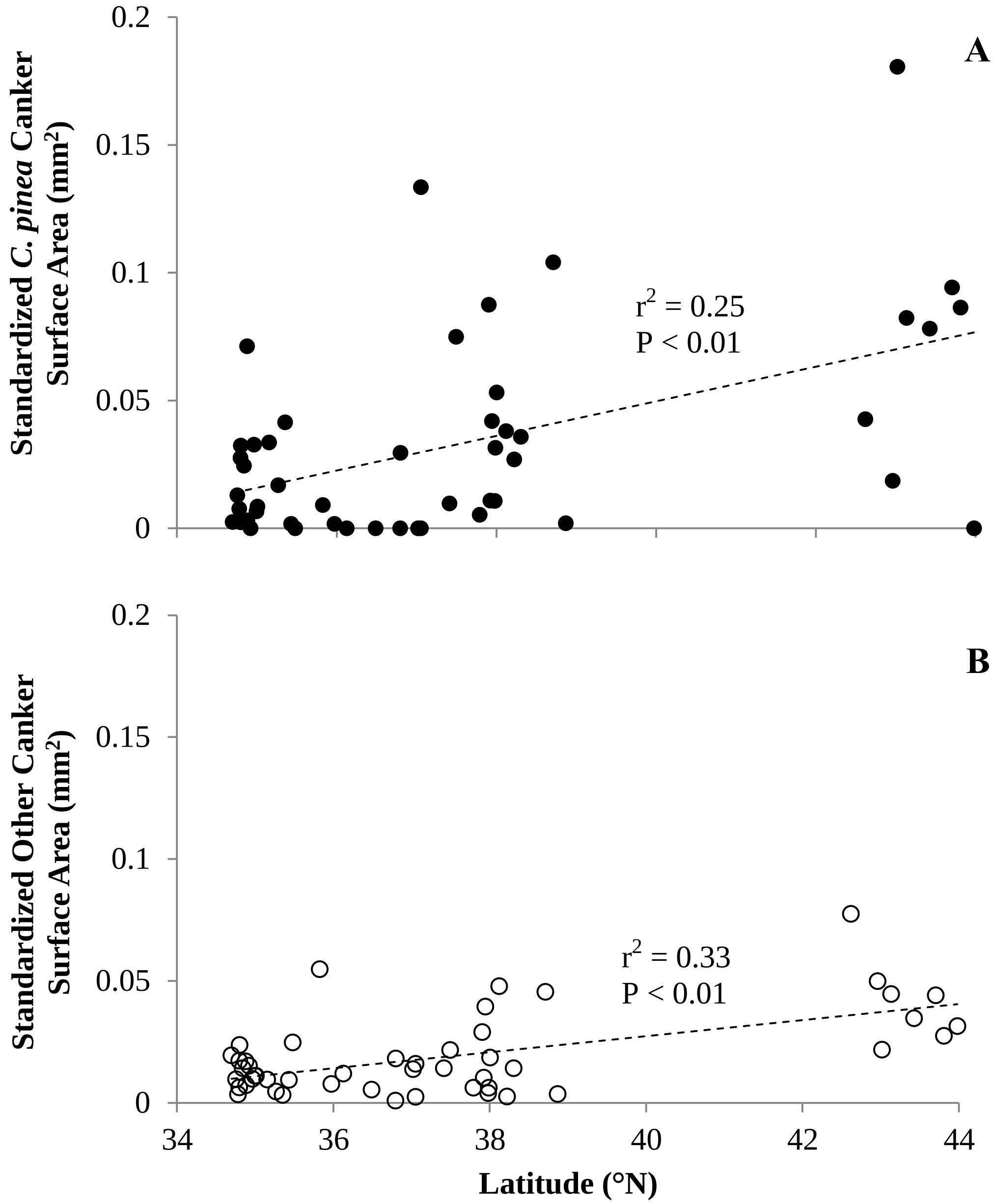
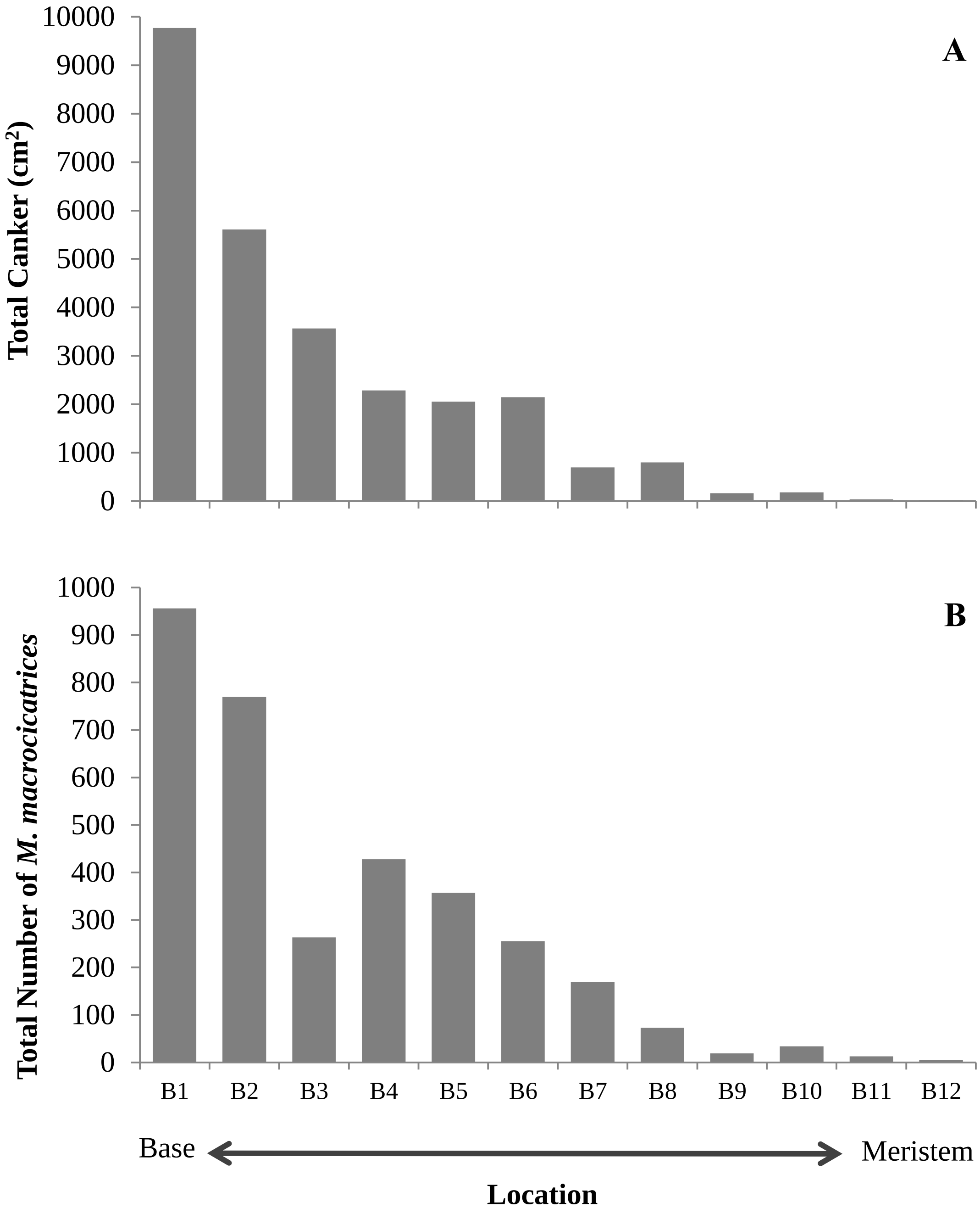

A
B
r² = 0.25
P < 0.01
r² = 0.33
P < 0.01
Standardized C. pinea Canker Surface Area (mm²)
Standardized Other Canker Surface Area (mm²)
Latitude (°N)
0
0.05
0.1
0.15
0.2
34
36
38
40
42
44

Figure 3.9



A
Total Canker (cm²)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
B
Total Number of *M. macrocicatrices*
1000
900
800
700
600
500
400
300
200
100
0
B1
B2
B3
B4
B5
B6
B7
B8
B9
B10
B11
B12
Base
Meristem
Location

Figure 3.10

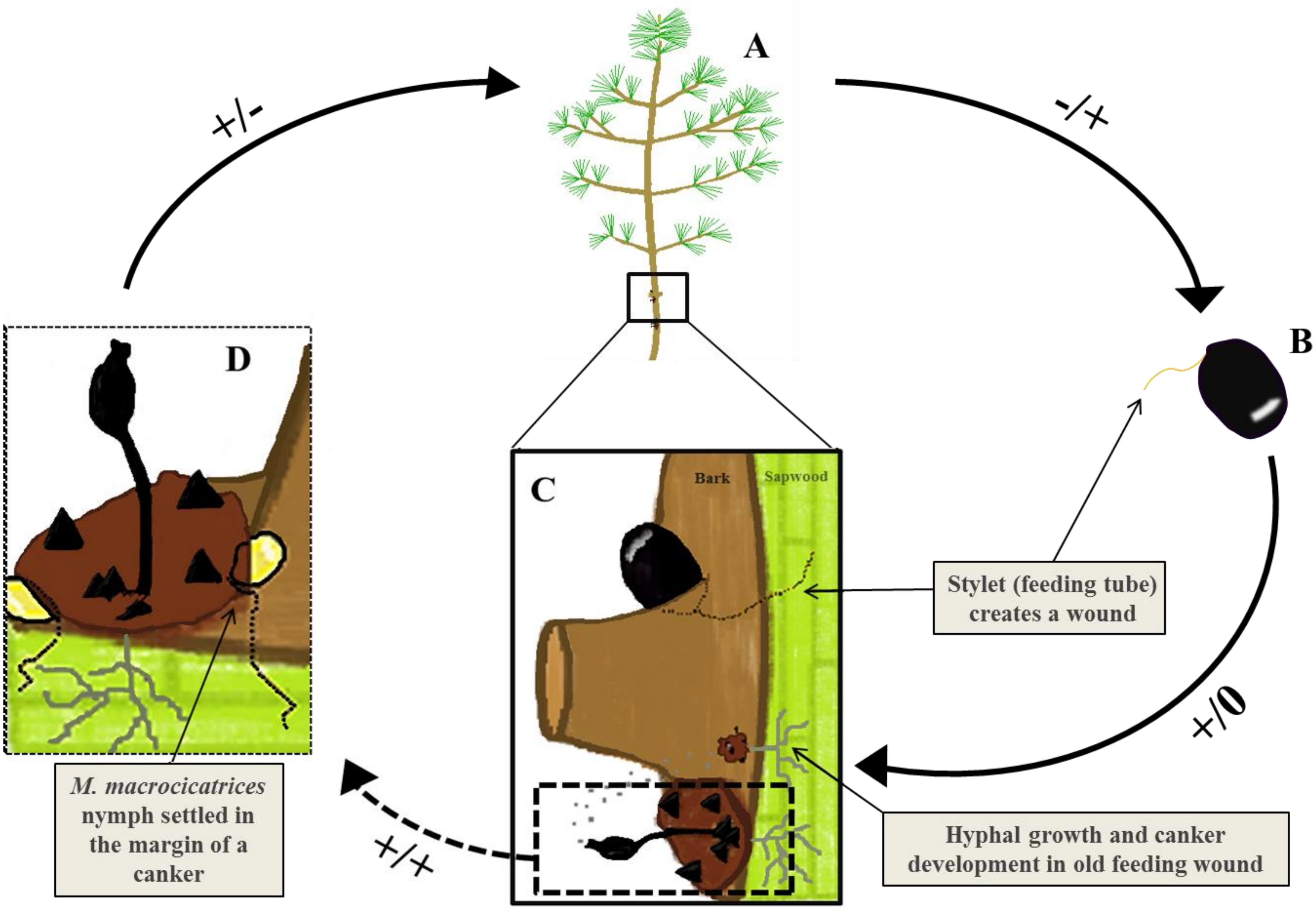

A
+/-
-/+
B
D
C
Bark
Sapwood
Stylet (feeding tube) creates a wound
+/0
Hyphal growth and canker development in old feeding wound
+/+
M. macrocicatrices nymph settled in the margin of a canker

Figure 3.11

CHAPTER 4

THESIS CONCLUSIONS

4.1 Thesis Conclusions

With symptoms of a pronounced bottom-up dieback becoming exceedingly common in eastern white pine (*Pinus strobus* L.) forests (Asaro 2011; Rose 2011; Rosenholm 2012), it has become important to evaluate eastern white pine dieback in the southern Appalachian Mountains, specifically the correlations among various abiotic and biotic conditions, *Matsucoccus macrocicatrices* Richards, cankers, and the dieback of eastern white pine.

Chapter two updated the range and severity of eastern white pine dieback, mapped the occurrence of *M. macrocicatrices* and *Caliciopsis pinea* Peck, assessed whether eastern white pine health was associated with particular abiotic and biotic conditions, and evaluated the mean health rating of eastern white pine in three size class categories (saplings, poletimber, and sawtimber). We hypothesized that eastern white pine mortality levels would be higher in higher latitudes (i.e., Virginia and West Virginia) versus lower latitudes (i.e., Georgia and South Carolina), eastern white pine health would be associated with abiotic and biotic factors, and sawtimber would be healthier than the poletimber or sapling size classes. Overall, results from this study indicate that, like we hypothesized, more eastern white pine dieback occurred in Virginia and West Virginia than Georgia and South Carolina. Furthermore, we determined that eastern white pine health rating was associated with the diameter at breast height (DBH) and density (square-root) of eastern white pine trees in the plots, which suggests that large diameter trees in less dense plots are healthier than small diameter trees in denser plots. Occurrence mapping determined that *M. macrocicatrices* and *C. pinea* were found at 85% and 87.5% of the 40 sites sampled in 2014, respectively, and *M. macrocicatrices* was found in 77% of sites sampled from 2011-2014.

In chapter three, we evaluated correlations among *M. macrocicatrices*, cankers, and the dieback of eastern white pine saplings by sampling 270 saplings from nine states (Georgia, Maine, Massachusetts, New Hampshire, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia) in the eastern U.S. We predicted that correlations would occur among all of the factors involved in the eastern white pine-*M. macrocicatrices*-canker complex. Results from this study indicate that correlations exist among the eastern white pine dieback, *M. macrocicatrices*, and cankers. We further posit that, like the relationships within the beech bark disease and Aleppo pine (*Pinus halepensis* Mill.)-Israeli pine bast scale (*Matsucoccus josephi* Bodenheimer and Harpaz)-*Sphaeropsis sapinea* Fr. complexes, the *M. macrocicatrices* scale insect feeds on the eastern white, thereby creating feeding wounds (infection courts) that the spores of canker-forming fungi take advantage of to establish on the trees (Houston 1994; Madar et al. 2005). Although the correlations between cankers and sapling dieback were stronger than the correlations between *M. macrocicatrices* and sapling dieback, *M. macrocicatrices* may still be considered a contributing factor to eastern white pine dieback due to its association with cankers.

Since eastern white pine remains one of the most economically and ecologically important conifer species in the Appalachian Mountains, it will be essential to monitor and evaluate eastern white pine dieback over the next few decades. The results from this study indicate that monitoring efforts and management strategies should not only focus on the cankers, but also on *M. macrocicatrices*. Further exploration of abiotic and biotic conditions will be necessary to predict dieback severity, model the impacts of eastern white pine dieback on forest ecosystem functions, and develop management strategies to best manage the tripartite, eastern white pine-*M. macrocicatrices*-canker complex.

4.2 Directions for Future Research

This study provides a foundation for future work necessary to understand the intricacies of the eastern white pine-*M. macrocicatrices*-canker complex. Future research may encompass the three major factors within the proposed tripartite complex: the eastern white pine host, scale insect (*M. macrocicatrices*), and fungal pathogens. The following research initiatives may be undertaken in the future:

1. Identify and run pathogenicity tests on non-*C. pinea* cankers to determine if any of the “other” cankers are pathogenic and should be closely monitored. Studies should be completed throughout the range of eastern white pine to provide a comprehensive understanding of the potential pathogens which exist and could potentially affect eastern white pine.
2. Expand the assessment of eastern white pine dieback to other parts of its range using similar methodology for easy comparison among the southeast, northeast, and north central regions in the U.S. Continue to monitor sites that have been established in the southern Appalachian region.
3. Monitor and update the occurrence of *M. macrocicatrices*, *C. pinea,* and other pathogenic fungi throughout the range of eastern white pine.

4. Evaluate other economically and ecologically important white pine species, including *Pinus monticola* Douglas and *Pinus albicaulis* Engelm. in the western U.S. and Canada, as well as *Pinus strobiformis* Engelm. in the southwestern U.S. and Mexico. *Matsucoccus macrocicatrices* is a white pine specialist, so it may be essential to understand if these other white pine species are affected by the same species of scale insect and fungal pathogens. Expansion to studies on white pine species outside of the North American continent may be a further objective.
5. Assess other age and size classes of eastern white pine to quantify the effects of *M. macrocicatrices*, fungal pathogens and cankers on the dieback of mature eastern white pine versus sapling- and seedling-sized trees. It may also be important to determine what size the trees must be to become susceptible to *M. macrocicatrices* and fungal pathogens.
6. Determine if *M. macrocicatrices* is native or non-native to the southern Appalachian Mountains and the eastern U.S., in general.
7. Attain a better understanding of the feeding mechanisms, reproductive strategies, and phenology of *M. macrocicatrices.*
8. Ascertain whether the relationship between *S. pinicola* and *M. macrocicatrices* is mutualistic (Watson et al. 1960), or if some degree of parasitism could be occurring which influences the maturation and emergence of *M. macrocicatrices*.

4.3 Literature Cited

Asaro, C. 2011. Virginia Department of Forestry Forest Health Review. 11 p.

Houston, D.R. 1994. Major New Tree Disease Epidemics: Beech Bark Disease. Annual Review of Phytopathology 32: 75-87.

Madar, Z., Z. Solel, and M. Kimchi. 2005. Enhancement of Sphaeropsis Canker of Aleppo Pine by the Israeli Pine Bast Scale. Phytoparasitica 33 (1): 28-32.

Rose, J.A. 2011. A Decline in State's White Pines Found. West Virginia Department of Agriculture Market Bulletin. http://www.wvagriculture.org/market_bulletin/Past_Issues

Rosenholm, G. 2012. Pine canker a growing concern in the East. USDA Forest Service News Release No. DFO-08-12. http://www.na.fs.fed.us/nanews/nastories/News%20Release%20Canker%20FINAL%20062012%20w%20template.pdf.

Watson, W.Y., G.R. Underwood and J. Reid. 1960. Notes on *Matsucoccus macrocicatrices* Richards (Homoptera: Margarodidae) and its Association with *Septobasidium pinicola* Snell in Eastern Canada. The Canadian Entomologist 92 (9): 662-667.

APPENDICES

Appendix A

Location of plots sampled in the southern Appalachian Mountains, U.S.

Plot Code	State	National Forest	Latitude (°N)	Longitude (°W)	Date Sampled
GA1.1	Georgia	Chattahoochee	34.69767	83.41489	1/31/2014
GA1.2	Georgia	Chattahoochee	34.6981	83.41369	1/31/2014
GA1.3	Georgia	Chattahoochee	34.69769	83.41444	1/31/2014
GA2.1	Georgia	Chattahoochee	34.922	83.25822	2/5/2014
GA2.2	Georgia	Chattahoochee	34.92447	83.26074	2/5/2014
GA2.3	Georgia	Chattahoochee	34.92589	83.26072	2/5/2014
GA3.1	Georgia	Chattahoochee	34.88304	83.56023	2/21/2014
GA3.2	Georgia	Chattahoochee	34.88084	83.56149	2/21/2014
GA3.3	Georgia	Chattahoochee	34.8836	83.55981	2/21/2014
GA4.1	Georgia	Chattahoochee	34.83934	83.76933	2/25/2014
GA4.2	Georgia	Chattahoochee	34.83883	83.7704	2/25/2014
GA4.3	Georgia	Chattahoochee	34.83243	83.75253	2/25/2014
GA5.1	Georgia	Chattahoochee	34.75518	83.89426	2/26/2014
GA5.2	Georgia	Chattahoochee	34.75542	83.89334	2/26/2014
GA5.3	Georgia	Chattahoochee	34.75405	83.89698	2/26/2014
GA6.1	Georgia	Chattahoochee	34.87796	84.70926	3/9/2014
GA6.2	Georgia	Chattahoochee	34.88338	84.69677	3/9/2014
GA6.3	Georgia	Chattahoochee	34.88667	84.69557	3/9/2014
GA7.1	Georgia	Chattahoochee	34.77939	84.32744	3/9/2014
GA7.2	Georgia	Chattahoochee	34.78133	84.32733	3/9/2014
GA7.3	Georgia	Chattahoochee	34.78075	84.31113	3/9/2014
GA8.1	Georgia	Chattahoochee	34.79816	84.18977	3/11/2014
GA8.2	Georgia	Chattahoochee	34.79807	84.18724	3/11/2014
GA8.3	Georgia	Chattahoochee	34.80824	84.18712	3/11/2014
NC1.1	North Carolina	Pisgah	35.48113	82.5915	6/16/2014
NC1.2	North Carolina	Pisgah	35.4807222	82.5903611	6/16/2014
NC1.3	North Carolina	Pisgah	35.4818889	82.593	6/16/2014
NC2.1	North Carolina	Pisgah	35.8245556	81.8466389	6/16/2014
NC2.2	North Carolina	Pisgah	35.8243056	81.8459167	6/16/2014
NC2.3	North Carolina	Pisgah	35.8239167	81.84825	6/16/2014
NC3.1	North Carolina	Nantahala	35.3526667	83.9072778	6/17/2014
NC3.2	North Carolina	Nantahala	35.3521389	83.9068333	6/17/2014
NC3.3	North Carolina	Nantahala	35.3521389	83.9058889	6/17/2014
NC4.1	North Carolina	Nantahala	35.0092778	83.2417778	6/17/2014

Plot Code	State	National Forest	Latitude (°N)	Longitude (°W)	Date Sampled
NC4.2	North Carolina	Nantahala	35.0086111	83.2416667	6/17/2014
NC4.3	North Carolina	Nantahala	35.0105	83.24325	6/17/2014
SC1.1	South Carolina	Sumter	34.96559	83.09306	3/19/2014
SC1.2	South Carolina	Sumter	34.96409	83.10079	3/19/2014
SC1.3	South Carolina	Sumter	34.96522	83.10308	3/19/2014
SC3.1	South Carolina	Sumter	34.79885	83.31249	4/15/2014
SC3.2	South Carolina	Sumter	34.79825	83.31364	4/15/2014
SC3.3	South Carolina	Sumter	34.79856	83.31208	4/15/2014
TN1.1	Tennessee	Cherokee	35.26812	84.33806	3/30/2014
TN1.2	Tennessee	Cherokee	35.27302	84.33478	3/30/2014
TN1.3	Tennessee	Cherokee	35.26833	84.33756	3/30/2014
TN2.1	Tennessee	Cherokee	35.15207	84.37422	3/30/2014
TN2.2	Tennessee	Cherokee	35.15321	84.37542	3/30/2014
TN2.3	Tennessee	Cherokee	35.15256	84.37408	3/30/2014
TN3.1	Tennessee	Cherokee	36.48812	82.08171	4/22/2014
TN3.2	Tennessee	Cherokee	36.48821	82.08543	4/22/2014
TN3.3	Tennessee	Cherokee	36.47717	82.10046	4/22/2014
TN4.1	Tennessee	Cherokee	36.12572	82.53853	4/22/2014
TN4.2	Tennessee	Cherokee	36.1306	82.53314	4/22/2014
TN4.3	Tennessee	Cherokee	36.13734	82.53151	4/22/2014
TN5.1	Tennessee	Cherokee	35.97279	82.85342	4/23/2014
TN5.2	Tennessee	Cherokee	35.96671	82.86375	4/23/2014
TN5.3	Tennessee	Cherokee	35.96572	82.86511	4/23/2014
TN6.1	Tennessee	Cherokee	35.43005	84.0629	4/23/2014
TN6.2	Tennessee	Cherokee	35.44091	84.04606	4/23/2014
TN6.3	Tennessee	Cherokee	35.44268	84.04337	4/23/2014
TN7.1	Tennessee	Cherokee	34.99749	84.63921	5/30/2014
TN7.2	Tennessee	Cherokee	34.99704	84.64357	5/30/2014
TN7.3	Tennessee	Cherokee	34.9942	84.63929	5/30/2014
VA1.1	Virginia	Jefferson	36.7933056	81.4958611	7/7/2014
VA1.2	Virginia	Jefferson	36.7925833	81.4969444	7/7/2014
VA1.3	Virginia	Jefferson	36.7936389	81.4975833	7/7/2014
VA2.1	Virginia	Jefferson	36.7996111	80.9839167	7/7/2014
VA2.2	Virginia	Jefferson	36.7988611	80.9838889	7/7/2014
VA2.3	Virginia	Jefferson	36.7983056	80.9846667	7/7/2014
VA3.1	Virginia	Jefferson	37.0190278	81.23375	7/8/2014
VA3.2	Virginia	Jefferson	37.0174722	81.2370278	7/8/2014
VA3.3	Virginia	Jefferson	37.0170556	81.23875	7/8/2014
VA4.1	Virginia	Jefferson	37.0527222	81.0696389	7/8/2014

Plot Code	State	National Forest	Latitude (°N)	Longitude (°W)	Date Sampled
VA4.2	Virginia	Jefferson	37.0538056	81.06875	7/8/2014
VA4.3	Virginia	Jefferson	37.0530278	81.0683611	7/8/2014
VA5.1	Virginia	Jefferson	37.0526944	80.8730278	7/8/2014
VA5.2	Virginia	Jefferson	37.0533889	80.8743056	7/8/2014
VA5.3	Virginia	Jefferson	37.0542222	80.8746667	7/8/2014
VA6.1	Virginia	Jefferson	37.4142222	80.5915278	7/9/2014
VA6.2	Virginia	Jefferson	37.4149722	80.5917222	7/9/2014
VA6.3	Virginia	Jefferson	37.4133056	80.5875833	7/9/2014
VA7.1	Virginia	Jefferson	37.4943333	80.1956667	7/9/2014
VA7.2	Virginia	Jefferson	37.4949444	80.1948056	7/9/2014
VA7.3	Virginia	Jefferson	37.4955556	80.1940833	7/9/2014
VA8.1	Virginia	Jefferson	37.7892778	79.7010278	7/9/2014
VA8.2	Virginia	Jefferson	37.7886389	79.7018333	7/9/2014
VA8.3	Virginia	Jefferson	37.7870833	79.7011944	7/9/2014
VA9.1	Virginia	George Washington	37.92225	79.7908611	7/10/2014
VA9.2	Virginia	George Washington	37.9217222	79.7898056	7/10/2014
VA9.3	Virginia	George Washington	37.9228611	79.7883889	7/10/2014
VA10.1	Virginia	George Washington	38.3053611	79.43025	7/10/2014
VA10.2	Virginia	George Washington	38.3047222	79.4314444	7/10/2014
VA10.3	Virginia	George Washington	38.3039167	79.4319444	7/10/2014
VA11.1	Virginia	George Washington	38.2213611	79.3239167	7/10/2014
VA11.2	Virginia	George Washington	38.2218056	79.3224444	7/10/2014
VA11.3	Virginia	George Washington	38.2203889	79.32175	7/10/2014
VA12.1	Virginia	George Washington	38.7119444	78.84325	7/11/2014
VA12.2	Virginia	George Washington	38.7124722	78.8441667	7/11/2014
VA12.3	Virginia	George Washington	38.7112222	78.8448333	7/11/2014
VA13.1	Virginia	George Washington	38.8666944	78.6855	7/11/2014
VA13.2	Virginia	George Washington	38.8676111	78.6848333	7/11/2014
VA13.3	Virginia	George Washington	38.8698333	78.6813333	7/11/2014
WV1.1	West Virginia	Monongahela	37.9878056	80.2177222	8/14/2014
WV1.2	West Virginia	Monongahela	37.9882222	80.2166667	8/14/2014
WV1.3	West Virginia	Monongahela	37.9866111	80.2176667	8/14/2014
WV2.1	West Virginia	Monongahela	37.9789722	80.2810556	8/14/2014
WV2.2	West Virginia	Monongahela	37.9786667	80.2799444	8/14/2014
WV2.3	West Virginia	Monongahela	37.9776667	80.2803611	8/14/2014
WV3.1	West Virginia	Monongahela	37.9041667	80.2516111	8/14/2014
WV3.2	West Virginia	Monongahela	37.9038611	80.2503611	8/14/2014
WV3.3	West Virginia	Monongahela	37.9034444	80.2498611	8/14/2014
WV4.1	West Virginia	Monongahela	38.0027778	80.0226111	8/14/2014

Plot Code	State	National Forest	Latitude (°N)	Longitude (°W)	Date Sampled
WV4.2	West Virginia	Monongahela	38.0038611	80.0250278	8/14/2014
WV4.3	West Virginia	Monongahela	38.0043333	80.0237222	8/14/2014
WV5.1	West Virginia	Monongahela	38.1191389	80.0119722	8/15/2014
WV5.2	West Virginia	Monongahela	38.1190278	80.01275	8/15/2014
WV5.3	West Virginia	Monongahela	38.1185556	80.0131667	8/15/2014
WV6.1	West Virginia	Monongahela	37.9423889	80.0742222	8/15/2014
WV6.2	West Virginia	Monongahela	37.9430833	80.0739722	8/15/2014
WV6.3	West Virginia	Monongahela	37.94375	80.0735278	8/15/2014

Appendix B

Location of sites where eastern white pine saplings were collected in the northeastern U.S.

Site Code	State	Latitude (°N)	Longitude (°W)	Date Sampled
ME1	Maine	43.426772	70.649808	9/19/2014
ME2	Maine	43.705993	70.674035	9/19/2014
ME3	Maine	43.809592	70.638708	9/19/2014
ME4	Maine	43.98127	70.93385	9/19/2014
MA1	Massachusetts	42.618196	71.61146	7/25/2014
NH1	New Hampshire	42.961983	71.884363	7/20/2014
NH2	New Hampshire	43.018726	71.326695	9/8/2014
NH3	New Hampshire	43.133605	71.493854	9/8/2014