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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Bachelor of Science, University of Missouri-Columbia, 2013

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2015
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August 2015
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.
ACKNOWLEDGEMENTS

Let me start by saying, in the process of assembling this thesis, I have had the opportunity to work with many amazing professionals. Please forgive me if I have overlooked anyone essential to this project.

First, I would like to thank all of the awesome people who took time out of their busy work schedules to help me with site selection, permits, field work, or statistical analyses. Without Dave Coyle, Chris Asaro, Brittany Barnes, Ansley Silva, Jaxk Reeves, WenQian Kong, Christiane Helbig, Michael and Florian Müller, Derek Robertson, Danielle Sank, Jason Rodrigue, Ted Oprean, Joan Brown, Virginia Gibbs, Chris Thomsen, John Wright, Dennis Anderson, Donald Garman, Lori Chamberlin, Isabel Munck, Jennifer Weimer, and Bill Ostrofsky, I would still be in the field measuring and counting trees or running analyses, and nowhere near completing my thesis. My sanity thanks you guys/gals for your hard work and willingness to help.

Next, I would like to thank my truly remarkable mentors, Drs. Kamal Gandhi, Rima Lucardi, and Nate Nibbelink, for giving me advice on everything from statistics and modeling, to careers and conferences. Thank you for pushing me to reach for the stars! To the newly minted Dr. Angela Mech, thank you for teaching me everything you know about eastern white pine and Matsucoccus, inspiring me to become a better teacher, humoring me when I came up with crazy questions about research and life, being my gym buddy and fellow Sternorrhynchist, and, through all else, still wanting to be my friend. To Michelle Cram, thank you, from the bottom of my heart, for spending
countless hours plating and identifying the hundreds of cankers I sent your way. You’re way too awesome for words!

Thank you to the Gandhi Forest Entomology Lab (with honorary members, Chip Bates and Kier Klepzig), Courtney Brissey, Ami Flowers, Dan Miller, and my undergrad “framily” who have been great moral support during this time. Finally, thank you to the USDA Forest Service Forest Health Evaluation Monitoring Grant, Virginia Department of Forestry, Sigma Xi Grants in Aid of Research, and the Daniel B. Warnell School of Forestry and Natural Resources for providing the funding which made this project possible.
TABLE OF CONTENTS

ACKNOWLEDGEMENTS.................................................................................................................. v

LIST OF TABLES............................................................................................................................... ix

LIST OF FIGURES ............................................................................................................................. x

CHAPTERS

1 THESIS INTRODUCTION AND LITERATURE REVIEW ................. 1

   1.1 Importance of Eastern White Pine .................................................. 1

   1.2 Dieback of Eastern White Pine .......................................................... 2

   1.3 Thesis Objectives ............................................................................ 4

   1.4 Literature Cited ............................................................................... 6

2 IMPACTS OF ABIOTIC AND BIOTIC SITE CONDITIONS ON THE
   HEALTH OF EASTERN WHITE PINE IN THE SOUTHERN
   APPALACHIAN MOUNTAINS ................................................................. 10

   2.1 Introduction ..................................................................................... 12

   2.2 Methods .......................................................................................... 15

   2.3 Results ............................................................................................. 22

   2.4 Discussion ....................................................................................... 24

   2.5 Acknowledgements ......................................................................... 28

   2.6 Literature Cited ............................................................................... 29
3 EFFECTS OF A SCALE INSECT-FUNGAL PATHOGEN COMPLEX ON
EASTERN WHITE PINE HEALTH IN THE APPALACHIAN
MOUNTAINS..................................................................................................46

3.1 Introduction...............................................................................................48
3.2 Methods.....................................................................................................53
3.3 Results.......................................................................................................59
3.4 Discussion.................................................................................................62
3.5 Acknowledgements...................................................................................66
3.6 Literature Cited........................................................................................67

4 THESIS CONCLUSIONS..............................................................................89

4.1 Thesis Conclusions................................................................................89
4.2 Directions for Future Research.................................................................91
4.3 Literature Cited........................................................................................93

APPENDICES

A Location of plots sampled in the southern Appalachian Mountains, U.S.......94

B Location of sites where eastern white pine saplings were collected in the
northeastern U.S.............................................................................................97
LIST OF TABLES

Table 2.1. Number of sites sampled per state based on the proportion of USDA Forest Service white/red/jack pine land (ha) ..........................................................36

Table 2.2. Occurrence of Matsucoccus macrocicatrices in the southern Appalachian region in sites sampled from 2011-2014 ..........................................................37

Table 2.3. Occurrence of Caliciopsis pinea in the southern Appalachian region in sites sampled in 2014 ..........................................................38

Table 2.4. The range (low and high) and mean (± SE) of measured abiotic and biotic factors (n = 120) ..........................................................39

Table 2.5. Fixed-effects models to predict eastern white pine health rating at the plot level (n = 120) ..........................................................40

Table 2.6. Mixed-effects model used to predict eastern white pine health rating at the plot level using fixed variables and the random effect of site (n = 120) .................41

Table 3.1. Genera-level identification of other cankers (n = 381) on the eastern white pine saplings collected in the southern Appalachian Mountains. .........................75
LIST OF FIGURES

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.................................................................43

Figure 2.2. Occurrence of *Matsucoccus macrocicatrices* from 2011-2014, and *Caliciopsis pinea* in 2014 in the southern Appalachian Mountains, U.S.................44

Figure 2.3. Average (± SE) eastern white pine health rating for different size classes (saplings, poletimber, and sawtimber).................................................................45

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.................................................................78

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.. ........................................................................................................79

Figure 3.3. Distribution of southeastern (six saplings/site) and northeastern (three saplings/site) collection sites for eastern white pine saplings.......................80

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....81
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..82

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..............83

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.....................................................84

Figure 3.8. Correlations between proportion of total canker and proportion of *Matsucoccus macrocicatrices* in the southeastern (O) and northeastern (▲) regions..............................................................................................................85

Figure 3.9. Standardized *Caliciopsis pinea* (A) and other canker (B) surface area (mm²) for each sampled site (n = 49) along the latitudinal range of eastern white pine ..86

Figure 3.10. Distribution of the total canker surface area (cm²) (A) and *Matsucoccus macrocicatrices* (B) from the base to the meristem of the eastern white pine saplings (n = 270). .........................................................................................................................................................87

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.................................................................88
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.) Carrière) 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 (*Erethizon 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, pole timber, and saw timber); 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


CHAPTER 2

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

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.) Carrière], 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 (< 2.54 cm 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 ($\leq 25\%$ 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 (m²/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 (WPTree½) and hardwoods (HWTree½) 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):

\[
\text{Transformed aspect} = \cos(45 - \text{azimuth}) + 1
\]

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 (≥ 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\(^{\frac{1}{2}}\), hardwood density, HWTree\(^{\frac{1}{2}}\),
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$^{1/2}$, 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$^{1/2}$ ($P < 0.001$, $t = 3.75$), 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 ($\pm$ SE) eastern white pine health rating ranged from 3.8 $\pm$ 0.03 for saplings, to 4.0 $\pm$ 0.05 for poletimber, and 2.9 $\pm$ 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 ($\pm$ 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 planipennis* 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; Vité 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.

2.5 Acknowledgements

We would like to thank the Virginia Department of Forestry and USDA Forest Service for helping us find sites, and providing the necessary permits for this project. Special thanks to Brittany Barnes, Ansley Silva, Christiane Helbig, Derek Robertson, and Danielle Sank from the University of Georgia; Jason Rodrigue, Ted Oprean, Joan Brown, and Virginia Gibbs from the USDA Forest Service; and Chris Thomsen, John Wright, Dennis Anderson, Donald Garman, and Lori Chamberlin from the Virginia Department of Forestry for assisting with field work, and to Jaxk Reeves and WenQian Kong for assistance with statistical analyses of the site data. Financial support for this project was provided by the USDA Forest Service Forest Health Evaluation Monitoring Grant, the Virginia Department of Forestry, Sigma Xi Grants in Aid of Research, and the Daniel B. Warnell School of Forestry and Natural Resources at the University of Georgia.
2.6 Literature Cited


Review of Phytopathology 32: 75-87.


McClure, M.S. 1989. Importance of weather to the distribution and abundance of


USGS Geosciences and Environmental Change Science Center. 2013.

http://esp.cr.usgs.gov/data/little/


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

<table>
<thead>
<tr>
<th>State</th>
<th>USDA Forest Service Land (ha)</th>
<th>Proportion</th>
<th># Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Georgia</td>
<td>10,592.00</td>
<td>0.19</td>
<td>8</td>
</tr>
<tr>
<td>North Carolina</td>
<td>6,013.60</td>
<td>0.11</td>
<td>4</td>
</tr>
<tr>
<td>South Carolina</td>
<td>3,443.90</td>
<td>0.06</td>
<td>2</td>
</tr>
<tr>
<td>Virginia</td>
<td>17,605.00</td>
<td>0.31</td>
<td>13</td>
</tr>
<tr>
<td>West Virginia</td>
<td>8,580.20</td>
<td>0.15</td>
<td>6</td>
</tr>
<tr>
<td>Tennessee</td>
<td>9,977.50</td>
<td>0.18</td>
<td>7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>56,212.20</strong></td>
<td><strong>1</strong></td>
<td><strong>40</strong></td>
</tr>
</tbody>
</table>
Table 2.2. Occurrence of *Matsucoccus macrocicatrices* in the southern Appalachian region in sites sampled from 2011-2014.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Georgia</td>
<td>8</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Kentucky</td>
<td>0</td>
<td>3</td>
<td>NA*</td>
</tr>
<tr>
<td>North Carolina</td>
<td>6</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>South Carolina</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Tennessee</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Virginia</td>
<td>2</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>West Virginia</td>
<td>2</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>21</strong></td>
<td><strong>10</strong></td>
<td><strong>34</strong></td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>State</th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Georgia</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>North Carolina</td>
<td>3</td>
<td>1</td>
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<tr>
<td>South Carolina</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Tennessee</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Virginia</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>West Virginia</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>35</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 2.4. The range (low and high) and mean (± SE) of measured abiotic and biotic factors (n = 120).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern White Pine Health Rating¹</td>
<td>1.65</td>
<td>4.5</td>
<td>3.35</td>
<td>0.07</td>
</tr>
<tr>
<td>Latitude</td>
<td>34.7</td>
<td>38.87</td>
<td>36.42</td>
<td>0.13</td>
</tr>
<tr>
<td>Slope (%)</td>
<td>1</td>
<td>50</td>
<td>15.89</td>
<td>1.02</td>
</tr>
<tr>
<td>Aspect</td>
<td>0</td>
<td>2</td>
<td>1.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Elevation (m)</td>
<td>316</td>
<td>897</td>
<td>633.6</td>
<td>14.63</td>
</tr>
<tr>
<td>Eastern White Pine Density (trees/ha)</td>
<td>32</td>
<td>3,121</td>
<td>547.11</td>
<td>44.01</td>
</tr>
<tr>
<td>WPTree¹/²</td>
<td>1</td>
<td>8.06</td>
<td>3.78</td>
<td>0.15</td>
</tr>
<tr>
<td>Hardwood Density (trees/ha)</td>
<td>96</td>
<td>3,918</td>
<td>1,092.33</td>
<td>61.39</td>
</tr>
<tr>
<td>HWTree¹/²</td>
<td>1.73</td>
<td>11.09</td>
<td>5.59</td>
<td>0.16</td>
</tr>
<tr>
<td>DBHA (cm)</td>
<td>4.19</td>
<td>98.3</td>
<td>26.91</td>
<td>1.83</td>
</tr>
<tr>
<td>Eastern White Pine Basal Area (m²/ha)</td>
<td>0.22</td>
<td>80.49</td>
<td>25.87</td>
<td>1.75</td>
</tr>
<tr>
<td>Hardwood Basal Area (m²/ha)</td>
<td>0.35</td>
<td>46.1</td>
<td>19.15</td>
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<tr>
<td><em>M. macrocicatrices</em></td>
<td>0</td>
<td>1</td>
<td>0.15</td>
<td>0.03</td>
</tr>
<tr>
<td><em>C. pinea</em></td>
<td>0</td>
<td>1</td>
<td>0.13</td>
<td>0.03</td>
</tr>
<tr>
<td>Eastern Hemlock</td>
<td>0</td>
<td>1</td>
<td>0.43</td>
<td>0.05</td>
</tr>
<tr>
<td>Other Pine</td>
<td>0</td>
<td>1</td>
<td>0.36</td>
<td>0.04</td>
</tr>
<tr>
<td>Rhododendron/Mountain Laurel</td>
<td>0</td>
<td>1</td>
<td>0.32</td>
<td>0.04</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Model #</th>
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<th>PAR</th>
<th>RMSE</th>
<th>RSQ</th>
<th>AIC</th>
<th>BIC</th>
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<td>1</td>
<td>0.7207</td>
<td>0.0000</td>
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<td>270.39</td>
</tr>
<tr>
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<td>SITE</td>
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<td>0.5279</td>
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</tr>
<tr>
<td>1a</td>
<td>DBHA, DBHA^2, DBHA^3</td>
<td>4</td>
<td>0.6294</td>
<td>0.2557</td>
<td>240.38</td>
<td>249.31</td>
</tr>
<tr>
<td>1b</td>
<td>WPTree^{1/2}</td>
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<td>0.6558</td>
<td>0.1781</td>
<td>243.27</td>
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</tr>
<tr>
<td>1c</td>
<td>LAT</td>
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<td>0.6807</td>
<td>0.1146</td>
<td>252.21</td>
<td>260.57</td>
</tr>
<tr>
<td>1d</td>
<td>ICP</td>
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<td>0.6944</td>
<td>0.0786</td>
<td>257.00</td>
<td>265.36</td>
</tr>
<tr>
<td>2</td>
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<td>5</td>
<td>0.5986</td>
<td>0.3326</td>
<td>224.28</td>
<td>241.01</td>
</tr>
<tr>
<td>3</td>
<td>DBHA, DBHA^2, DBHA^3, WPTree^{1/2}, LAT</td>
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<td>0.4023</td>
<td>213.05</td>
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<tr>
<td>4</td>
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<td>0.4102</td>
<td>213.46</td>
<td>235.76</td>
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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).

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<td>Residual</td>
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<table>
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<th>Fixed Effects</th>
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<th>P*</th>
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<tr>
<td>(Intercept)</td>
<td>-2.7244</td>
<td>1.7324</td>
<td>-1.5727</td>
<td>0.12</td>
</tr>
<tr>
<td>WPTree$^{1/2}$</td>
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<td>0.0430</td>
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<tr>
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<td>0.005</td>
</tr>
</tbody>
</table>

*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
Figure 2.2
Figure 2.3

Growing Stock Classification

Mean (± SE) Eastern White Pine Health Rating

Sapling (2.54 – 12.45 cm)
Poletimber (12.7 – 22.61 cm)
Sawtimber (> 22.86 cm)
CHAPTER 3

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

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
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 ≥ 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 ($\chi^2 = 0.05; P >$
Another way to view these values is that 66% of the collected \textit{M. macrocicatrices} were associated with \textit{C. pinea} cankers, 29% were associated with other cankers, and only 5% were found without a canker, so, overall, 95% of the collected \textit{M. macrocicatrices} were found to be associated with cankers on the saplings. Mean (± SE) \textit{C. pinea} canker size (mm$^2$) was 206.8 ± 16.1, while mean other canker size was 36.6 ± 2.1. \textit{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 \textit{M. macrocicatrices} and dead nodes on saplings ($P < 0.001$, $r_s = 0.46$, Figure 3.7B), and the proportions of \textit{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 \textit{Phaeomoniella} spp., 5% were \textit{Phomopsis} spp., 4% were \textit{Chaetophoma} spp., 3% were \textit{Pestalotiopsis} spp., and 2% were \textit{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 \textit{Penicillium} spp. (Table 3.1).

\textbf{3.3.3 Eastern white pine-\textit{M. macrocicatrices}-canker relationship in the northeastern region}

A total of 929 individual \textit{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 (cm²) on each sapling was 277.9 ± 43.9, and ranged from 25.6 cm² to 812.6 cm² 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 (χ² = 4.67; *P* < 0.05), as well as other cankers and no canker (χ² = 8.66; *P* < 0.01), but there were no differences between *C. pinea* and other cankers (χ² = 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 (mm²) 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*ₜ = 0.62, Figure 3.7A), the proportions of *M. macrocicatrices* and dead nodes on white pine saplings (*P* < 0.01, *r*ₜ = 0.44, Figure 3.7B), and the proportions of *M. macrocicatrices* and total cankers on eastern white pine saplings (*P* < 0.01, *r*ₜ = 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 (cm²) 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 (cm²) 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*.

### 3.5 Acknowledgements

We would like to thank the USDA Forest Service, New Hampshire Division of Forests and Lands, and Maine Forest Service for collecting and shipping saplings from Massachusetts, New Hampshire, and Maine, respectively. Special thanks to Isabel Munck, Jennifer Weimer, Bill Ostrofsky, and their helpers for collecting the samples from the field. Financial support for this project was provided by the USDA Forest Service, Forest Health Protection, Evaluation Monitoring Grant, the Virginia Department of Forestry, Sigma Xi Grants in Aid of Research, and the Daniel B. Warnell School of Forestry and Natural Resources at the University of Georgia.
3.6 Literature Cited


gen. nov. and *Phaeomoniella* species associated with wood necroses of *Prunus* trees. Persoonia 24: 60-80.


Soil Orders Map of the U.S.. National Soil Survey Center, Lincoln, NE USA.

USGS Geosciences and Environmental Change Science Center. 2013.

http://esp.cr.usgs.gov/data/little/


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

<table>
<thead>
<tr>
<th>Fungal Genera</th>
<th>Number of Isolates</th>
<th>% of Total Isolates</th>
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</tr>
<tr>
<td>Pestalotiopsis</td>
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</tr>
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<td>Phaeomoniella</td>
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<td>20</td>
</tr>
<tr>
<td>Phomopsis</td>
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<td>5</td>
</tr>
<tr>
<td>Other genera</td>
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<td>31</td>
</tr>
<tr>
<td>Unknown genera</td>
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<td>9</td>
</tr>
<tr>
<td>Contamination (Mold)</td>
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</tr>
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<td>No Fungal Growth</td>
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<td>4</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>381</strong></td>
<td><strong>100</strong></td>
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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 3.4
Figure 3.6

Mean (± SE) # of M. macrocicatrices

- C. pinea
- Other Canker
- No Canker

Legend:
- ■ Southeast
- □ Northeast
Figure 3.7

Proportion of Dead Nodes (Dieback) vs. Proportion of Canker

- **A**
  - Proportion of M. macrocicatrices
  - $r^2 = 0.25$, $P < 0.001$
  - $r^2 = 0.33$, $P < 0.05$

- **B**
  - Proportion of $M. macrocicatrices$
  - $r^2 = 0.07$, $P < 0.001$
  - $r^2 = 0.20$, $P < 0.01$

Southeast vs. Northeast:

- Southeast (circles)
- Northeast (triangles)
Figure 3.8

Proportion of Canker

Proportion of M. macrocicatrices

Southeast
Northeast

$\text{r}^2 = 0.25$
$P < 0.001$

$\text{r}^2 = 0.29$
$P < 0.01$
Figure 3.10
Figure 3.11

M. macrocicatrices nymph settled in the margin of a canker

Stylet (feeding tube) creates a wound

Hyphal growth and canker development in old feeding wound
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 strobus* 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 susceptbile 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


http://www.wvagriculture.org/market_bulletin/Past_Issues


## APPENDICES

### Appendix A

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

<table>
<thead>
<tr>
<th>Plot Code</th>
<th>State</th>
<th>National Forest</th>
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<th>Longitude (°W)</th>
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Appendix B

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

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<th>Site Code</th>
<th>State</th>
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<th>Longitude (°W)</th>
<th>Date Sampled</th>
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