FACTORS IMPACTING PEST DAMAGE ON *PINUS STROBUS* L.: IMPLICATIONS FOR MANAGEMENT

By

Katherine Minnix

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ABSTRACT

Pinus strobus L. is an iconic forest species native to eastern North America. Managing for high-quality *P. strobus* timber is complicated by *Pissodes strobi* Peck, which colonizes and kills terminal leaders, causing bole defects. *Pinus strobus* is also impacted by Caliciopsis canker. This emerging disease, caused by the native fungal pathogen *Caliciopsis pinea* Peck, causes shallow bole and branch cankers, dieback, and sapling mortality. Caliciopsis canker disease was detected in Michigan in 2016, but the distribution and severity of the disease in the state is not well known.

Management strategies for *P. strobi* often reduce *P. strobus* growth. To evaluate trade-offs between growth and *P. strobi* caused defects, long-term *P. strobus* stands were established under four different regeneration tactics: monoculture, shelterwood, even-aged mixed-species stand with varying density, and even-aged mixed-species stand with varying hardwood competition (Chapter 1). Trees in these stands were assessed 17 to 19 years after they were planted. Mean annual growth rate of *P. strobus* was eight times greater in the monoculture than in the shelterwood, however incident rate of bole defects was 64% in the monoculture and 6% in the shelterwood. The even-aged mixed-species stands yielded markedly different results with *P. strobus* reaching the overstory at the variable density stand but suffering up to 90% mortality at the varying hardwood competition stand.

A survey of 66 *P. strobus* stands across northern Michigan found defects in *P. strobus* boles in 85% of stands and 11% of surveyed *P. strobus* (Chapter 2). *Pinus strobus* crown class and size class were predictive of bole defects but stand density factors (i.e., basal area, trees per hectare, stems per hectare) did not affect the likelihood of defects. Caliciopsis canker disease was widespread throughout the surveyed area. Signs of Caliciopsis canker disease were found in 47% of stands and 7% of surveyed *P. strobus*. The disease was most frequently found on *P. strobus*

saplings, suggesting Caliciopsis canker has greater impact on regeneration. Lower *P. strobus* basal area was associated with a higher likelihood of Caliciopsis canker signs and symptoms, likely because lower basal area was more conducive to regeneration.

During the stand surveys, bark samples with *Caliciopsis* spp. ascocarps were collected from *P. strobus* stems and branches (Chapter 3). From these, 37 isolates of *Caliciopsis* spp. were isolated, 35 of which were identified as *C. pinea* by sequencing the ITSrDNA gene region. The other two isolates were a potentially undescribed species of *Caliciopsis*, *C.* sp. 1. Pathogenicity tests were conducted on *P. strobus* excised branches and live seedlings. On excised branches, both species produced cankers larger in area and deeper than the control. In live seedlings, *C. pinea* produced cankers larger in area than the control while *C.* sp. 1 produced deeper but not larger cankers than the control. *Caliciopsis* species were re-isolated from canker margins following both pathogenicity trials. This study confirmed *C. pinea* as a pathogen of *P. strobus* and found that it was more virulent than *C.* sp. 1. In live seedlings, *C.* sp. 1. was more effective in colonizing down into the sapwood rather than the living cambium suggesting it is a weak pathogen or saprophyte, rather than a primary disease agent.

These studies provide new insight into two native damage agents of *P. strobus*. Quantitative data on trade-offs between growth, competition, and *P. strobi* damage could help managers weigh different silviculture options (Chapter 1). Surveys found both damage agents are widespread throughout northern Michigan and provide a baseline for future efforts to track damage over time (Chapter 2). Pathogenicity tests showed *Caliciopsis pinea* is the primary cause of Caliciopsis canker disease, but at least one other species may contribute to the pathogen load (Chapter 3). Future studies are needed to elucidate the epidemiology of this emerging disease and to better inform management decisions.

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INTRODUCTION

Pinus strobus L. (eastern white pine) is an economically, ecologically, and culturally important forest species native to eastern North America (Wendel and Smith 1990). As an intermediately shade tolerant species, *P. strobus* grows in both pure stands and in mixed stands with hardwoods or other conifers (Carey 1993). *Pinus strobus* is an important habitat and food source to birds and mammals (Rogers and Lindquist 1992). As the tallest species in eastern North American forests, the tops of supercanopy *P. strobus* are common nesting places for bald eagles (*Haliaeetus leucocephalus* L.) and hollow bases provide denning sites for black bears (*Ursus americanus* Pal.) (Uprety et al. 2013).

Extensive logging of *P. strobus* forests from the mid-1800's to early 1900's necessitated reforestation efforts in the Great Lakes region (Ziegler 2010; Barnett and Burns 2012). Restoring high quality *P. strobus* was in part hindered by white pine weevil (*Pissodes strobi* Peck, Coleoptera: Curculionidae), a native insect that can kill up to four years of upper canopy growth in a single growing season (Wallace and Sullivan 1985). Loss of the terminal leader reduces height growth by an average of 40-60% for that year, while multiple weevil attacks over the course of a rotation (80-100 years) can reduce sawtimber volume by 20-60% and tree height by up to 3 m (Katovich and Morse 1992). Injury from *P. strobi* can result in loss of apical dominance and can lead to bole defects, including crooked stems, forks, and multiple leaders. Damaged *P. strobus* can outgrow minor defects but boles may still contain bark-encased knots and compression wood, which degrade timber value (Brace 1971).

Advances in managing white pine weevil have renewed interest in regenerating *P. strobus* in recent decades (Ostry et al. 2010). Silvicultural strategies can reduce weevil damage by either increasing intraspecific competition to encourage young *P. strobus* to quickly recover

apical dominance (Ostry et al. 2010; Pubanz et al. 1999) or by regenerating *P. strobus* in partial shade as this results in slender terminal leaders that are less likely to be colonized (Stiell and Berry 1985). These advances, however, have coincided with the emergence of Caliciopsis canker disease which is caused by the native fungal pathogen *Caliciopsis pinea* Peck. This disease causes excessive resin production, branch and stem cankers, branch dieback, and sapling mortality (Munck et al. 2015). In 2016, Caliciopsis canker disease was confirmed in Michigan (Minnix and Sakalidis 2018) following anecdotal evidence of dieback and mortality in *P. strobus* regeneration (O'Brien 2007). The extent and severity of the disease throughout the state, however, was unknown.

For my dissertation, I researched two native pests of *P. strobus*: *Pissodes strobi* and Caliciopsis canker disease. In Chapter 1, I evaluated *P. strobus* regeneration for trade-offs among growth rates, the likelihood of *P. strobi* damage, the ecosystem services likely to be accrued over time and specific management goals. To evaluate these trade-offs, long-term *P. strobus* stands were established in 1998-2000 under four different regeneration tactics: monoculture; shelterwood; even-aged, mixed-species stand with varying density; and even-aged, mixed-species stand with varying hardwood competition. Revisiting these areas approximately 17 to 19 years after planting provided a unique opportunity to assess tree growth along with bole defects likely to have resulted from *P. strobi* attacks. Quantitative information on the trade-offs associated with these different silvicultural strategies can inform decisions related to *P. strobus* regeneration and projected growth.

In Chapter 2, I surveyed 66 stands across northern Michigan for bole defects resembling *P. strobi* injury and signs and symptoms of Caliciopsis canker disease. This study further aimed to identify site (i.e., soil type, slope, and drainage level) and stand level (i.e., basal area, trees per

ha, and stems per ha) factors associated with bole defects or Caliciopsis canker disease. This was the first extensive survey of Caliciopsis canker in the state and established a baseline for the impacts of this emerging disease on Michigan forests.

During the surveys, bark samples containing *Caliciopsis* spp. ascocarps were collected. Those samples were used to isolate cultures of *C.* spp. DNA was then isolated from pure cultures and the ITSr gene region was sequenced to identify the isolates to species. While 35 of the isolates were *C. pinea*, two were identified as a potentially new species of *Caliciopsis*. I then conducted pathogenicity tests on excised branches and live seedlings to evaluate the virulence of a novel species on *P. strobus* in comparison to the known pathogen *C. pinea*.

CHAPTER 1:

Growth and condition of *Pinus strobus* L. established under different regeneration conditions in 1998-2000

Introduction

Pinus strobus L. (eastern white pine) is an economically and ecologically important forest species across much of eastern Canada and the northeastern U.S. (Wendel and Smith 1990). Extensive logging of *P. strobus* forests across the Great Lakes region of Michigan, Wisconsin, and Minnesota occurred between the mid 1800's and early 1900's (Ziegler 2010). Major reforestation efforts were undertaken in the mid 1930's when the US Forest Service established nurseries and produced billions of tree seedlings for planting by the Civilian Conservation Corps (Barnett and Burns 2012). Efforts to re-establish high-quality *P. strobus* forests, however, were largely limited by two pests: white pine blister rust and white pine weevil.

White pine blister rust, a disease caused by the invasive fungal pathogen *Cronartium ribicola* J. C. Fisch, invaded the Great Lakes region between 1913 and 1917 (Hummer 2000). Extensive efforts were undertaken starting in the 1930s to eradicate *Ribes* spp., the alternate host of the invasive fungus, and regulate *Ribes* spp. cultivation (Geils et al. 2010). Today, white pine blister rust is effectively managed by regulations that restrict *Ribes* cultivation and avoiding *P. strobus* planting in high hazard areas where environmental factors are conducive to infection (White et al. 2002). Genetically resistant stock is also available for reforestation projects (David et al. 2012; Ostrofsky et al. 1988; Riker and Kouba 1940; White et al. 2002).

White pine weevil, *Pissodes strobi* Peck (Coleoptera: Curculionidae), native to much of North America, also frustrated efforts to re-establish timber producing forests of *P. strobus*. Weevil larvae feed beneath the bark on terminal leaders of *P. strobus*, as well as other pines and

spruces. Young, vigorously growing pines with thick terminal leaders are most likely to be colonized (Belyea and Sullivan 1956; Ostry 2000). Up to four years of upper canopy growth can be killed in a single growing season (Wallace and Sullivan 1985), resulting in the loss of apical dominance. Competition among lateral shoots can lead to deformities including forks or crooked stems and "stag-headed" or "cabbage" trees with multiple leaders. These defects typically render *P. strobus* trees unsaleable, particularly if the damage affects the lowest and most valuable log within 5.5 m above ground (Brace 1971). When the terminal leader is killed by *P. strobi*, height growth for that year is reduced by an average of 40-60% (Katovich and Morse 1992). Multiple attacks over the course of a rotation (80-100 years) can reduce tree height by up to 3 m, sawtimber volume by 20-60% (Katovitch and Morse 1992) and the value of sawlogs by an average of 25% (Brace 1971).

Managing *P. strobus* in forested settings remains challenging in the Great Lakes region and beyond. Tactics to reduce *P. strobi* damage to *P. strobus* regeneration focus on two general silvicultural strategies. First, young trees can be encouraged to quickly recover apical dominance by planting a high density of stems in an open field such as in reforestation efforts or when planting seedlings into a recent clearcut (Ostry et al. 2010). Young *P. strobus* that are exposed to full or nearly full sunlight are typically most susceptible to *P. strobi* attack because of their vigorous growth and stout terminal leaders (Livingston et al. 2019). When young, open-grown pines are attacked by *P. strobi*, competition for light in a high-density stand encourages re-establishment of apical dominance relatively quickly, presumably reducing the likelihood of severe bole defects (Pubanz et al. 1999).

A second silvicultural tactic to reduce *P. strobi* damage involves regenerating *P. strobus* in shaded or partially shaded conditions. Adult female weevils, who lay individual eggs in

feeding punctures on terminal leaders in spring, preferentially select stout, vigorous leaders at least 4 mm in diameter for feeding and oviposition (Sullivan 1961; Wallace and Sullivan 1985). Pines growing in shade produce slender terminal leaders that are less desirable for oviposition by female *P. strobi* (Stiell and Berry 1985). Shade also results in relatively cool temperatures which are not preferred by adult *P. strobi* and appear to be linked to higher winter adult mortality (Taylor et al. 1996).

Options for regenerating *P. strobus* under shade can include planting *P. strobus* below an existing canopy of overstory trees, then eventually removing the overstory to release the pines, ideally without injury (Katovich and Morse 1992; Messier et al. 1999). Alternatively, in even-aged, mixed-species stands, fast-growing hardwood basal sprouts can provide partial shade to young *P. strobus* (Stiell and Berry 1985). In either case, however, reduced *P. strobi* damage may be offset by relatively slow growth of young *P. strobus* resulting from low light availability, leading to extended rotations (Stiell and Berry 1985). There is also a risk that hardwood regeneration may overtop and suppress growth of *P. strobus* (Leak et al. 2020).

Regenerating *P. strobus* involves considering potential trade-offs among growth rates, the likelihood of *P. strobi* damage, the ecosystem services likely to be accrued over time and specific management goals. In this study, we assessed *P. strobus* trees established in four experimental stands in 1998-2000 under different regeneration strategies. These strategies included *P. strobus* seedlings planted (1) at three densities as a monoculture in an open field, (2) beneath an oak canopy that provided varying levels of shade, and (3) following hardwood clearcuts to establish even-aged, mixed-species stands. Revisiting these areas approximately 20 years after planting provided a unique opportunity to assess tree growth along with bole defects likely to have resulted from *P. strobi* attacks. Quantitative information on the trade-offs

associated with these different silvicultural strategies can inform decisions related to *P. strobus* regeneration and projected growth.

Methods

This long-term project to assess *P. strobus* regeneration strategies began in 1998 as a collaborative effort between Michigan State University (MSU) and the Michigan State Department of Natural Resources (MDNR), with support from the USDA Forest Service (Williams 2002). Three regeneration strategies were evaluated including planting *P. strobus* seedlings at three densities into an open field in Delta County, MI (Monoculture; Figure 1.1, Table 1.1) yielding single species blocks. Seedlings were also planted beneath an oak overstory with varying levels of canopy cover in Kalkaska County, MI (Shelterwood). Finally, seedlings were planted in two hardwood stands following clearcut harvests, creating even-aged, mixed-species stands. In one of these stands, located in Cass County, MI, *P. strobus* was planted at two densities (Mixed-species stand planted at varying densities). In the other, in Kalkaska County, MI, herbicide was applied to a portion of the area to control hardwood regeneration (Mixed-species stand with varying hardwood competition). Stand establishment and evaluation methods are described for each strategy below. Site conditions (i.e., soil type, climate data) are described in Table 1.1.

Monoculture with three planting densities

Stand preparation and planting: To assess effects of density on tree growth and form, *P. strobus* seedlings were planted in 1998 at the MSU Upper Peninsula Forestry Innovation Center near Escanaba, Delta County, Michigan (Figure 1.2). The site consisted of an 8.1 ha area of grasses and herbaceous vegetation on Onaway-Ossineke fine sandy loams (70% of site) and Charlevoix sandy loam (30% of site) (NRCS 2025). In 1997, glyphosate was applied to control

vegetation followed by periodic mowing (Williams 2002). Seedlings were acquired from multiple nurseries in the Great Lakes region in spring 1997 and then transplanted into nursery beds at the Tree Research Center, MSU, East Lansing, Michigan for one year (Williams 2002). The 1-1 seedlings were lifted, bagged, and transported to the Escanaba site in May 1998 (Williams 2002).

A total of 23,500 seedlings were planted in four replicated blocks, each two ha in size, divided into three plots (12 total plots) (Williams 2002). Each plot was planted at one of three densities: 1680 (low), 2198 (intermediate), and 2989 (high) trees per ha (TPH) (Williams 2002). Blister rust resistant seedlings (5,000), provided by the USDA Forest Service Oconto River Nursery in White Lake, Wisconsin, were planted in seven to nine rows of each plot (Williams 2002). Four or five additional rows of *P. strobus* seedlings were planted around each of the 12 plots to serve as buffers between adjacent plots or open spaces (Williams 2002). At the time of planting, the blister rust resistant seedlings were lighter in color and smaller in height and diameter than the standard seedlings (Willams 2002). To accommodate the growing trees, the stand was mechanically thinned in January 2015 by removing alternate rows of trees, creating alleys in each plot.

Tree Evaluation: In June 2017, we recorded size and assessed bole defects of 40 to 62 trees in each of the 12 plots. In June 2019, we returned and assessed bole defects for 32 to 40 additional trees per plot in rows and alleys not surveyed in 2017. To assess size, four trees (standard stock) in adjacent rows at three or four points along three alleys in the interior of each plot were measured and recorded as live or dead. Rust resistant *P. strobus* were similarly surveyed at three to four points along two alleys. We also measured four border trees (standard stock) on the outer two rows of three alleys. For each tree, live or dead, we measured diameter at

breast height (DBH, 1.3 m high) using calipers and height using an extendable height pole. Bole defects on the lowest (basal) log (between 0.5 and 5.4 m above ground) were qualitatively ranked as 0 if the basal log appeared straight with no visible defects; 1 if one or two minor crooks were present but likely to be outgrown; and 2 if the tree was forked, severely crooked, or had other permanent defects likely to make the basal log unsaleable. Height and the type (crook, fork; Figure 1.3) of the lowest bole defect were also recorded. In 2019, we recorded the height and type of the lowest three defects on each tree (live or dead). We also tallied the number of gaps encountered in the rows we surveyed as evidence of trees that had died and were removed in previous years.

To quantify radial growth, we used increment borers to extract cores perpendicular to the tree that extended from the bark to just past the pith. One core was extracted from six representative live trees at stump height (0.5 m) in each plot that appeared healthy and similar to other trees in the plot, including two standard, two rust resistant, and two border trees. Six additional trees were cored in each plot in 2019 using the same selection process and methods as in 2017.

Radial Growth Analysis: To estimate annual radial growth rates, we calculated the area of annual growth rings, which also represents the annual basal area increment (BAI). Cores were dried, mounted, sanded with up to 600 grit sandpaper, and crossdated using standard dendrochronological techniques (Speer 2010). The list method was used to visually crossdate the cores and identify potential missing or false rings (Yamaguchi 1991). Cores were digitally scanned at a resolution of 1200 dpi and annual ring widths were measured using CooRecorder v 7.3 and Cdendro v 7.3 (Cybis Dendrochronology, Sweden). Visual crossdating was verified using COFECHA, a statistical crossdating program (Holmes 1983, Grissino-Mayer 2001).

CooRecorder v 9.0.1 was used to estimate the distance to the pith from the earliest visible ring on each core, which in turn was used to calculate the inner bark radius of the tree at the location where the core was extracted. Annual basal area increment (BAI) was calculated for each ring on each core using the R package dplR (Bunn 2008).

Statistical Analyses: After checking for normality using residual plots, generalized linear mixed models (GLMMs) followed by Type-III ANOVA were used to test for effects of planting density on *P. strobus* DBH and height, and for differences in DBH or height among tree types (standard stock, rust resistant stock, border trees). Pairwise comparisons with Tukey's adjustment were used when factors were significant ($\alpha = 0.05$). To determine if planting density or tree type affected the likelihood of bole defects (ranked 0, 1, or 2), we used proportional odds models with random intercepts and blocks as random factors. All analyses were conducted using SAS v 9.4 (SAS Institute, Cary, NC).

Oak shelterwood with varying canopy cover

Stand Preparation: To assess the effects of canopy cover on *P. strobus* growth and form, 29,000 seedlings were planted at a density of 2198 TPH (evenly spaced) in May 1999 beneath an established but variable overstory of mature *Quercus* spp. (oak) growing on Rubicon-Graycalm sands in a 16-ha stand on state forest land. Planting occurred following a selection harvest (1998-1999) in the stand near Lake City, Michigan (Williams 2002; NRCS 2025). The *P. strobus* seedlings were purchased in April 1999, root pruned, packed in sphagnum moss, and placed in cold storage until planted in May. One month following planting, triclopyr herbicide (Garlon™, Dow AgriSciences, Indianapolis, IN) was applied to control *Populus* spp. (aspen) and *Acer* spp. (maple) stump sprouts present throughout the stand.

Tree Assessment: To measure growth and assess the form of *P. strobus* in this stand, we

systematically established 11 circular plots with a 10 m radius by randomly selecting alternate cells from a grid of 24 similarly sized cells overlaid on an aerial image of the stand (Google Earth Pro 7.3 2017). Height and DBH of all *P. strobus* (≥ 2.5 cm DBH) trees in each plot were measured and visually evaluated for bole defects in August 2017 using the same methods as at the monoculture. Other tree species within plots were tallied by species and DBH recorded (≥ 2.5 cm DBH). Diameter-length increment cores were collected at stump height (0.5 m) from four representative *P. strobus* in each plot. Cores were processed and analyzed using the same methods as described above. Overstory canopy cover was estimated in each plot using a handheld densitometer (Geographic Resource Solutions, Arcata, CA) at 3 m intervals from the plot center to the plot perimeter in each cardinal direction and along a 10 m transect running between the aspects. Approximately 30 densitometer observations were recorded for each plot. Percent canopy cover was then calculated by species.

Statistical Analyses: We used GLMMs with random intercepts and plots as random factors followed by Type-III ANOVA to evaluate effects of canopy cover (percentage) and total basal area (BA) on *P. strobus* DBH and height. To determine if either canopy cover or BA influenced the likelihood of bole defects, we used proportional odds models with random intercepts and plots as random factors.

Mixed-species stand planted at varying densities

Stand preparation: Effects of planting density on *P. strobus* growth and bole defects were evaluated in an even-aged, mixed-species stand at the MSU Fred Russ Forest in Cass County, Michigan. This mixed-species stand was established by planting *P. strobus* seedlings at two densities, 526 and 1028 TPH, following a clearcut harvest in April 1998 of a 53-year-old stand comprised of *Quercus rubra* L. (red oak), *Liriodendron tulipifera* L. (tulip tree), *Juglans nigra* L.

(black walnut), *Prunus serotina* Ehrh. (black cherry), and *Fraxinus americana* L. (white ash) (Williams 2002). A total of 3,000 seedlings were machine planted in a 4 ha site on Oshtemo sandy loam soil in May 1998: 2,000 seedlings at 1028 TPH and 1,000 seedlings at 526 TPH (NRCS 2025; Williams 2002).

Tree assessment: We overlaid a 16-cell grid on an aerial image of the stand (Google Earth Pro 7.3 2017), randomly selected eight cells and established a circular plot with a 10 m radius in the center of each selected cell. In June 2017, tree DBH was measured for all tree species within the plots. Heights and bole defects were recorded for *P. strobus* ≥ 2.5 cm DBH. Increment cores were extracted from four representative *P. strobus*, processed, and analyzed using the same methods as at the oak shelterwood stand described above. Heights of *P. strobus* that were > 7.5 m tall, however, were obscured by foliage from neighboring trees in June. Therefore, we returned to the plots in December 2019 and measured height of 6 to 17 *P. strobus* (> 10 cm DBH) near the center of each plot with a laser hypsometer (Forestry 550, Nikon Corp., Tokyo, Japan). To determine which plots corresponded to the original planting design, we assumed plots with more than 526 *P. strobus* per ha were in areas originally planted at 1028 TPH (Plots 1-5) while the remaining plots were assumed to be in areas originally planted at 526 TPH (Plots 6-8).

We calculated relative importance values (RIV) for all trees (≥ 10 cm DBH) tallied in plots (Kent and Coker 1992). The RIV of a species represents the sum of its relative frequency (percentage of plots in which it is present), dominance (percentage of its BA relative to total BA of all species), and density (percentage of stems relative to the total number of stems tallied).

Statistical analyses: We used GLMMs with random intercepts and plots as random factors followed by Type-III ANOVA to evaluate effects of planting density on *P. strobus* DBH

and height. To determine if planting density affected the likelihood of bole defects, we used proportional odds models with random intercepts and plots as random factors.

Mixed-species stand with varying hardwood competition

Stand Preparation: To evaluate effects of competition from hardwood regeneration on *P. strobus* growth and bole defects, *P. strobus* seedlings were planted in spring 2000 at a constant density of 2198 TPH across a 4 ha stand on Kalkaska sand (NRCS 2025) following a clearcut harvest in 1999 of *Populus* spp., *Acer* spp., and other hardwoods on state forest land near Kalkaska, Michigan (Williams 2002). In 2002, two years after planting, triclopyr herbicide was applied to control hardwood basal sprouts on half of the stand.

Tree Assessment: In July 2017, we overlaid a grid of 18 similarly sized cells on an aerial image of the stand (Google Earth), selected nine alternating cells, and established a circular plot with a 12 m radius in the center of each selected cell. Larger plots were used at this stand because the *P. strobus* density in 2017 appeared to be considerably lower than in the other stands. In each plot, trees were measured, evaluated, and cored using methods described above. Bole defects were evaluated on *P. strobus* trees ≥ 5 cm DBH and RIVs were calculated for all tree species measured > 10 cm DBH.

Statistical Analysis: We were unable to confirm which plots were in areas where hardwood sprouts were treated with herbicide, so we used the total basal area of hardwood species in each plot as a proxy for hardwood competition. To test effects of hardwood competition on *P. strobus* DBH and height, we used GLMMs with random intercepts and plot as a random factor followed by Type-III ANOVA. A log transformation was applied to normalize DBH values prior to analyses. A proportional odds model with random intercepts and plot as a random factor was used to assess effects of total hardwood BA on the likelihood of bole defects.

Results

Monoculture with three planting densities

Tree growth: Planting density negatively affected radial growth ($F_{2,559}$ = 29.62, P < 0.01) but had no effect on height growth ($F_{2,559}$ = 1.27, P = 0.28) of *P. strobus* in the monoculture. In low density plots (1680 TPH), *P. strobus* DBH was 9% larger than the DBH of trees planted at the intermediate density (2198 TPH) (t = -3.63, P < 0.01) (Table 1.2). Similarly, DBH of *P. strobus* planted at the intermediate density was 10% larger than DBH of trees planted at the high density (2989 TPH (t = -3.75, P < 0.01).

Across all blocks, DBH of live *P. strobus* ranged from 5.3 to 27.9 cm and most (83%) were pole-sized, ranging from 12.5 to 25 cm DBH in 2017. Saplings (DBH < 12.5 cm) accounted for 11% of *P. strobus* in the low, 15% in the intermediate, and 21% in the high density plots. Of the seven trees with a DBH larger than 25 cm, three were growing on the borders (outer two rows) of the plots and six of the trees had obvious bole defects. We tallied a total of 25 dead *P. strobus* at the observation points within plots; these were excluded from statistical analyses. Dead trees ranged from 1.8 to 16.5 cm in DBH and 80% were saplings. An additional 54 gaps in the rows were observed where *P. strobus* had died and been removed in past years.

Total height of live *P. strobus* across all blocks and planting densities ranged from 4.0 to 12.3 m. Results of linear regression showed DBH was related to tree height, although the slope of that relationship and the amount of variability in height explained by DBH (R^2) varied with planting density (Figure 1.4). Growth of standard and rust resistant planting stock was similar; neither DBH (t = -1.74, P = 0.19) nor height (t = -0.79, P = 0.71) of *P. strobus* differed. However, trees planted in the interior rows of the plots were taller and thinner than trees planted on the borders. Standard *P. strobus* stock planted on plot borders had larger diameters than the

standard stock trees in the interior rows (t = 2.37, P = 0.05) and the rust resistant stock trees (t = 3.50, P < 0.01). Standard *P. strobus* planted on plot borders were approximately 0.5 m shorter than the standard stock in the interior rows (t = -4.42, P < 0.01) and the resistant stock (t = -3.15, P < 0.01). There were no interactions between planting density and *P. strobus* stock (standard, rust resistant) or planting location (interior, border) for either DBH ($F_{4,553}$ = 1.66, P = 0.16) or height ($F_{4,553}$ = 1.35, P = 0.25).

Bole defects: Overall, 64% of *P. strobus* trees had at least one bole defect in the basal log and 35% had at least one severe defect. Trees in plots planted at the low density were more likely to have severe defects in the basal (lowest) log (0.5-5.4 m) than trees planted at intermediate density (t = 2.94, P < 0.01) or high density (t = 3.44, P = 0.01), which had similar probabilities of sustaining bole defects (t = 0.47, P = 0.64). Bole ratings indicated 60%, 60%, and 68% of the basal logs had at least one defect in the high, intermediate, and low planting density plots, respectively (Table 1.2). Crooks (e.g., bole defect rating of 1), the most common defect, were present at similar rates on the basal logs of trees in each of the planting densities. Severe crook and forks (e.g., bole defect rating of 2), however, affected basal logs in the low density plots at a 10% higher rate than the high and intermediate density plots.

Rust resistant and standard *P. strobus* had similar likelihoods of sustaining bole defects (t = -0.87, P = 0.39), but trees on the border (standard stock) were more likely to have severe defects than interior trees, including standard stock (t = -3.85, P < 0.01) and resistant stock (t = -2.59, P = 0.01). Among standard, rust resistant, and border tree types, 58%, 62%, and 73% of basal logs had at least one obvious defect, respectively. Severe crooks and forks affected trees planted on the border at a 10% higher rate than interior standard and resistant *P. strobus*. There were no interactions between bole defects and tree stock or location ($F_{4,1100}$ = 1.89, P = 0.11).

Increment Core Analysis: Radial growth rates, represented by annual basal area increment (BAI), did not differ among planting densities until 2007, nine years after planting (Figure 1.5). Radial growth of *P. strobus* planted in the high density plots (2127 ± 114 mm^2) stagnated in 2008. Similar reductions in annual radial growth occurred in 2009 for trees planted in the intermediate density plots (2478 ± 154 mm^2), and in 2011 for trees in the low density plots (2194 ± 182 mm^2). The most pronounced difference in BAI among planting densities was in 2010: radial growth of *P. strobus* in the intermediate density plots (2423 ± 188 mm^2) was 27% higher than trees in the high density plots (1906 ± 159 mm^2). Similarly, trees in the low density plots (3166 ± 233 mm^2) had 31% higher radial growth than those in the intermediate density plots. Similar patterns emerged when comparing BAI among rust resistant stock, standard stock planted interiorly, and standard stock planted on the border (Figure 1.5). All three groups had similar levels of radial growth through 2007 when growth rates stagnated for both resistant (2297 ± 127 mm^2) and standard stock planted interiorly (2432 ± 93 mm^2) in 2007 while BAI for standard stock planted on the border continued to increase through 2010 (3498 ± 243 mm^2). During the 2016 growing season following the row thinning in January 2015, average BAI increased 51% across all *P. strobus* regardless of planting stock, density, or location. Radial growth continued to increase annually through 2018, the most recent year for which we have data.

Oak shelterwood with varying canopy cover

Tree Assessment: *Pinus strobus* DBH in this stand ranged from 2.5 to 9.1 cm and height ranged from 1.4 to 7.6 m (Table 1.3). *Pinus strobus* DBH was strongly associated with tree height (Figure 1.6). Within plots, *P. strobus* density ranged from 44 to 80% of the original planting density (2198 TPH). Three oak species were present throughout the stand: northern red

oak (*Quercus rubra* L.), white oak (*Q. alba* L.), and northern pin oak (*Q. ellipsoidallis* E.J. Hill). Up to 12 oak trees per plot (10 m radius) were tallied and oak canopy cover per plot ranged from 14 to 69%. Oak DBH ranged from 10.4 to 55.4 cm, averaging 24.0 ± 3.1 to 42.7 ± 3.0 cm within plots. Oak BA within plots ranged from 0 to 32 m^2/ha; the 16% oak canopy cover of one plot was solely from adjacent trees. Aspen (*Populus* spp.) and maple (*Acer* spp.) saplings were also present throughout the stand. Up to 63 saplings were tallied within individual plots and combined aspen and maple canopy cover per plot ranged from 6 to 52% (Table 1.3). Canopy cover of these two species was inversely related to oak canopy cover (Figure 1.6).

Neither DBH nor height of *P. strobus* trees were affected by oak cover ($F_{1,427}$ = 1.31, P = 0.25 and $F_{1,427}$ = 0.71, P = 0.40, respectively) (Figure 1.7). Only 6% of the *P. strobus* trees in the plots had any substantial bole defects and bole ratings were unaffected by oak cover ($F_{1,426}$ = 3.70, P = 0.06) or total canopy cover ($F_{1,426}$ = 0.02, P = 0.88). Of the bole defects observed, 59% were minor crooks, 18% of were moderate crooks and 23% were forks. Oak basal area also had no effect on *P. strobus* DBH ($F_{1,427}$ = 0.02, P = 0.89), height ($F_{1,427}$ = 0.08, P = 0.78), or likelihood of bole defects ($F_{1,426}$ = 0.58, P = 0.45).

Increment Core Analysis: Annual BAI increased slowly from 2000 (7.5 ± 1.0 mm^2) to 2004 (Figure 1.8) and then rose steadily from 2005 to 2012 (436 ± 22.6 mm^2) as growth rates stabilized, reaching a peak BAI of 473 ± 28.7 mm^2) in 2015. In 2016, the most recent year for which we have data, the mean annual BAI for *P. strobus* planted in this stand (324 ± 17.9 $mm^{2)}$ was only 12% of the growth rate for the *P. strobus* trees in the monoculture (2733 ± 117 mm^2).

Mixed-species stand planted at varying densities

Tree Assessment: Mean *P. strobus* DBH varied among plots (Table 1.4) but was not affected by planting density ($F_{1,147}$ = 0.05, P = 0.82). Similarly, mean height varied among plots

for *P. strobus* (≥ 10 cm DBH) but was also not affected by planting density ($F_{1,67}$ = 2.26, P = 0.14). Across the stand, *P. strobus* DBH ranged from 2.5 to 23.4 cm; 32% of trees were less than 10 cm in DBH and 53% were pole sized (12.5 to 25.0 cm). Tree height ranged from 2.9 to 15.6 m but in summer 2017, when measurements were initially recorded with a height pole, 74% of *P. strobus* were taller than the 7.6 m pole. Crooks or forks were tallied for 30 to 70% of *P. strobus* within individual plots but basal area did not affect the likelihood of bole defects ($F_{1,136}$ = 0.53, P = 0.47). Overall, 51% of *P. strobus* had no obvious bole defects, 24% had crooks, and 25% had forks or other severe defects. Bole defects occurred in *P. strobus* of all DBH and height sizes at this stand. Within plots, *P. strobus* density ranged from 53-77% of original planting density. Multiple tree species regenerated along with the planted *P. strobus* seedlings. Species with the highest relative importance values (Kent and Coker 1992) included northern red oak (*Quercus rubra*) and tulip poplar (*Liriodendron tulipifera*) (Table 1.5).

Radial Growth Analysis: Seedlings were planted in this stand in 1998, the same year monoculture was established. Basal annual increment (BAI) of the *P. strobus* trees was similar at the two stands through 2006, when growth of the trees planted in the mixed-species stand peaked at 2382 ± 182 mm^2 and then stagnated (Figure 1.8). Radial growth also stagnated at the variable density monoculture stand in 2011 and the BAI for both stands was again comparable until 2014. At that point the monoculture was row thinned, and growth rates substantially increased while annual growth rates continued to decline at the mixed-species stand with variable density. In 2016, the most recent year we have data for this stand, the mean annual BAI (727 ± 91.9 mm^2) was 27% of the growth rate of *P. strobus* in the monoculture.

Mixed-species stand with varying hardwood competition

Tree Assessment: In 2017, *P. strobus* density in plots ranged from 155 to 420 TPH

indicating a survival rate of only 7 to 19%. Overall *P. strobus* at this stand ranged from 2.0 to 16.3 cm in DBH; 26% of the stems were ≤ 2.5 cm while 6% were pole-sized (> 12.5 cm). *Pinus strobus* height ranged from 0.9 to 8.2 m across the stand; 28% of the stems were less than 3 m tall while 29% were greater than 5 m tall. The 2002 application of herbicide to control hardwood regeneration did not affect *P. strobus* DBH ($F_{1,114}$ = 0.00, P = 0.99) or total height ($F_{1,112}$ = 0.05 P = 0.83) measured in 2017.

Bole defects were evaluated only on *P. strobus* stems ≥ 5.0 cm DBH, which included 51% of the stems in the plots. Bole defects were present on 70 to 100% of *P. strobus* per plot (Table 1.6). Across the stand, 13% of *P. strobus* evaluated had no observable bole defects in the basal log, 42% had one or more crooks, and 45% had forks or other severe defects in the basal log. Application of herbicide to hardwood sprouts in 2002 did not affect the likelihood of bole defects in *P. strobus* ($F_{1,53}$ = 1.48, P = 0.23).

Similar to the mixed-species stand with variable density, different tree species regenerated alongside the planted *P. strobus* in this stand. Sugar maple (*Acer saccharum*) and American beech (*Fagus grandifolia*) (Table 1.7) had the highest RIVs and both were higher than the RIV of *P. strobus*.

Radial Growth Analysis: Annual BAI for this stand followed a similar trend as the oak shelterwood stand until 2009 (Figure 1.7). At that point, BAI for *P. strobus* in this stand gradually rose, reaching a peak of 1076 ± 81.3 mm^2 in 2015. In 2016, the most recent complete growth year available, BAI for this stand was 36% of the BAI at the monoculture, 137% of the mixed-species stand with variable density, and 25% of the BAI at the oak shelterwood stand.

Discussion

Pinus strobus can be successfully regenerated using a variety of techniques, providing

managers with a range of options that can be integrated with other management objectives related to wildlife habitat or other ecosystem services. Our study quantified trade-offs between *P. strobus* growth and bole defects, presumably caused by *P. strobi*, associated with multiple regeneration methods. We found that interspecific competition was inversely related to radial growth but an increased incidence of *P. strobi* damage, i.e., defects in the lowest and most valuable log, was associated with relatively higher rates of tree growth. In the monoculture, mean annual radial growth of *P. strobus* was more than eight times greater than the growth of *P. strobus* planted in a shelterwood. More than 30% of *P. strobus* in the monoculture, however, had severe bole defects while only 6% of the small *P. strobus* growing in the shelterwood had any visible defect. *Pinus strobus* growth varied greatly between the two even-aged, mixed-species stands. At the mixed-species stand with variable planting density, *P. strobus* were successfully competing with hardwood species and were a part of the overstory. In the mixed-species stand with varying hardwood competition, however, *P. strobus* were heavily browsed and overtopped by hardwood regeneration, resulting in nearly 90% seedling mortality, illustrating how easily hardwood stump sprouts, even when treated with an early herbicide application, can outcompete *P. strobus* under the right conditions. Further discussion of the individual stands continues below.

Monoculture with variable density

Monocultures reduce or eliminate competition from other species, maximizing *P. strobus* growth and reducing timber rotations but may also create a favorable environment for *P. strobi* and other specialized pests (Liu et al. 2018). Trade-offs between *P. strobus* growth and the likelihood of bole defects were especially evident in the replicated blocks in the Delta County stand where *P. strobus* faced no interspecific competition. *Pinus strobus* planted in the low

density plots were on average 19% larger in DBH than those planted in the high density plots but had a 13% greater incidence rate of severe bole defects. These results are consistent with a Wisconsin study of 17 *P. strobus* plantings with 30 to 150-year-old trees that reported the incidence of weevil injury was inversely related to total stem density (Pubanz et al. 1999). A study in New England on 18-year-old trees also recorded more *P. strobi* related injuries on *P. strobus* planted at 2679 TPH compared with *P. strobus* growing at 8467 TPH following a direct seeding (Graber 1988).

Higher planting densities and intraspecific competition for light presumably encouraged *P. strobus* to re-establish apical dominance relatively quickly following the loss of the terminal leader (Stiell and Berry 1985). We noted that *P. strobus* trees in intermediate density plots were not more likely to sustain bole defects than trees in the high density plots. This indicates that an intermediate planting density (2198 TPH) may be optimal for achieving high radial growth while avoiding bole defects.

Pinus strobus trees bordering the plots and adjacent to open areas had larger diameters but were shorter and more likely to have bole defects than *P. strobus* planted in the interior rows of the blocks. Increased incidence of *P. strobi* damage on border trees reflects the increased exposure to sun compared with that experienced by interior trees, resulting in thicker terminal leaders or higher bark temperatures on terminal leaders, both of which are attractive to *P. strobi* (Sullivan 1960; Wallace and Sullivan 1985; Wilkinson 1983). Additionally, increased light availability presumably reduced the competitive pressure for border trees to re-establish apical dominance quickly following *P. strobi* colonization.

The *P. strobus* trees with resistance to white pine blister rust did not differ in size or incidence of bole defects from standard stock that would presumably be susceptible to infection.

There were no discernible differences in BAI over the course of the study, despite the notably smaller size of the resistant seedlings at the time of planting (Williams 2002). A *P. strobus* restoration study across forests in areas presumed to be at high hazard for white pine blister rust across the Great Lakes region found growth of rust resistant stock was higher than susceptible stock (Ostry 2000). In that study, however, trees from susceptible stock sustained higher rates of blister rust infection, which accounts for the better growth of resistant stock (Ostry 2000). White pine blister rust was not present in any of our study sites, and we observed no differences between rust resistant trees and susceptible trees in terms of tree growth or *P. strobi* injury. This stand continues to be managed and comparing final size and merchantable volume of mature trees among planting densities will provide more information on the trade-offs between *P. strobus* growth and defects attributable to *P. strobi.*

Oak shelterwood with variable canopy density

Advantages of shelterwood harvest methods can include minimizing *P. strobi* injury to young *P. strobus* while diversifying tree age and vertical structural diversity in a stand. High levels of canopy cover, however, stunted *P. strobus* growth. At the shelterwood stand we evaluated, *P. strobus* seedlings were underplanted following a selection harvest of mature oaks that established a range of canopy cover across the stand (0 to 100%) (Williams 2002). When we evaluated *P. strobus* trees 17 years after planting, canopy cover provided by overstory oaks ranged from 16 to 69% within plots but canopy cover did not affect *P. strobus* size (DBH, height) or the likelihood of bole defects. In contrast, Katovitch and Morse (1992), examined young *P. strobus* naturally regenerated in a Wisconsin shelterwood with varying oak density. They found pines were larger where total basal area was relatively low (up to 7 m^2/ha) but had higher rates of bole defects than similarly-aged *P. strobus* growing where basal area was

moderate (11-16 m^2/ha) or high (23-28 m^2/ha) (Katovich and Morse 1992). These differences were presumably related to light availability in the stands. In our study, a selection harvest between 1998 and 1999 removed individual trees so that oak canopy cover ranged from 0 to 100% (Williams 2002). Among plots in 2017, oak BA ranged from 0 to 32 m^2/ha and oak canopy cover ranged from 14 to 69% but total canopy cover ranged from 46 to 84%. Hardwood regeneration, primarily stump sprouts of red maple (*Acer rubrum*, L.) and bigtooth aspen (*Populus grandidentata*, Michx.), along with lateral in-growth of overstory oak canopies, captured gaps left by the selection harvest.

Only 6% of the *P. strobus* in this stand had visible evidence of bole defects 17 years after planting. While minor damage caused by *P. strobi* may have been outgrown, the slender terminal leaders on the shaded trees were likely too small to attract ovipositing adult weevils or support *P. strobi* larval feeding (Sullivan 1961; Stiell and Berry 1985). These results differ markedly from a 1995-2004 study in Ontario, Canada where 23% of *P. strobus* planted beneath 60% canopy cover provided by red pine (*Pinus resinosa* Aiton) had sustained weevil damage eight years after planting (Major et al. 2009). The discrepancy between our results and those of the Ontario study presumably reflects the different overstory composition; more light likely penetrated red pine canopies that the hardwood canopy in our site (Canham et al. 1994). As the *P. strobus* in our stand were older and larger at the time of measurement than those in the Major et al. study (2009), it is possible that they outgrew some of the minor evidence of previous *P. strobi* damage (Pubanz 1999). Additionally, Major et al. (2009) counted weevil attack as presence of *P. strobi* damage. This may overstate *P. strobi* effects as not all attacks result in bole defects (Marty and Mott 1964). Local density of weevil populations also varies across the range of *P. strobus*, which could also account for differences in *P. strobi* damage incidence. For instance, in open-grown *P.*

strobus stands in northern Wisconsin, the incidence rate of *P. strobi* damage was much higher than in stands in southern Wisconsin (Goulding et al. 1988).

Given that overall rate of bole defects recorded in our stand was minimal, canopy cover of 46% appears to be sufficient at preventing weevil damage in *P. strobus*. Nevertheless, competition and reduced light availability caused the underplanted *P. strobus* to grow very slowly. These trees were on average less than one-third the diameter and less than two-thirds the height of the *P. strobus* growing in the monoculture. While a shelterwood of oak or other hardwoods will reduce weevil damage on *P. strobus*, removing the overstory 5-10 years after planting and controlling hardwood regeneration is likely needed to release pines (Lancaster and Leak 1978). *Pinus strobus* that are less than 30 years old with live crown ratios of at least 30% typically respond well to thinning (Wendel and Smith 1990).

Mixed-species stands

Planting *P. strobus* following a hardwood clearcut to create an even-aged, mixed-species stand can reduce *P. strobi* injury if the regenerating hardwoods provide adequate shade to create microclimate conditions and tree characteristics unsuitable or undesirable for *P. strobi*. Competition with hardwoods, however, can result in intense suppression and even mortality of young *P. strobus*. We evaluated two mixed-species stands. In the first, *P. strobus* planting density varied but did not affect DBH, height, or likelihood of bole defects. These pines competed well with the hardwood species present (*Q. rubra, L. tulipifera, R. pseudoacacia, P. grandidentata,* and *P. serotina*) and many had reached the overstory 19 years after planting. Despite the hardwood competition, the mean DBH of *P. strobus* in this stand was about 75% of the mean DBH of those in the monoculture and more than twice the mean DBH of *P. strobus* in the oak shelterwood. Although half of the *P. strobus* in this stand were pole-sized, growth rates

had stagnated.

The second even-aged mixed-species stand tested the effects of reducing hardwood competition on the growth and incidence of bole defects on *P. strobus*. Treating young hardwood sprouts with herbicide two years after planting *P. strobus* appears to have been effective in reducing hardwood basal area; three of the surveyed plots had a much lower hardwood basal area (2.7-3.4 m^2/ha) than the other six plots (6.3-13.6 m^2/ha). This reduction in hardwood density, however, did not appreciably enhance *P. strobus* growth (diameter or height) nor was there a detectable effect of the herbicide treatment on bole defects 17 years after planting. Size (DBH) of *Pinus strobus* in this stand was similar to *P. strobus* in the oak shelterwood stand and approximately half the DBH of trees in the even-aged, mixed-species stand with variable planting density. Despite their suppressed size, over 85% of the surviving *P. strobus* stems had bole defects. Given the size and canopy position (suppressed or intermediate) of the *P. strobus*, however, we suspect the damage was caused by deer (*Odocoileus virginianus* Zimmerman) browsing on the young pines. *Pinus strobus* mortality was high: only 7 to 19% of stems remained from the initial planting, suggesting a single herbicide application was not adequate to prevent hardwoods from outcompeting *P. strobus*. In a previous study, controlling herbaceous and hardwood regeneration for two consecutive years following planting increased *P. strobus* diameter growth by 50% compared to a single year of treatment (Pitt et al. 2009). If regeneration was controlled for three years, *P. strobus* diameter growth was 90% higher than in untreated plots (Pitt et al. 2009). Such control measures, however, are unrealistic on a large scale.

Pinus strobus competed well with hardwoods in one of the mixed-species stands, but suffered suppression and nearly 90% mortality at the other. This discrepancy is likely due to site and stand differences as the relative competitiveness of *P. strobus* compared to other species can

change based on site conditions and stand dynamics (Kobe 1996). *Pinus strobus* may have had more of a competitive edge at variable planting density stand because of the slower growing hardwood species present. Sugar maple, which had the highest RIV in the variable hardwood competition stand, grow more rapidly than red oak, which had the highest hardwood RIV at the variable planting density stand (Johnson 1971). Additionally, the variable density stand was established on well drained, sandy loam soil while the varying hardwood competition stand was established on excessively drained, sandy soil. *Pinus strobus* generally has a competitive advantage over other native species on poor sandy soils but also competes well on sandy loams where the species is more productive (Mader 1985; Wendel and Smith 1990). Alternatively, browse could have contributed to *P. strobus* suppression and mortality at the varying hardwood competition site. White-tailed deer commonly browse on *P. strobus* as a winter food source and have been known to browse seedlings to the ground, causing growth deformities and high rates of mortality (Katovich et al. 2004; Krueger and Puettmann 2004). Managers considering regenerating *P. strobus* in even-aged, mixed-species stands may need to include hardwood suppression treatments for multiple years after planting. In areas with large populations of white-tailed deer, protection from browse may also be necessary. Selection harvests may also be needed 10-15 years after planting to release *P. strobus*, even if they initially compete well with hardwoods.

Conclusion

Pinus strobus is able to grow under a wide range of regeneration conditions, each of which is associated with trade-offs among competition, growth, and pest damage. Our study found that in an open-grown monoculture, a planting density of 2198 TPH produced larger *P. strobus* than a planting density of 2989 TPH without increasing the potential for *P. strobi*

damage. While monocultures are excellent at maximizing tree growth, mixed-species forests may provide other resources that outweigh timber production, such as increased wildlife diversity, reduced risk to specialized pests, and greater recreational value (Huuskonen et al. 2021). Regenerating *P. strobus* with other species, either with a shelterwood or in an even-aged, mixed species stand, will reduce tree growth and *P. strobi* damage, but competition will require management to protect pines and facilitate growth.

Tables

Table 1.1: Location, site variables, and planting methods for stands in Michigan where *Pinus strobus* regeneration was evaluated in 2017 and 2019.

	Monoculture	Shelterwood	Mixed-species stand variable density	Mixed-species stand variable hardwood competition
Year Planted	1998	1999	1998	2000
Location	Delta County 45.7700°N, 87.1902°W	Kalkaska County 44.5126°N, 85.0487°W	Cass County 42.0072°N, 85.9604°W	Kalkaska County 44.8366°N, 85.0640°W
Size (ha)	8	16	4	4
Soil name(s)[1]	Onaway-Ossineke fine sandy loams; Charlevoix sandy loam	Rubicon-Graycalm sands	Oshtemo sandy loam	Kalkaska sand
Drainage level[1]	Moderately well drained; somewhat poorly drained	Somewhat excessively drained	Well drained	Somewhat excessively drained
Slope[1]	0-6%	0-35%	0-2%	0-6%
Mean, min, max annual temperature (°C)[2]	5.8	6.4	9.5	6.4
	0.3	0.8	4.6	0.9
	11.4	12.1	14.3	11.8
Mean annual ppt (mm)[3]	781	871	1017	871
No. seedlings planted	18,500 standard; 5,000 rust resistant	29,000	3,000	8,800
Regeneration strategy	Randomized block design: 4 blocks with 3 densities: 1680, 2198, 2989 trees per ha (TPH)	Varying levels of overstory shade (0-100%); Planted at 2198 TPH	Planted at 2 densities: 526, 1028 TPH after a hardwood clearcut	Planted at 2198 TPH after a hardwood clearcut; Basal sprouts in half the site treated with herbicide in 2002

[1]Web Soil Survey (NRCS 2025)
[2]PRISM Climate Group 2024
[3]PRISM Climate Group 2022

Table 1.2: Initial planting densities (trees per ha) and summary of size (height and diameter at 1.3 m) and bole rating measured in 2017 on *Pinus strobus* planted in 1998 at three densities in an 8.1 ha monoculture (Delta County, MI).

Planting density (TPH)	Tree type[1]	Mean (± SE) DBH[2] (cm)	Max; Min DBH (cm)	Mean (± SE) height[3] (m)	Min; Max height (m)	No. trees[4]	Bole Rating [5] (Percent Ps)		
							0	1	2
Low (1680)		18.2 ± 0.3^{a}	5.3; 27.9	9.3 ± 0.1^{a}	5.1; 12.0	206; 387	32	28	40
Intermediate (2198)		16.7 ± 0.3^{b}	5.6; 25.1	9.5 ± 0.1^{a}	4.0; 12.2	169; 357	40	28	32
High (2989)		15.1 ± 0.3^{c}	6.6; 24.4	9.2 ± 0.1^{a}	5.5; 12.3	190; 369	40	32	28
	Standard	16.6 ± 0.2^{B}	5.3-26.7	9.5 ± 0.2^{A}	5.1; 12.3	299; 552	42	27	31
	Border	17.7 ± 0.4^{A}	5.8; 27.9	9.0 ± 0.2^{B}	4.0; 12.0	127; 283	27	33	40
	Resistant	15.9 ± 0.4^{B}	5.6-24.4	9.4 ± 0.2^{A}	6.0; 12.2	139; 278	37	31	32

[1] Resistant trees refers to seedlings propagated from trees believed to be resistant to white pine blister rust; border refers to trees from standard stock. Border trees were planted on the outer two rows of each plot, adjacent to open space. Standard trees were planted at least two rows from an open space or an adjacent plot.
[2] Values in this column denoted by the same letter are not significantly different at $\alpha = 0.05$.
[3] Values in this column denoted by the same letter are not significantly different at $\alpha = 0.05$.
[4] Number of trees measured and number of trees visually assessed for bole defects.
[5] Bole ratings of 0, 1, and 2 denote trees with no observable defects or a slight crook, trees with one or more moderate crooks, and trees with severe crook(s) and/or fork(s) in basal log (0.5 – 5.4 m), respectively.

Table 1.3: Summary of percent canopy cover by genera and *Pinus strobus* (Ps) size and bole rating by plot in the 16.2 ha oak shelterwood stand (Kalkaska County, MI) surveyed in 2017.

	% Cover								Bole Rating [1] (Percent Ps)		
Plot	Oak	Maple & Aspen	Mean (± SE) DBH (cm)	Max DBH (cm)	Mean (± SE) height (m)	Max height (m)	No. Ps	Ps per ha	0	1	2
1	39	18	5.2 ± 0.3	8.4	5.1 ± 0.2	7.4	32	1146	97	3	0
2	50	17	5.3 ± 0.2	8.1	5.0 ± 0.2	7.6	45	1528	98	2	0
3	55	12	4.7 ± 0.2	8.9	4.6 ± 0.2	7.0	38	1401	97	0	3
4	69	6	5.1 ± 0.2	8.1	4.7 ± 0.2	7.2	44	1592	98	0	2
5	26	52	5.9 ± 0.3	8.4	5.2 ± 0.2	7.2	29	987	72	21	7
6	50	10	5.9 ± 0.3	8.9	5.3 ± 0.2	7.6	35	1337	94	0	6
7	16	36	5.5 ± 0.3	8.4	5.4 ± 0.2	7.1	42	1592	98	2	0
8	65	19	5.2 ± 0.2	7.9	5.2 ± 0.2	7.3	42	1528	98	2	0
9	14	32	5.2 ± 0.2	9.1	4.7 ± 0.1	7.6	45	1560	98	0	2
10	53	25	4.0 ± 0.2	6.4	4.0 ± 0.1	6.0	45	1751	98	0	2
11	39	33	4.8 ± 0.2	8.9	4.6 ± 0.2	7.5	40	1432	90	8	2

[1] Bole ratings of 0, 1, and 2 denote trees with no defects or a slight crook, one or more moderate crooks, and trees with severe crook(s) and/or fork(s) in lower log (0.5 – 5.4 m), respectively.

Table 1.4: Summary of total plot density and *Pinus strobus* (Ps) density, size, and form (bole rating) at the 4.0 ha mixed-species and variable planting density stand (Cass County, MI) in 2017.

	BA (m^2 per ha)		No. Stems		No. Stems per ha					Bole Rating [2] (Percent Ps)		
Plot	Total	Ps	Total	Ps	Total	Ps	Density [1]	Mean DBH ± SE (cm)	Max DBH (cm)	0	1	2
1	29.1	13.9	73	22	2324	700	High	15.5 ± 0.8	22.6	59	23	18
2	21.2	11.1	88	25	2801	796	High	12.7 ± 0.7	19.1	44	40	16
3	17.7	9.4	77	17	2451	541	High	13.5 ± 1.4	23.4	71	12	18
4	27.4	8.6	117	21	3724	668	High	11.6 ± 1.2	19.6	43	24	33
5	19.5	9.9	155	24	4934	764	High	11.2 ± 1.1	21.1	54	21	25
6	19.7	5.1	134	12	4265	382	Low	11.6 ± 1.6	21.1	55	27	18
7	18.3	6.2	113	12	3597	382	Low	13.5 ± 1.4	22.4	67	17	17
8	24.6	6.2	92	11	2928	350	Low	14.3 ± 1.5	19.6	30	20	50

[1] *P. strobus* planting density: high = 1028 trees per ha, low = 526 stems per ha

[2] Bole ratings of 0, 1, and 2 denote trees with no defects or a slight crook, one or more moderate crooks, and trees with severe crook(s) and/or fork(s) in lower log (0.5 – 5.4 m), respectively.

Table 1.5: Number, size, and density of live trees (≥ 10 cm DBH) for *Pinus strobus* and the five co-occurring tree species with the highest relative importance values (RIVs)[1] recorded in a 4 ha mixed-species stand (Cass County, MI) in 2017.

Species	No. plots	No. trees	No. trees per ha	Mean DBH (cm) ± SE	Min; Max DBH (cm)	Basal area (m^2 per ha)	RIV
Pinus strobus	8	100	397.9	15.6 ± 0.3	10.2; 23.4	8.0	175
Quercus rubra	8	98	390.0	15.4 ± 0.3	10.2; 22.9	7.6	173
Liriodendron tulipifera	8	33	131.3	18.9 ± 0.8	12.7; 27.9	3.9	131
Robinia pseudoacacia	4	13	51.7	12.9 ± 0.5	10.2; 15.7	0.7	58
Populus grandidentata	4	7	27.9	16.5 ± 1.5	12.7; 22.9	0.6	56
Prunus serotina	4	7	27.9	13.3 ± 0.8	10.4; 17.8	0.4	55

[1] Relative importance values represent the sum of its relative frequency (percentage of plots in which it is present), dominance (percentage of its BA relative to total BA of all species), and density (percentage of trees relative to the total number of trees tallied), following Kent and Coker (1992).

Table 1.6: Summary of total and hardwood (HW) density and *Pinus strobus* (Ps) density, size, and form at the 4 ha mixed-species with varying hardwood competition stand (Kalkaska, MI) in 2017.

Plot	BA (m^2 per ha)			# Stems		Density (stems per ha)		Mean ± SE DBH (cm)	Mean ± SE Height (m)	Bole Rating[1] (percent Ps)		
	Total	HW	Ps	Total	Ps	Total	Ps			0	1	2
1	7.0	6.3	0.8	116	7	2564	155	10.1 ± 0.9	5.9 ± 0.4	0	57	43
2	14.2	13.6	0.7	96	8	2122	177	8.4 ± 1.9	4.9 ± 0.7	0	0	100
3	6.3	6.3	0.0	102	14	2255	309	4.9 ± 0.6	4.3 ± 0.4	40	40	20
4	8.9	8.4	0.5	99	10	2188	221	7.4 ± 1.6	4.9 ± 0.7	20	60	20
5	7.6	7.6	0.0	123	13	2719	287	4.9 ± 0.6	4.1 ± 0.3	20	80	0
6	3.1	2.7	0.4	106	16	2343	354	6.3 ± 0.9	4.2 ± 0.3	0	50	50
7	3.2	2.8	0.4	88	9	1945	199	9.1 ± 1.0	4.8 ± 0.2	0	50	50
8	5.4	3.4	2.0	81	17	1790	376	9.5 ± 1.0	6.1 ± 0.4	29	21	50
9	10.4	10.2	0.2	156	19	3448	420	5.4 ± 1.2	4.3 ± 0.6	0	43	57

[1] Bole ratings of 0, 1, and 2 denote trees with no defects or a slight crook, one or more moderate crooks, and trees with severe crook(s) and/or fork(s) in lower log (0.5 – 5.4 m), respectively.

Table 1.7: Number, size, and density of live trees (≥ 10 cm DBH for *Pinus strobus* and the five co-occurring tree species with the highest relative importance values (RIVs)[1] recorded in a 4 ha mixed-species stand with varying hardwood competition (Kalkaska, MI) in 2017.

Species	No. plots	No. trees	No. trees per ha	Mean DBH (cm) ± SE	Min; Max DBH (cm)	Basal area (m^2 per ha)	RIV
Pinus strobus	7	19	46	12.1 ± 0.5	10.2; 16.3	0.6	101
Acer saccharum	5	40	98	19.3 ± 1.3	10.2; 38.6	3.4	135
Fagus grandifolia	5	25	61	18.0 ± 1.5	11.2; 42.2	1.8	101
Populus grandidentata	4	25	61	11.9 ± 0.5	10.2; 21.3	0.7	86
Acer rubrum	3	4	9	21.3 ± 6.2	11.7; 39.4	0.4	43
Ulmus americana	3	5	12	17.9 ± 2.2	13.2; 23.9	0.3	42

[1] Relative importance values represent the sum of its relative frequency (percentage of plots in which it is present), dominance (percentage of its BA relative to total BA of all species), and density (percentage of trees relative to the total number of trees tallied), following Kent and Coker (1992).

Figures

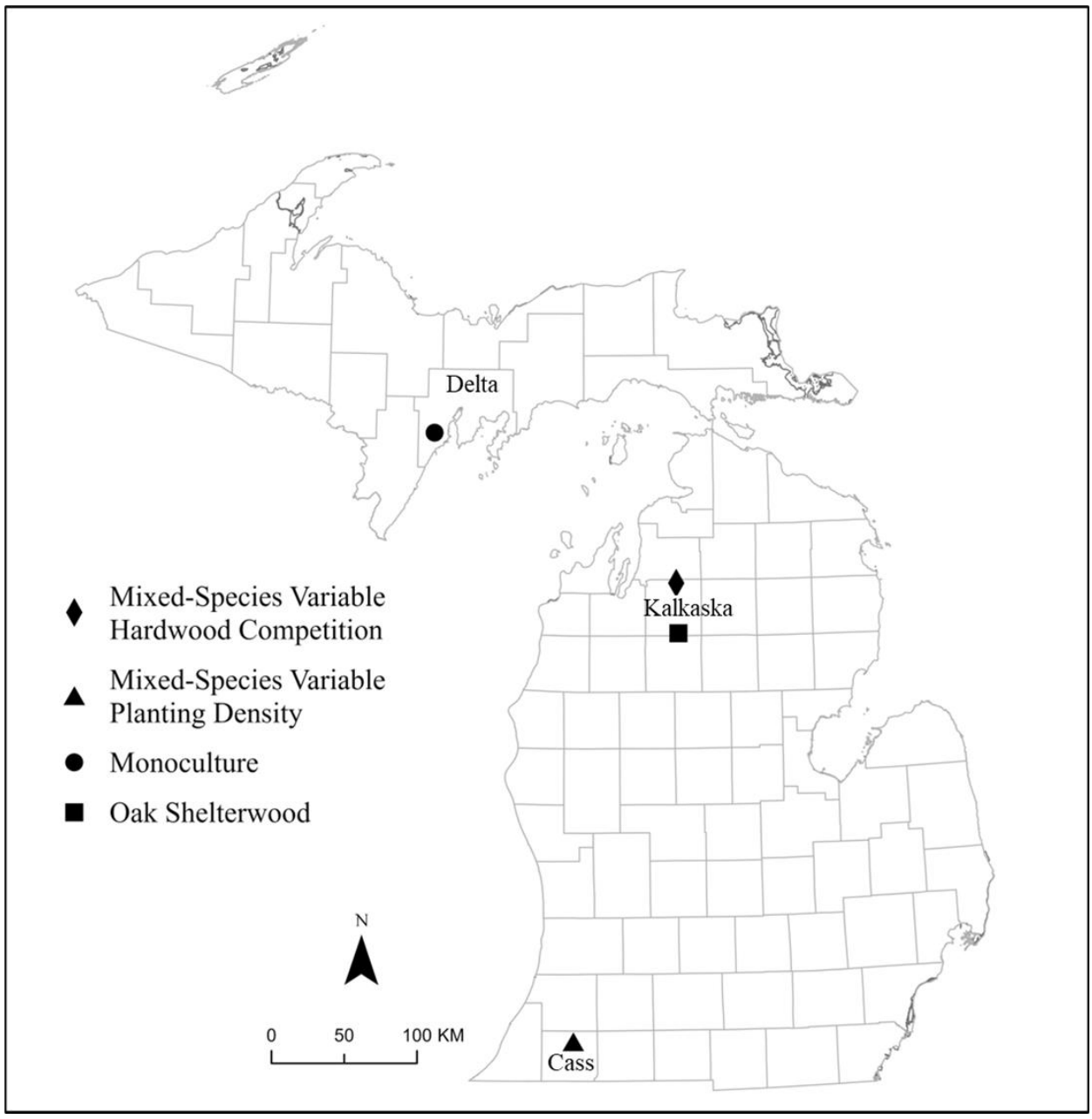

Figure 1.1: Location, including county, of *Pinus strobus* regeneration stands established in 1998-2000 and surveyed in 2017-2019.

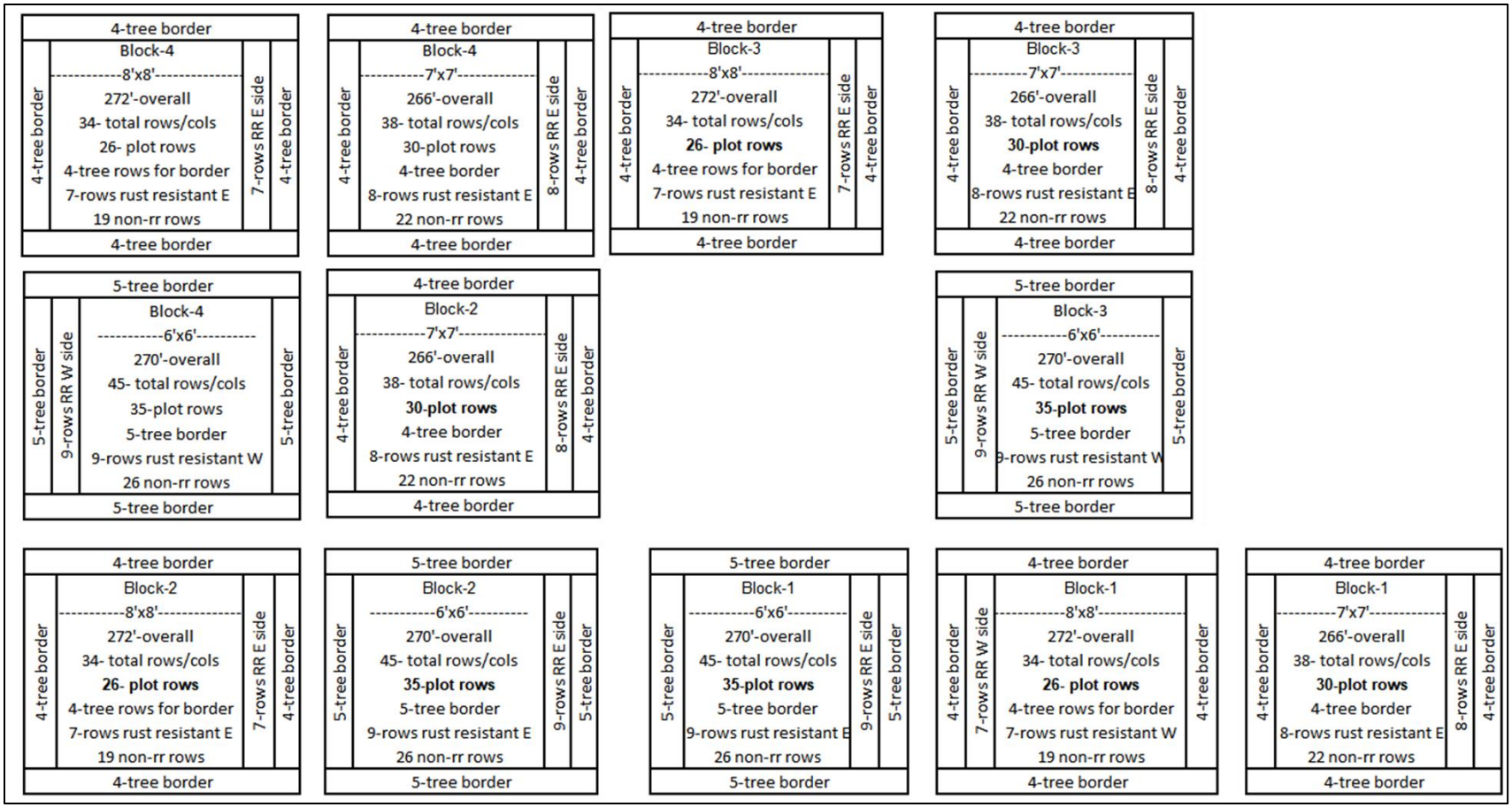

Figure 1.2: Site map rendering of open planted *Pinus strobus* in 1998 in Delta County, MI, adapted from Williams (2002). North faces up, but the map is not drawn to scale (space between plots and blocks varies). In January 2015, all plots were row thinned such that alternate rows of trees were removed. Three planting densities (high, medium, and low) were replicated in four blocks.

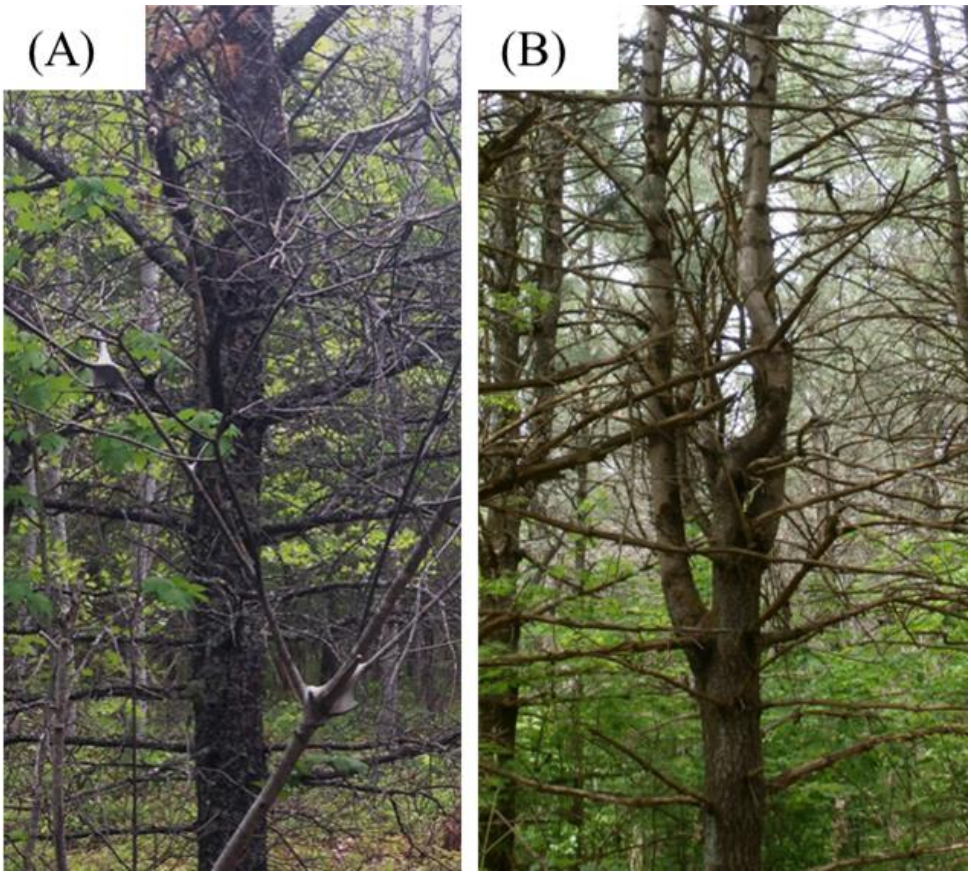

Figure 1.3: Examples of bole defects associated with *Pissodes strobi* injury: crook (A) and fork(s) (B).

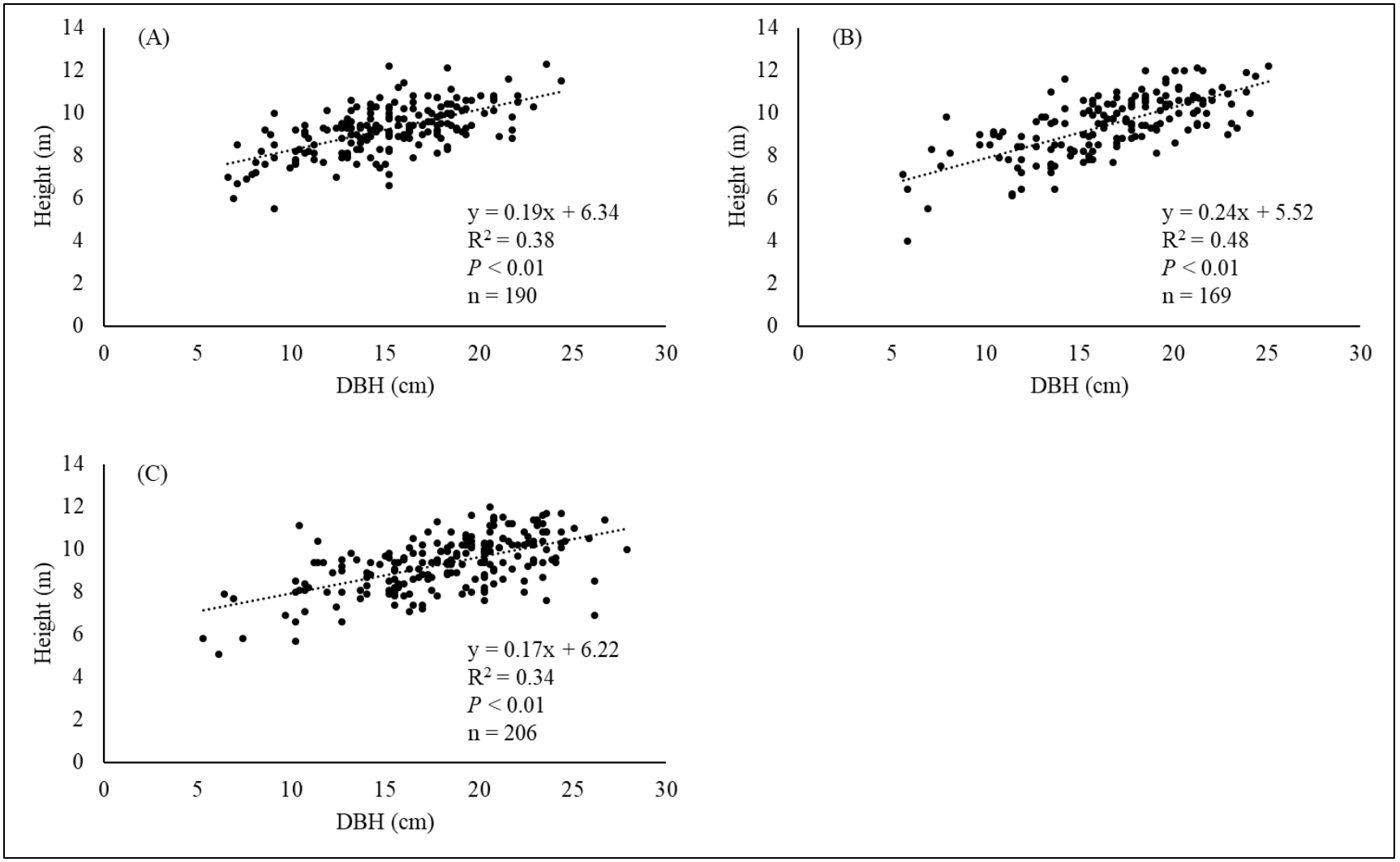

Figure 1.4: Linear regressions for height and DBH of *Pinus strobus* planted in replicated blocks at three densities: 2989 TPH (A), 2198 TPH (B), and 1680 TPH (C).

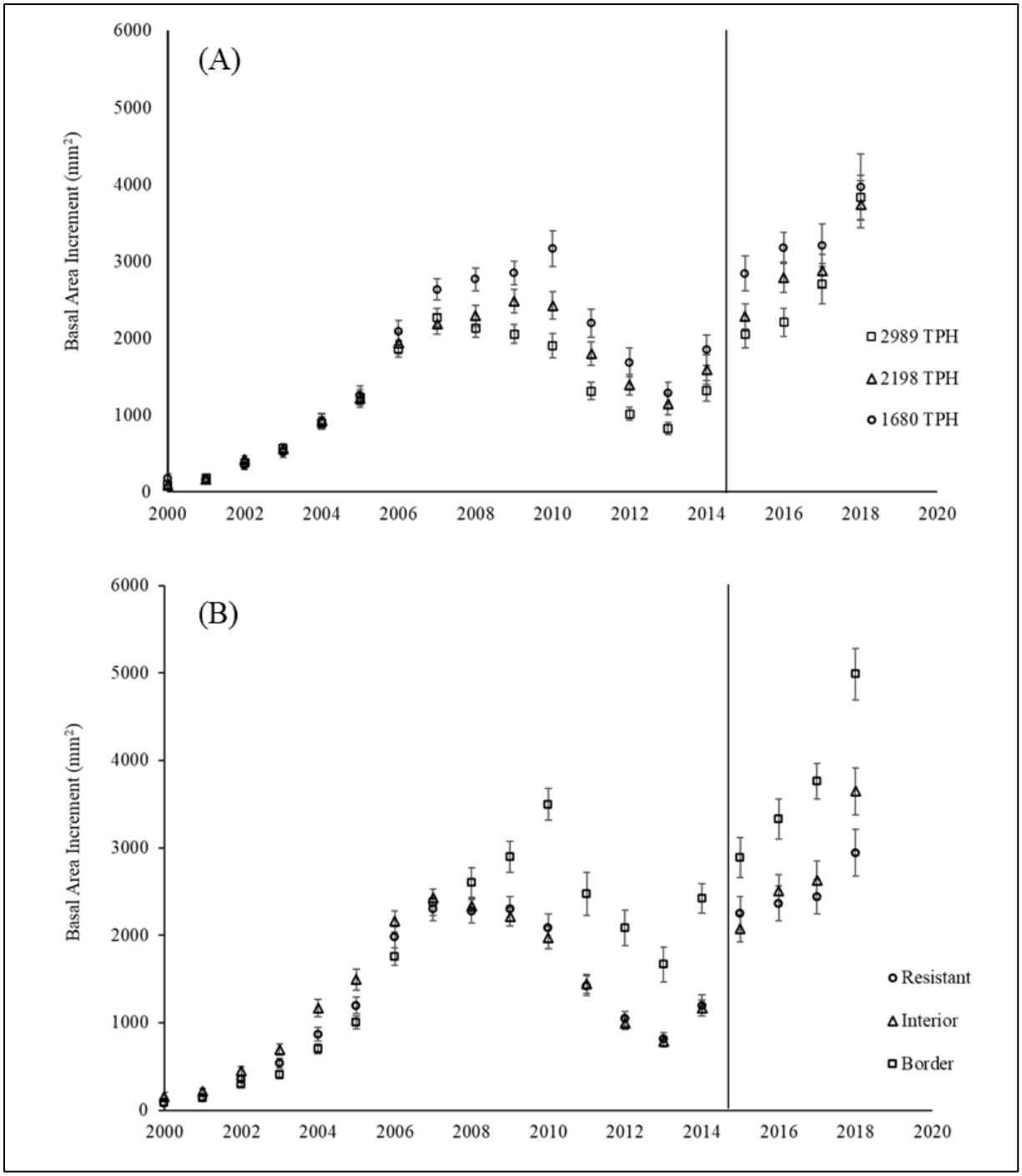

Figure 1.5: Mean (±SE) basal area increment (mm^2) of *Pinus strobus* planted in 1998 at three densities in replicated 1.2 ha blocks (A) and with stock resistant to white pine blister rust, standard stock planted in interior rows and standard stock planted in border rows (B) in a monoculture in Delta County, MI. The vertical lines indicates when all plots were row-thinned in January 2015, which reduced tree densities by half.

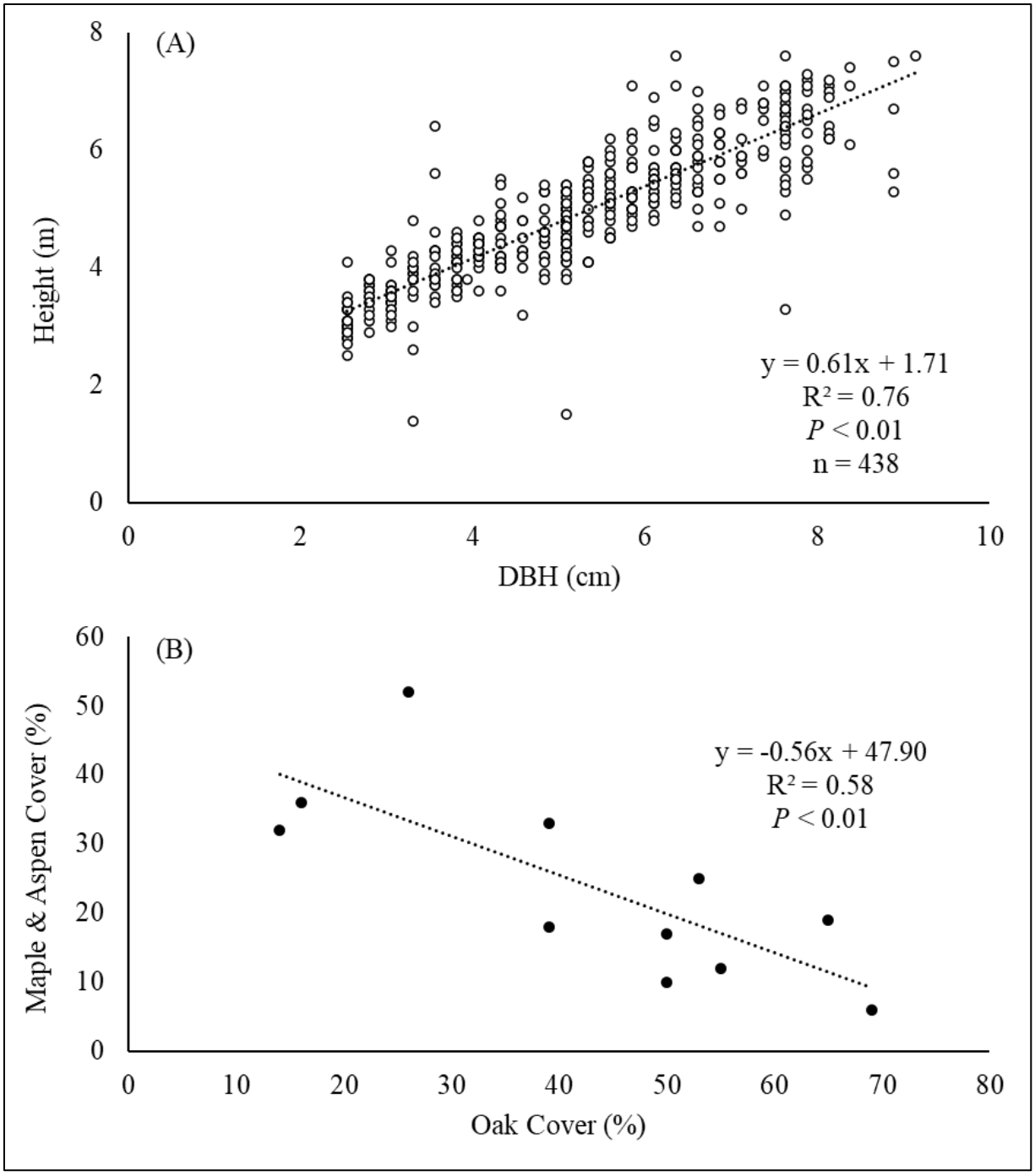

Figure 1.6: Linear regressions of tree DBH on *Pinus strobus* height (A) and percent cover of overstory oaks on percent cover of maple and aspen stump sprouts (B) recorded in 2017 in 11 plots where *Pinus strobus* was planted in 1999 in Kalkaska County, MI following a selection harvest of mature oaks.

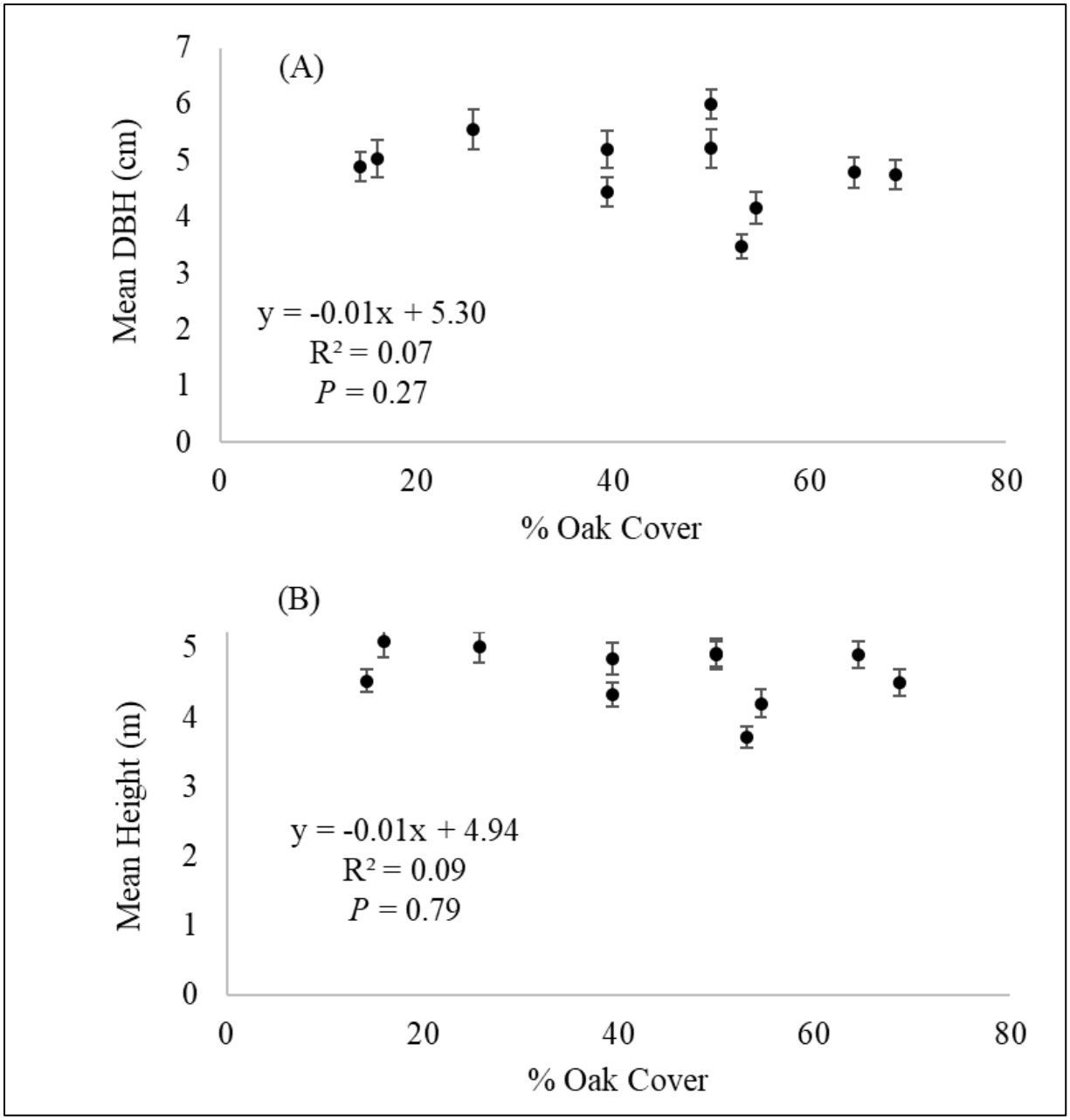

Figure 1.7: Linear regressions of *Pinus strobus* DBH (A) and height (B) on percent cover of overstory oaks recorded in 2017 in 11 plots where *Pinus strobus* was planted in 1999 in Kalkaska County, MI following a selection harves of mature oaks. Error bars denote standard errors.

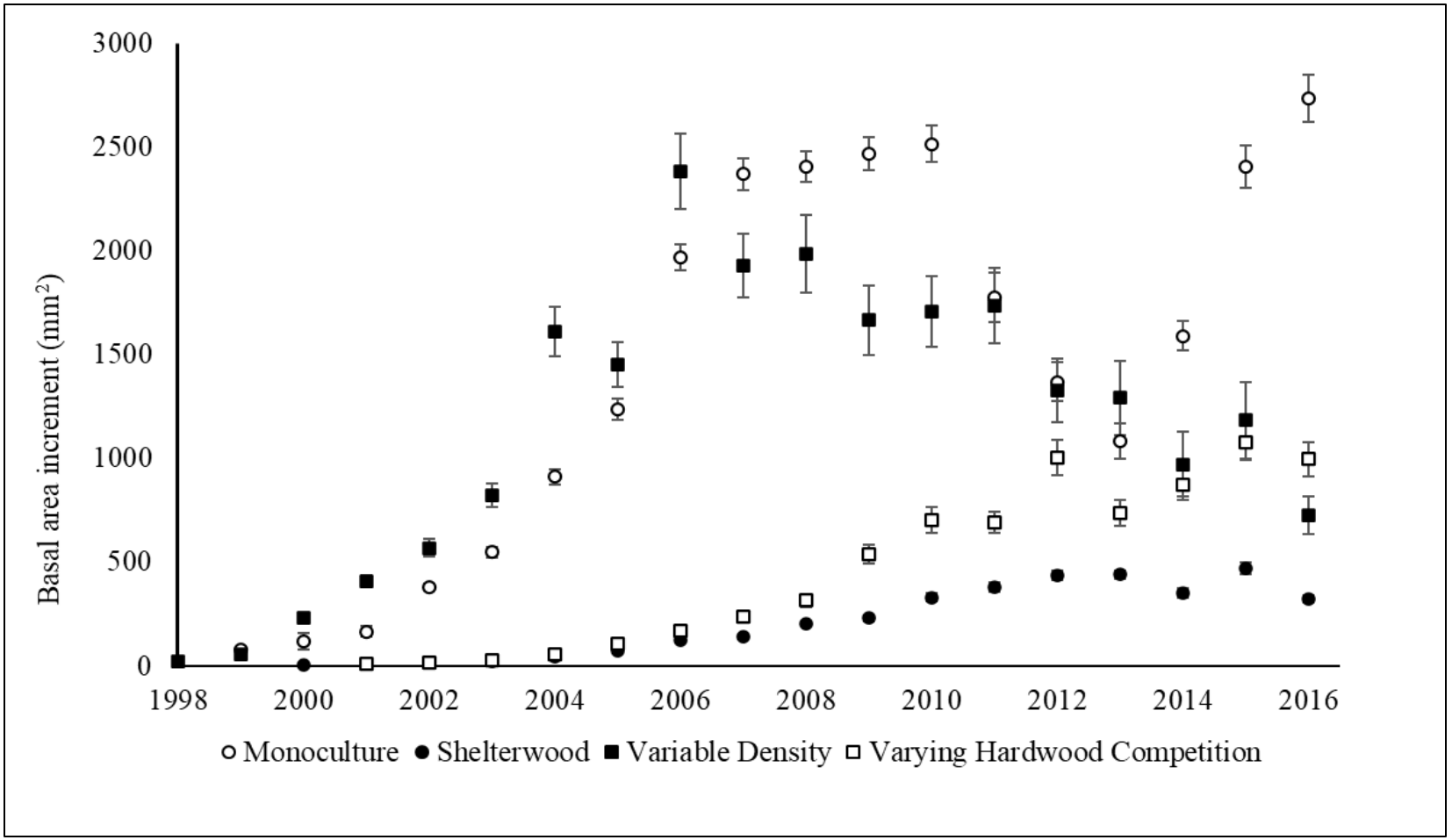

Figure 1.8: Mean annual radial growth rates (basal area increments; mm^2) of *Pinus strobus* in a Monoculture (planted 1998; n = 144 cores); Shelterwood (planted 1999; n = 44 cores); Mixed-species stand with variable planting density (planted 1998; n = 32 cores); and Mixed-species stand with varying hardwood competition (planted 2000; n = 36 cores). Mean values were calculated from all increment cores collected within each site. Error bars denote standard errors and are smaller than marker size where not visible.

CHAPTER 2:

Extent and severity of Caliciopsis canker disease and white pine weevil damage on *Pinus Strobus* L. in Michigan

Introduction

Pinus strobus L. (eastern white pine) is an iconic forest species native to eastern North America (Wendel and Smith 1990). A major component of five forest types, *P. strobus* grows in pure stands and alongside hardwoods or other conifers in mixed stands (Carey 1993). Managing *P. strobus* for timber has historically been hindered by white pine weevil (*Pissodes strobi* Peck, Coleoptera: Curculionidae), a native insect that colonizes and kills the terminal leader of *P. strobus* and other conifers (Wallace and Sullivan 1985). Loss of the terminal leader results in the loss of apical dominance and can lead to bole deformities including crooked stems, forks, and multiple leaders. Damaged *P. strobus* occasionally appear to outgrow minor defects but often still contain bark encased knots and other deformities such as compression wood (Brace 1971). Silvicultural strategies can reduce *P. strobi* caused injury by regenerating *P. strobus* either at high densities to encourage the young trees to quickly recover apical dominance (Ostry et al. 2010; Pubanz et al. 1999) or in partial shade which results in slender terminal leaders that are less likely to be colonized (Stiell and Berry 1985).

Renewed interest in regenerating *P. strobus* in recent decades (Ostry et al. 2010) has coincided with the emergence of new threats. Previously insignificant damage agents are now causing a wide range of symptoms including branch dieback, defoliation, and severe resinosis in *P. strobus* of all age and size classes in New England and the southern Appalachian Mountains (Costanza et al. 2018). Most notable of these is the native fungal pathogen *Caliciopsis pinea* Peck which causes Caliciopsis canker, a disease associated with excessive resin production,

branch and stem cankers, branch dieback, and sapling mortality (Munck et al. 2015).

Recently, anecdotal evidence of dieback and mortality associated with shallow cankers in *P. strobus* regeneration along the AuSable and Manistee Rivers in Michigan began in 2006 (O'Brien 2007). Caliciopsis canker disease was confirmed in an impacted *P. strobus* stand in 2016. Many of the *P. strobus* in the stand had resinosis associated with shallow bole cankers and branch flagging and dieback, but the extent and severity of the disease elsewhere in the state was not understood (Minnix and Sakalidis 2018). Also unknown was whether Caliciopsis canker disease was responsible for all the reported dieback and mortality or if there were other contributing factors.

We surveyed stands throughout northern Michigan for bole defects consistent with *P. strobi* injury and signs and symptoms of Caliciopsis canker disease. This study further aimed to identify site (e.g., soil, slope, and drainage) and stand level factors (e.g., density) associated with *P. strobi* caused bole defects or Caliciopsis canker disease. As *P. strobi* is a native insect with a wide host range and geographic distribution (Belyea and Sullivan 1956), bole defects were hypothesized to be widespread. Defects were also hypothesized to be less likely in higher density stands and sawtimber trees because increased competition can reduce *P. strobi* caused injury (Belyea and Sullivan 1956) and *P. strobus* can superficially outgrow minor defects (Brace 1971). Early reports of *P. strobus* dieback noted young trees were most impacted, especially along two river corridors (MDNR 2008), so Caliciopsis canker was hypothesized to be in isolated areas and most commonly on regeneration (< 12.5 cm DBH). Because previous surveys in other states have found increased Caliciopsis canker incidence and severity in stands with dry soils (Munck et al. 2016), we also hypothesized that the disease would be more commonly found on excessively drained soils.

Methods

Site Selection

Cover type inventory data for Michigan forests were obtained from the Michigan Department of Natural Resources (MDNR) and United States Forest Service (USFS) and mapped using ArcMap v. 10.6 (Esri 2018) (MDNR 2018; USFS 2014; USFS 2015). Cover type data for stands managed by MDNR or USFS were filtered to include only stands greater than 4 ha in size with at least 50% *P. strobus* in the overstory. The survey area included much of the northern Lower Peninsula (LP) and the eastern Upper Peninsula (UP) as few *P. strobus* stands were located in southern Michigan and time and funding constraints prevented us from surveying the UP west of the Hiawatha National Forest. Remaining *P. strobus* stands were then sorted by dominant tree size (sawtimber, pole, or sapling) and then overlaid with a 16.1 x 16.1 km grid. Grid cells containing *P. strobus* were randomly selected for sampling and one stand within each grid cell was systematically chosen. A total of 70 stands, all within 0.8 km from a navigable road (to limit travel time), in 29 counties were surveyed. Four of these stands were dropped from analyses either because of insufficient *P. strobus* stems or recent logging activity. Based on cover type data, the 66 stands surveyed spanned a range of size classes; 41 were classified as sawtimber stands (≥ 25 cm mean DBH), 22 were classified as pole-timber stands (12.5 – 24.9 cm mean DBH), and three were classified as sapling stands (< 12.5 cm mean DBH) (MDNR 2018; USFS 2014; USFS 2015). Additionally, 43 stands (33 in the LP and 10 in the UP) were managed by MDNR and 23 (14 in the LP and 9 in the UP) by the USFS.

Plot and Transect Set-up

Prior to visiting the field, each stand chosen for surveying was mapped in ArcMap and overlaid with a 10 x 10 m grid. To ensure our data were representative of the respective stand,

circular fixed-radius plots were placed in random grid cells so that plots were at least 300 m away from each other and 25 m from plot edges. The number of plots depended on the size of the stand: three plots were established for stands up to 16 ha in size. Another plot was installed for every additional 4 ha in area up to a maximum of eight plots. For each stand, an additional back-up plot location was selected but was only surveyed if another plot was deemed unsuitable (ex. underwater or lacking at least five *P. strobus*). Plots were circular and typically had a 10 m radius. We aimed to survey five to ten *P. strobus* per plot, so this radius was occasionally adjusted to 5 m or 15 m depending on *P. strobus* density. In addition to circular plots, 2 m wide linear transects were established extending 25, 50, 75, or 100 m from one plot edge in the direction of another plot and along stand borders (25, 50, 75, or 100 x 2 m). Interior transects began outside a plot boundary and ran in the direction to the next plot, so the number of interior transects was one less than the number of fixed-radius plots. Border transects (one transect for each of two different stand edges) were centered at 3 m from the edge and preferentially placed on edges that were along roads. Both types of transects were surveyed in 25 m increments until at least five *P. strobus* (≥ 12.5 cm DBH) were recorded or until the transect reached 100 m long.

Data collection

Cover type data for MDNR specified whether stands had been planted, naturally regenerated, or if the regeneration method was unknown. Data for USFS stands, however, did not specify regeneration method. Regeneration method for USFS stands were recorded as planted if the *P. strobus* were aligned in rows and recorded as unknown if rows were not obvious. To capture plot density and tree light availability, variables collected in each plot and transect for trees ≥ 12.5 cm DBH included: species, DBH, alive or dead, and crown class. Crown class was defined as suppressed when trees had no direct light exposure from above or the sides,

intermediate for trees with light exposure from the sides but not above, and dominant for trees with light exposure from above. Live and dead trees < 12.5 cm DBH were tallied by species. Tallies were typically done at the whole plot level, but occasionally a sub-plot was created when regeneration density was particularly high.

Additionally, *P. strobus* within fixed radius plots and transects were surveyed for bole defects or direct evidence of *P. strobi* injury and for signs and symptoms of Caliciopsis canker disease. Any injury that kills the top leader, whether from browse or frost damage, can lead to bole defects, so we were unable to distinguish between causal agents. We recorded bole defects resembling *P. strobi* caused injuries (i.e., crooks, forks, and stag-head or cabbage forms; Figure 1.3) on the lowest (basal) log (0.6-3.6 m) for all *P. strobus* ≥ 12.5 cm DBH.

To evaluate Caliciopsis canker disease, we recorded the presence of signs (i.e., *Caliciopsis* spp. ascocarps) and symptoms: percent needles flagged; number of dead branches retaining dead needles; and resinosis that did not appear to be associated with wounds, insect boring, white pine blister rust, or Armillaria root rot (Figure 2.1). Resin streaks were counted according to the methods of Munck et al. (2015): on one aspect of each *P. strobus*, we visually divided the tree bole into thirds (lower, middle, and upper) and recorded the number of resin streaks in each third up to ten resin streaks per section. We then repeated this assessment on the opposite aspect of the tree. *Pinus strobus* < 12.5 cm DBH were typically tallied using the same methods as described above. If a plot contained signs or symptoms of Caliciopsis canker disease, however, all *P. strobus* ≥ 2.5 cm DBH in that plot were evaluated for disease. Resinosis and dieback can be caused by multiple damage agents including insects and other diseases. Symptoms were only considered to be associated with Caliciopsis canker disease if ascocarps were found on at least one surveyed *P. strobus* in the stand.

Soil and slope data: To test if soil type, drainage level, or slope affected the likelihood of Caliciopsis presence, spatial data for slope and soil data (i.e., classification and drainage level) were downloaded from the Soil Survey Geographic (SSURGO) Database and overlaid on shapefiles of the surveyed stands (NRCS 2020). Slopes for plots and transects were classified as low (< 12%), moderate (12 to 18%), high (> 18%), or NA if a transect crossed over different slope classifications (Table 2.1). Soil classes from were also condensed and plots and transects were classified as Sandy for the soil classes sand, sandy loam, or sandy peat; Loamy for the soil classes loam, loamy fine sand, and loamy sand; Organic; or NA if a transect crossed over polygons for different soil classes. Drainage codes range from 1 for subaqueous to 8 for excessively well drained. These drainage codes were condensed and plots and transects were classified as low drainage for codes 1-3, moderate drainage for codes 4-6, high drainage for codes 7-8, or NA is a transect crossed over polygons for different drainage classifications.

Data analysis: Prior to analyses, we calculated the following density factors from measurements of live trees > 12.5 cm DBH: basal area (BA), *P. strobus* basal area (BA_PIST), trees per ha (TPH), and *P. strobus* trees per ha (TPH_PIST). Tallies of live trees of all sizes were used to calculate stems per ha (SPH) and *P. strobus* stems per ha (SPH_PIST). To standardize orders of magnitude, all density data were scaled to a mean of zero prior to analyses.

All statistical analyses were run in R v. 4.4.2 (The R Foundation for Statistical Computing, Vienna, Austria, 2024) using generalized linear mixed models (GLMM) fit by maximum likelihood with a binomial distribution. Data for *P. strobi* injury were analyzed using a GLMM with presence of bole defects as the response variable and land management agency (MDNR or USFS), crown class, size class, BA, TPH, and SPH as predictors and stands and plots nested in stands as random factors. Analyses for bole defects only included *P. strobus* that were

≥ 12.5 cm. Data for bole defects were analyzed at the tree level because we were able to identify all the bole defects from the ground. Data for Caliciopsis canker disease were analyzed using a GLMM with presence of *Caliciopsis* spp. ascocarps as the response variable and land management agency (MDNR or USFS), BA_PIST, TPH_PIST, SPH_PIST, soil type, slope, and drainage level as predictors and stand as a random factor. Data for Caliciopsis canker symptoms were analyzed using a GLMM with presence of symptoms (i.e., flagging, resinosis, or dieback) as the response variable and the same predictors and random factor as the GLMM for ascocarps. Data for Caliciopsis canker disease signs and symptoms were analyzed on the plot or transect level because the ascocarps were more cryptic and could only be detected around eye level. Plots were only considered symptomatic if ascocarps were found within the stand, even if they were not found in the plot, and at least one *P. strobus* had symptoms of Caliciopsis canker disease.

Total basal area and *P. strobus* basal area were highly correlated (0.91), so only one set of density values was used for each analysis. Total density values were used for analysis of bole defects because competition from other species has previously been shown to reduce *P. strobi* injury (Chapter 1). Density of *P. strobus*, however, could affect inoculum levels for Caliciopsis canker disease, so *P. strobus* density values were used in the ascocarps and symptom analyses (Munck et al. 2016). Crown class and size class were not analyzed in the ascocarps and symptom analyses because these tree level values did not scale up to the plot level.

Results

Caliciopsis Canker Disease

Caliciopsis canker disease was widespread throughout the survey area with *Caliciopsis* spp. ascocarps present in 66% of surveyed counties and 47% of the surveyed stands (Figure 2.2, Table 2.2; Chapter 2 Supplement). Ascocarps were often sparsely distributed throughout the

stands. Where present, their frequency ranged from 1 to 61% of *P. strobus* stems (mean = 15%) per stand. In 11 of the stands with Caliciopsis canker, ascocarps were observed on a single *P. strobus* stem. Symptoms associated with Caliciopsis canker were observed more frequently than ascocarps. In stands where ascocarps were present, the frequency of symptoms ranged from 0 to 61% of *P. strobus* stems (mean = 26%) per stand. Caliciopsis canker ascocarps were observed in 20% of surveyed plots while associated symptoms were observed in 28% of surveyed plots. Of the 4874 evaluated *P. strobus* stems, 7% had Caliciopsis canker ascocarps and 13% had symptoms associated with the disease. Caliciopsis canker disease was found most frequently on regenerating *P. strobus* < 12.5 cm DBH. Of the 335 *P. strobus* with ascocarps, 4% were sawtimber, 28% were pole-sized, and 68% were saplings (< 12.5 cm DBH). Of the 619 symptomatic *P. strobus*, 8% were sawtimber, 28% were pole-sized, and 64% were saplings. While symptoms were observed more frequently than signs (i.e., ascocarps), severe symptoms were rare. Six percent of stems with ascocarps were asymptomatic. Of the stems that were symptomatic, with or without ascocarps, 5% had at least one dead branch, 33% had five or more resin streaks and only 5% had significant levels of flagging (> 10 % of the live crown).

Caliciopsis canker ascocarps and associated symptoms were more likely to be present in plots with lower *P. strobus* basal area (Table 2.3). Neither land management agency (USFS or MDNR), soil class, slope, drainage, TPH_PIST, nor SPH_PIST were predicative of the presence of *Caliciopsis* spp. ascocarps or symptoms.

Bole Defects

Defects consistent with *P. strobi* injury affected one or more *P. strobus* bole in 93% of surveyed counties, 85% of surveyed stands (Table 2.2; Chapter 2 Supplement), 40% of plots, and were observed on 11% of *P. strobus* surveyed. Crooks were slightly more common (6% of *P.*

strobus evaluated) than forks and other more serious defects (5% of *P. strobus* evaluated). Where bole defects were present, affected *P. strobus* ranged from 1 to 9% of trees (mean = 4%) per stand, and 0 to 100% of trees (mean = 14%) per plot. In 50% of the affected plots, bole defects occurred on less than 25% of *P. strobus*. In 7% of affected plots, more than 75% of *P. strobus* had at least one bole defect.

Bole defects were as likely to occur on MDNR managed land as USFS managed land. Tree level variables were predictive of bole defects while stand density variables were not (Table 2.3). Neither BA, TPH, nor SPH predicted the likelihood of bole defects while size class and crown class did. Sawtimber trees were more likely to have bole defects than pole-sized trees (Z =-3.29, $P < 0.01$) and trees classed as suppressed or intermediate were more likely to have bole defects than those that were dominant ($Z = 3.36$, $P < 0.01$ and $Z = 3.52$, $P < 0.01$, respectively). Trees with a suppressed crown position were not more likely to have bole defects than those that were intermediate ($Z = 1.11$, $P = 0.80$).

Discussion

To our knowledge, this is the first survey of the distribution of *Caliciopsis pinea* and the severity of Caliciopsis canker disease in Michigan. Caliciopsis canker was widespread throughout Michigan but caused notable damage in only a few stands. Regeneration was affected most frequently, and the disease appears to be acting to "thin from below" by infecting relatively small or suppressed *P. strobus*. Ascocarps were only found on 15 sawtimber trees and few of the larger trees were symptomatic. These results are, in part, supported by two studies from New England and one from the southern Appalachian Mountains that reported pole-sized *P. strobus* were more likely to have Caliciopsis canker symptoms than mature, sawtimber trees (Munck et al. 2016; Munck et al. 2015; Schulz et al. 2018a). As we were only able to look for ascocarps at

eye level, *Caliciopsis* spp. incidence could be underreported here but symptoms were easily identified across the entirety of the tree. Symptoms may have been overreported as insect damage and other diseases can also cause branch flagging and resinosis.

We did not identify specific site level factors consistently associated with Caliciopsis canker disease, but we did find signs and symptoms of Caliciopsis canker were more likely to occur in plots with lower densities of *P. strobus*. This result was surprising as a previous study in New England reported Caliciopsis canker disease was more likely to occur in stands with greater *P. strobus* TPH (Munck et al. 2015). That study reported a mean density of 311 TPH_PIST for stands with Caliciopsis canker and a mean density of 220 TPH_PIST for stands without Caliciopsis canker (Munck et al. 2015). The *P. strobus* densities recorded for our stands were higher: mean TPH_PIST was 354 for plots with *Caliciopsis* spp. ascocarps and 542 for plots without *Caliciopsis* spp. ascocarps (Table 2.2). Caliciopsis canker disease is more prevalent in New England, however, as symptoms were noted in 80% of 62 surveyed *P. strobus* stands (Munck et al. 2016). Caliciopsis canker also affected a larger proportion of pole-sized and sawtimber *P. strobus* in New England; 20% of surveyed *P. strobus* in sawtimber stands and 66% in pole-timber stands were symptomatic (Munck et al. 2016). In contrast, only 6% of pole-sized and sawtimber *P. strobus* surveyed in Michigan were symptomatic. Lower BA_PIST may be associated with a higher likelihood of Caliciopsis canker in Michigan because lower densities are more conducive for saplings, which were more frequently affected by the disease in this study. A previous study on natural *P. strobus* seed production noted seedfall was 36% lower in a stand with 42.9 m^2/ha BA than a stand of 27.6 m^2/ha BA (Graber 1970). This suggests the plots without signs or symptoms of Caliciopsis canker (mean BA of 47.9 – 49.8 m^2/ha) produced fewer seeds and thus fewer saplings than the plots with Caliciopsis canker signs or symptoms

(mean BA of 32.9 – 33.7 m^2/ha). Higher density stands could also impede *P. strobus* seedling development by obstructing sunlight (Wendel and Smith 1990). Plots with Caliciopsis canker may have been more hospitable to saplings as they less frequently (63%) had a BA above 22.9 m^2/ha, a density associated with increased *P. strobus* sapling suppression and mortality, than plots without Caliciopsis canker (85%).

Munck et al. (2016) also found that Caliciopsis canker symptoms were less likely to occur on trees in stands on loamy soils than dry or shallow soils. We did not, however, find any associations between Caliciopsis canker disease and soil type, drainage, or slope. The majority of our surveyed stands were on low slopes with sandy, excessively drained soil. The sample size of other soil types was likely too small and variable to detect any differences.

Bole defects were more widespread across the landscape but only affected a small percentage of the *P. strobus* we surveyed. All bole defects resembling *P. strobi* injury were analyzed in this study, but some of the defects may have been caused by browse or frost injury. Previous studies have found that increased stand density reduces incidence of *P. strobi* caused bole defects (Katovich and Morse 1992; Pubanz et al. 1999).. In this study, however, neither total basal area, trees per hectare, nor stems per hectare were associated with the likelihood of bole damage. These results differ from those of a Wisconsin study of 17 *P. strobus* plantings with 30 to 150-year-old trees that reported the incidence of weevil injury was inversely related to total stem density (Pubanz et al. 1999). Pubanz et al. (1999) also noted bole defects in more than 80% of the *P. strobus* trees surveyed while we observed defects in only 11% of *P. strobus* trees surveyed. The low rate of injury explains why we did not find significant density factors associated with the likelihood of bole defects. Our survey included stands that were planted and stands that were naturally regenerated. The wide range of site and stand factors may have

introduced additional variables that may have muted the effects of stand density on bole defect incidence.

Bole defects were more likely to occur in sawtimber *P. strobus* than those that were pole-sized, a surprising result as larger trees are more likely to have overgrown past defects (Brace 1971). In our survey, pole-sized trees were more likely than sawtimber *P. strobus* to have intermediate or suppressed crown positions. Bole defects were also more likely to occur in intermediate or suppressed trees than dominant trees. This result was unexpected as dominant and co-dominant trees have access to more light and are more likely to produce larger terminal leaders that would presumably be attractive to *P. strobi* (Sullivan 1961).

While Caliciopsis canker disease and white pine weevil were widely distributed across northern Michigan, only a small proportion of the *P. strobus* we surveyed were impacted. Low levels of weevil damage in the basal log could be indicative of forest management success. Low levels of Caliciopsis canker disease, however, may still represent early warning signs of an emerging disease. This is especially true for the few stands that had high percentages of symptomatic *P. strobus*. *Caliciopsis pinea* has been described as "not uncommon" in eastern forests and is not an introduced species (McCormack 1936; Ray 1936) but reports of declining *P. strobus* and regeneration mortality are relatively recent, suggesting environmental conditions have become more conducive to disease (O'Brien 2007). Emerging diseases caused by native pathogens, such as Caliciopsis canker, are on the rise globally due to changing climatic conditions and are expected to cause an unprecedented increase of epidemics (Burgess et al. 2022). Currently, Caliciopsis canker disease is causing more damage in New England than it is in Michigan (Munck et al. 2015). Based on our surveys, Caliciopsis canker appears to be acting as a "thin from below" agent in Michigan where the disease primarily affects saplings. Regional

differences in disease impacts may reflect an earlier outbreak of Caliciopsis canker, first recorded as causing meaningful damage in New Hampshire in 1997 (Lombard 2003), which has allowed the disease to spread and intensify in New England longer than it has in Michigan. Alternatively, differences in disease activity could reflect regional differences in fire history, forest condition (including management and regeneration practices), or climatic variables. Monitoring Caliciopsis canker over time in Michigan and perhaps other Lake States will inform managers and provide accurate estimates of the potential impacts of this disease in Michigan forests.

Tables

Table *2.*1: Description and frequency of soil class, slope, and drainage values modified from Soils Survey Geographic Database (SSURGO) data (NRCS 2020).

Parameter	Classification	Description	Plot Frequency
Soil Class	Sand	Soils classified as sand, sandy loam, or sandy peat	88%
	Loam	Soils classified as loam, loamy fine sand, or loamy sand	10%
	Organic	Soils classified as organic	2%
Slope	Low	Maximum slope is up to 12%	81%
	Moderate	Maximum slope is 13 - 18%	14%
	High	Maximum slope is > 18%	5%
Drainage	Low	Soils classified as subaqueous, very poorly drained, or poorly drained	8%
	Moderate	Soils classified as somewhat poorly drained, moderately well drained, or well drained	24%
	High	Soils classified as somewhat excessively drained or excessively drained	68%

Table 2.2: Percent of counties and stands in the survey area where bole defects, *Caliciopsis* spp. ascocarps, and Caliciopsis canker disease symptoms (i.e., resinosis, branch flagging, branch dieback) were observed or not observed. The stands are also broken down by each management agency, dominant size class (sawtimber: ≥ 25 cm mean DBH, pole: 12.5 – 24.9 cm mean DBH, sapling: < 12.5 cm mean DBH), and stand type. Means and standard errors are listed for the following plot density variables: total basal area (BA), *Pinus strobus* basal area (BA_PIST), total trees per ha (TPH), *P. strobus* trees per ha (TPH_PIST), total stems per ha (SPH), and *P. strobus* stems per ha (SPH_PIST).

		Bole Defects		Caliciopsis Ascocarps		Caliciopsis Symptoms	
		Observed	Not Observed	Observed	Not Observed	Observed	Not Observed
Counties (n = 29)		93%	7%	66%	34%	66%	34%
Stands	All (n = 66)	83%	17%	47%	53%	47%	53%
Agency	MDNR (n = 43)	88%	12%	51%	49%	51%	49%
	USFS (n = 23)	74%	26%	39%	61%	39%	61%
Size Class	Sawtimber (n = 41)	78%	22%	44%	56%	44%	56%
	Pole (n = 22)	91%	9%	50%	50%	50%	50%
	Sapling (n = 3)	100%	0%	67%	33%	67%	33%
Stand Type	Planted (n = 14)	86%	14%	21%	79%	21%	79%
	Natural (n = 26)	85%	15%	54%	46%	54%	46%
	Unknown[1] (n = 26)	81%	19%	54%	46%	54%	46%
Plot Density Variables (Mean ± SE)	BA	45.5 ± 2.0	44.6 ± 1.6	32.9 ± 2.0	47.9 ± 1.5	33.7 ± 1.7	49.8 ± 1.6
	BA_PIST	36.3 ± 2.0	33.6 ± 1.6	22.9 ± 1.8	37.5 ± 1.4	22.6 ± 1.5	39.7 ± 1.6
	TPH[2]	752 ± 27	668 ± 21	554 ± 26	737 ± 19	573 ± 22	756 ± 21
	TPH_PIST	569 ± 25	465 ± 19	354 ± 22	542 ± 18	368 ± 19	563 ± 19
	SPH[3]	3414 ± 201	3844 ± 236	3271 ± 240	3787 ± 196	3109 ± 177	3931 ± 221
	SPH_PIST	1046 ± 71	1071 ± 131	1005 ± 79	1075 ± 104	999 ± 64	1087 ± 118
	Plots (n)	201	315	104	413	156	361

[1]Cover type data did not specify if the stand was planted or naturally regenerated and *P. strobus* were not aligned in rows.
[2]Trees per ha only include stems ≥ 12.5 cm DBH.
[3]Stems per ha includes woody stems of any size.

Table 2.3: Type III test for fixed-effect model results for the likelihood for presence of the following: 1) bole defects associated with *Pissodes strobi* damage, 2) signs (i.e., ascocarps) of Caliciopsis canker disease, and 3) symptoms (i.e., resinosis, flagging, branch dieback) of Caliciopsis canker disease. Bold *P*-values denote significant predictors ($P < 0.05$).

Observation	Fixed-Effect	X^2-test	*df*	*P*-Value
Bole Defects	Basal Area	0.6839	1, 3741	0.4082
	Trees per ha	1.6756	1, 3741	0.1955
	Stems per ha	0.7430	1, 3741	0.3887
	Size Class	10.8355	1, 3741	**0.0010**
	Crown Class	16.4944	2, 3740	**0.0003**
	Land Management Agency	0.0000	1, 3741	0.9972
Caliciopsis Ascocarps	*Pinus strobus* Basal Area	5.4874	1, 477	**0.0192**
	Pinus strobus Trees per ha	0.8192	1, 477	0.3654
	Pinus strobus Stems per ha	1.2747	1, 477	0.2589
	Soil Class	0.8970	2, 476	0.6386
	Slope	1.2054	2, 476	0.5473
	Drainage	0.8308	2, 476	0.6601
	Land Management Agency	0.5088	1, 477	0.4757
Caliciopsis Symptoms	*Pinus strobus* Basal Area	8.6914	1, 477	**0.0032**
	Pinus strobus Trees per ha	2.7952	1, 477	0.0945
	Pinus strobus Stems per ha	3.0889	1, 477	0.0788
	Soil Class	3.4911	2, 476	0.1745
	Slope	0.3022	2, 476	0.8598
	Drainage	0.0621	2, 476	0.9694
	Land Management Agency	0.5000	1, 477	0.4795

Figures

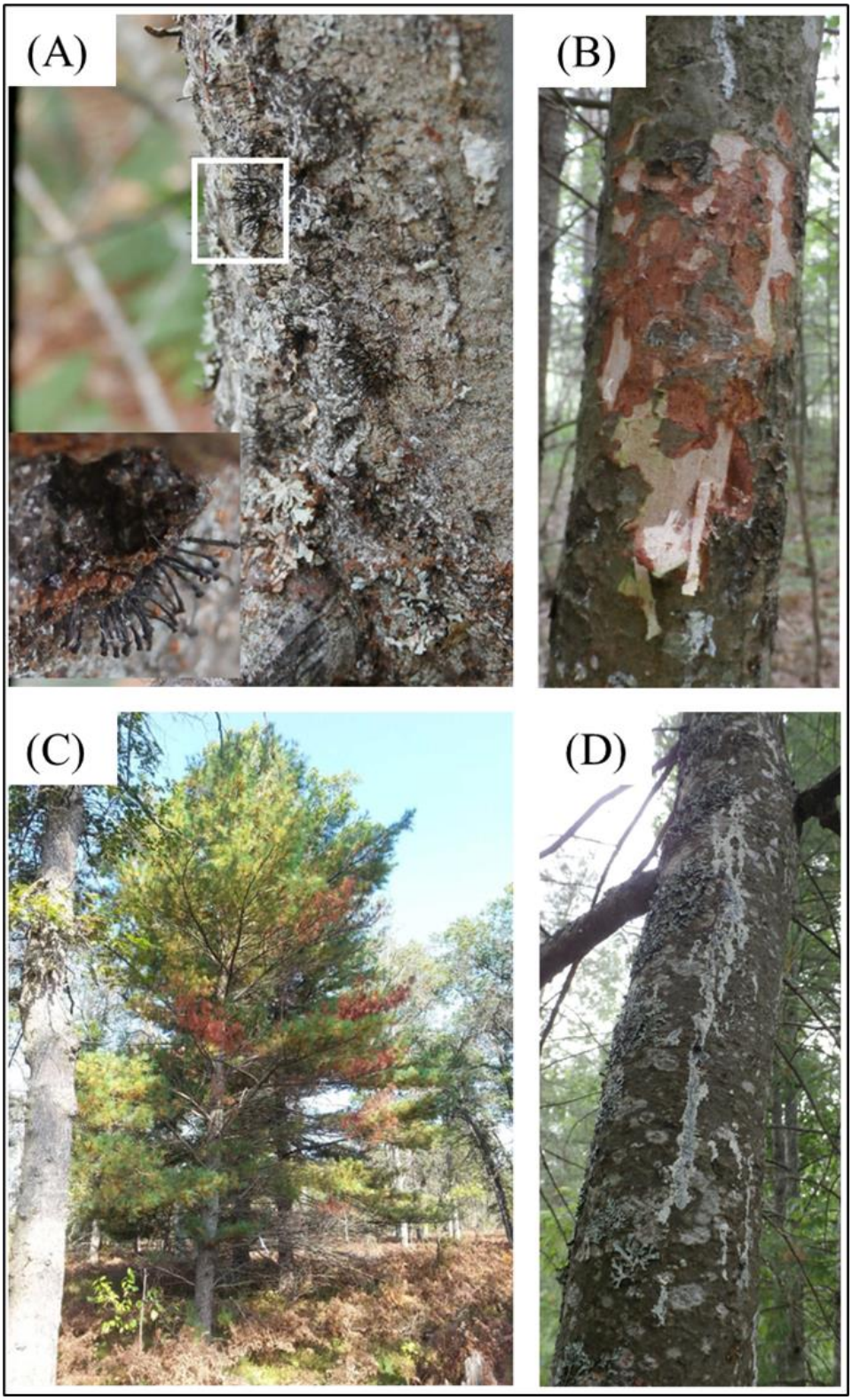

Figure 2.1: Examples of signs and symptoms of Caliciopsis canker disease: ascocarps (A), reddish, shallow cankers (B), branch flagging (C), and resinosis (D).

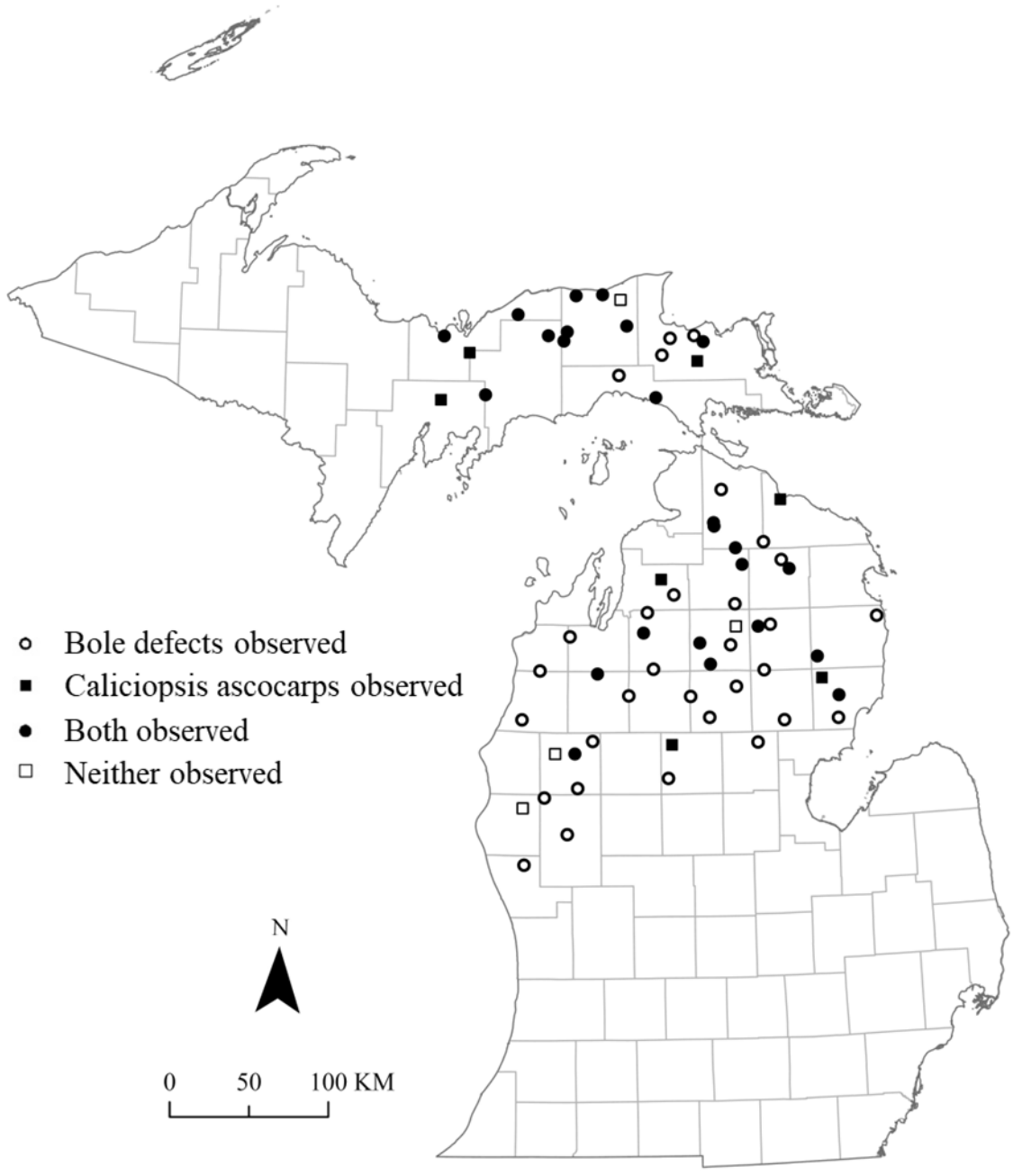

Figure 2.2: *Pinus strobus* stands in Michigan surveyed in 2018-2019 for bole defects, presumably caused by *Pissodes strobi*, and Caliciopsis canker disease.

CHAPTER 3:

Distribution and pathogenicity of *Caliciopsis* species on *Pinus strobus* L. in Michigan

Introduction

Forests across the globe are currently experiencing unprecedented disturbances relating to changing conditions including climate, land-use, and introduction of invasive pests (Ramsfield et al. 2016). These changes have resulted in more frequent and severe droughts, wildfires, and insect outbreaks (Ramsfield et al. 2016). Changing environmental conditions have also been contributed to emerging diseases caused by native pathogens that have historically caused negligible damage but are now increasing in severity across host ranges (Burgess et al. 2022). Caliciopsis canker, first reported in 1880, is an example of an emerging disease caused by a native pest that has increased in severity over the last 30 years and is now causing widespread damage (Peck 1880; Costanza et al. 2018).

Caliciopsis canker disease is caused by pathogenic *Caliciopsis* species, members of the globally distributed genus *Caliciopsis* (Coryneliaceae) that contains saprophytic, biotrophic, and pathogenic species. *Caliciopsis* species primarily colonize woody tissue but have also been found on oak galls and have been used as biocontrol agents of dwarf mistletoe (Garrido-Benavent and Pérez-Ortega 2015; Ramsfield et al. 2009; Sierra and Cifuentes 1998). Several *Caliciopsis* species are known to cause cankers on conifers. For example, *Caliciopsis orientalis* A. Funk forms cankers on eastern hemlock (*Tsuga canadensis* L.) and *Caliciopsis pseudotsuga* Fitzp. forms cankers on Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and grand fir (*Abies grandis* (Dougl.) Lindl.) (Funk 1963). Additionally, *Caliciopsis moriondi* N. Luchi causes cankers and resin production on *Pinus nigra* J.F. Arnold, *P. radiata* D. Don, and *P. resinosa* Aiton in Europe and North America (Migliorini

et al. 2020). *Caliciopsis pinea* Peck has similarly been found on several species of conifers in North America including pine species such *Pinus strobus* L., *P. nigra,* and *P. monticola* Dougl. ex D. Don, as well as some firs (*Abies* spp.) (Luchi et al. 2018).

Caliciopsis canker disease caused by the native pathogen *C. pinea* is characterized by two types of perennial cankers. The most common are reddish, sunken, but shallow, cankers that are round and sharply delineated (Ray 1936). The second type of canker forms just below branch whorls and resembles roughened bark (Ray 1936). Mycelia overwinters in the cankers and forms a cushion-like stroma that pushes through to the outside of the bark and produces spermagonia (small, black, and globose fruiting structures that are 100-150 µm in diameter) and spine-like ascocarps that are 1-2 mm long (McCormack 1936; Ray 1936). The ascospores mature inside the ascocarps in late winter and early spring and are then disseminated by wind and rain (Ray 1936). Ascocarps remain present on the cankers year-round (Funk 1963).

The cankers can coalesce and girdle branches. Mortality in sapling and pole sized trees can occur from stem girdling as well. Surveys in New Hampshire, Maine, and Massachusetts found that 70% of *Pinus strobus* stands had signs and symptoms of Caliciopsis canker disease (Munck et al. 2015) and that high density *P. strobus* growing on poor soils were the most likely to have high disease rates (Munck et al. 2016). In New England and the Appalachians, Caliciopsis canker disease has been associated with mortality of seedlings, saplings, and pole timber and degradation of sawtimber (Asaro et al. 2018; Munck et al. 2015; Schulz et al. 2018a).

Given the negative impacts Caliciopsis canker can have on pine populations, understanding the current distribution of the disease is critical. We surveyed *P. strobus* stands throughout northern Michigan (Chapter 2) and isolated cultures from *Caliciopsis* spp. ascocarps to determine the distribution of *Caliciopsis* spp. We then conducted pathogenicity tests on

excised branches and live seedlings to evaluate the virulence of a novel species on *Pinus strobus* in comparison to the known pathogen *C. pinea*.

Methods

Sample collection and isolation

Bark samples with *Caliciopsis* spp. ascocarps were collected from *P. strobus* stems and branches during a survey of *P. strobus* stands in northern Michigan from 2018-2019 (Chapter 2). Tissue from canker margins was surface sterilized in 1% bleach (Clorox, Oakland, CA) for 4 minutes, rinsed in sterile water for 1 minute, blotted with sterile paper towels and plated on pine needle agar (PNA) made with eastern white pine needles and 1.7% water agar (Bacto Agar, Difco, Sparks, MD, US) (Blodgett et al. 2003). Three to four ascocarps collected from the margins of an individual canker were placed inside the lid of an inverted petri dish, containing PDA (potato dextrose agar; 39 g in 1 L distilled water, Difco™ PDA, Sparks, MD, USA amended with streptomycin (50 mg/mL) and ampicillin (50 mg/mL). After 3-4 days emerging cultures potentially resembling *Caliciopsis* spp. were subcultured onto fresh PDA plates. To promote a sparser mycelia growth form, cultures resembling *Caliciopsis* spp. were transferred onto 1.7% water agar and grown at room temperature for 7-10 days. To obtain pure cultures, single hyphal tips were transferred to PDA.

DNA extraction, PCR amplification, and sequencing

Due to difficulties extracting high-quality DNA, three types of mycelia were used (fresh, frozen, or freeze-dried) were used to extract DNA from the isolates. Freeze dried mycelium was obtained by growing cultures in 50 mL potato dextrose broth (24 g in 1 L distilled water, Difco™ PDA, Sparks, MD, USA) a mended with streptomycin (50 mg/mL) and ampicillin (50 mg/mL) (PDB) for 10 days at room temperature (approximately 20°C) on a rotary shaker table

(120 rpm) under ambient light. Mycelium was harvested from the broth using vacuum filtration and was rinsed three times with sterile water and freeze dried at -80°C for 48 hours. Frozen mycelium was obtained by growing cultures on PDA for 7-10 days at room temperature and ambient light. The mycelia from these isolates were scraped from the agar and stored at -20°C (hereafter referred to as "frozen"). Fresh mycelium was used immediately after harvesting for DNA extraction.

DNA was extracted from 10-20 mg of mycelia using OmniPrep™ for Fungi (Geno Technology Inc., St. Louis, MO, USA) according to manufacturer's instructions with the following exceptions. Before grinding mycelia, a total 500 µl of Genomic Lysis Buffer (Geno Technology, Inc.) was added to each tube containing mycelia following incubation at room temperature (20°C) for 30 min. Mycelium was ground in a 1.5 mL Eppendorf® tube using a pestle and then homogenized either using an ultrasonic sonicator (Sonic Dismembrator Model 100, Fisher Scientific, Waltham, MA, USA) at 22.5 kHz for 60 s or using freeze/thaw cycles in liquid nitrogen (-80°C) and a heating block (60°C). The final DNA pellet was resuspended in 30 µL 1X Tris-EDTA buffer and stored at -20°C.

Isolates were identified to species level by amplifying the internal transcribed spacer (ITSrDNA) gene region using polymerase chain reactions (PCR) conducted in an ABI 2720 thermo cycler (Applied Biosystems, Foster City, CA, USA). The PCR reaction mixture contained 5 µL of 5x GoTaq Flexi colorless buffer (Promega Corp., Madison, WI, USA), 4 µL of Mg^{2+} (25mM) (Promega), 0.5 µL of dNTP (100 mM) (Invitrogen, Carlsbad, CA, USA), 1.0 µL of primer ITS1-F (10 mM; White et al. 1990), 1.0 µL of primer ITS4 (10mM; Gardes and Bruns 1993), 0.125 µL of GoTaq® Flexi polymerase (5U μL^{-1}) (Promega), and DNA (50 ng μL^{-1}), to a final volume of 25 µL (Table 3.1). Amplification conditions were as follows: initial

denaturation at 95°C for 5 min, 35 cycles of 94°C for 90 s, annealing temperature 56°C for 60 s, and extension at 72°C for 2 min, followed by a final extension of 72°C for 10 min (Migliorini et al 2020). PCR products were resolved through agarose (0.75% agarose dissolved in 0.5X Tris-glacial acetic acid-EDTA buffer, pH 7.5) gel electrophoresis at 93 volts in and visualized (Gel Doc XR+, Bio-Rad Laboratories, Inc., Hercules, CA, USA).

PCR products were purified using Qiagen PCR purification kit (Qiagen, Valencia, CA, USA) following manufacturer's instructions and sequenced by the MSU-Research Technology Support Facility on an ABI 3730xl platform (Applied Biosystems, Foster City, CA, USA). Forward and reverse sequences for the ITSrDNA gene region were aligned and manually edited using Geneious Prime (ver 2020.2.2). Sequences were used to identify the isolate to species level using the Basic Local Alignment Search Tool (BLAST; NCBI 2025; Altschul 1990).

Pathogenicity Trial: Excised Branch Assay

To complete Koch's postulates for the novel *Caliciopsis* species (*C.* sp. 1) and compare its virulence with *C. pinea*, pathogenicity tests were conducted on *P. strobus in vitro* (excised stem assay) and *in vivo* (stem inoculation of seedlings). Two pathogenicity trials were conducted on *P. strobus* branch segments (30 cm length, 1.2-2.9 cm diameter). For the first trial, 45 branch segments were collected from nine different apparently asymptomatic trees from a *P. strobus* stand at MSU's Tree Research Center, Okemos, MI, USA. For the second trial, five branch segments were collected from each of 10 different apparently asymptomatic trees from a *P. strobus* stand on MSU's main campus (East Lansing, MI, USA), a total of 50 branch segments. To prevent desiccation, branches were dipped in paraffin wax after harvesting to seal the ends and the ends of secondary branches three times over two days. Branch segments were randomly assigned to one of five inoculation treatments: inoculation with isolates MIFCC_1002 (*C. pinea*),

MIFCC_1034 (*C. pinea*), MIFCC_1033 (*C.* sp. 1), MIFCC_1000 (*C.* sp. 1), and negative control (sterile PDA).

Prior to inoculation of each trial, *Caliciopsis* isolates were grown on PDA for 7-10 days at room temperature (20-25°C). In a preliminary experiment, we found canker development was more consistent on branches that were inoculated twice compared to those inoculated once, thus two incisions were made on each branch at approximately 2 cm from the wax on either end. All incisions (i.e., inoculation points) were made superficially to expose the phloem using a sterile scalpel. Inoculation points were positioned distant to each other to prevent cankers from aggregating during canker development. A 2 mm agar plug colonized with mycelium was placed on each inoculation point facing the phloem. Each agar plug was covered with the remaining piece of bark and lightly sealed with parafilm (Denville Scientific, Holliston, MA, USA) by placing the parafilm around the entire circumference of the branch. Inoculated branches were placed in 7.6 L plastic bags (one branch from each treatment per bag) and stored in an opaque storage bin at ambient room temperature (20-25°C) for 22 days.

Pathogenicity Trial: Stem inoculation of seedlings

A total of 100 two-year-old *P. strobus* (30-45 cm tall) containerized seedlings (0.9 L) were purchased from a nursery located in Ottawa County, Michigan and transported to the greenhouses at MSU's Tree Research Center. Upon arrival, seedlings were immediately transplanted in 3.4 L RootMaker® pots (RootMaker® Products Company, LLC., Huntsville, AL, USA) using soilless media (Suremix Perlite, Michigan Grower Products, Inc., Galesburg, MI, USA). Prior to potting, pots were sterilized with Green-Shield® (BASF Corp, Research Triangle Park, NC, USA) according to manufacturer's instructions and potting mix was steam-treated in a steamer bin for 8 hours, allowed to cool for 16 hours, and then steam-treated for another 8 hours.

This protocol raised the soilless media temperature to 90°C on a previous cycle (C. Esch, per. comm, Nov. 2020). To control mite infestations, seedlings were sprayed immediately after inoculation and again four weeks later with abamectin (Avid 0.15 EC; Syngenta International AG, Basel, Switzerland) according to manufacturer's instructions. To control aphids, seedlings were sprayed with an insecticidal soap (Safer® Brand Lancaster, PA, USA) according to manufacturer's instructions.

Following planting, seedlings were allowed a 30-day acclimation period prior to inoculation. Seedlings were then inoculated once about 2 cm above the soil line using the same methods and isolates as the excised stem trials. The root collar diameter of each seedling was measured at the time of inoculation. Seedlings were arranged in a randomized complete design with 10 replicates of five treatments. Two pathogenicity trials were run concurrently in separate greenhouses: North and South. The temperature in the North greenhouse ranged from 14 to 30°C (mean 20°C) and the temperature in the South greenhouse ranged from 14 to 33°C (mean 19°C), measured with a HOBO UX100 data logger (Onset Computer Corporation, Bourne, MA, USA).

Canker assessment for excised branch and live seedling assays

For the excised branch assay, all excised branches were removed from storage bin after 22 days. After the removal of parafilm from each canker to reveal the inoculation point, each branch was cut roughly in half at the inoculation site using loppers that were sanitized with 70% ethanol between cuts on different branches. The depth of each canker was measured with electronic calipers. Each canker was then exposed by carefully removing the outer layer of the bark and the margin of each canker were traced on transparent film with a permanent marker. For the live seedling assay, seedlings were harvested at the root collar after 13 weeks for the South greenhouse and 14 weeks for the North greenhouse. Cankers were exposed and measured

using the same methods as in the excised branch study.

For cankers resulting from both assays, the trace of each canker on the film was scanned using an Epson Perfection V500 Photo scanner (Epson America Inc., Long Beach, CA, USA). Prior to scanning, a 10 mm line was drawn on each transparent film to use as a scale bar, and each scale bar was used to convert pixel distances to mm distances. The area of each canker image was measured in mm^2 by tracing the outlines with the free hand tool using ImageJ (Sakalidis et al. 2021; Rasband 2018).

Re-isolation

To complete Koch's postulates, *Caliciopsis* spp. were re-isolated from canker margins using the canker isolation method previously described. To confirm successful re-isolation, morphology of the re-isolated cultures was visually compared to that of the morphology of the inoculation cultures.

Statistics

Data analysis was conducted in R version 4.4.2 (R Foundation for Statistical Computing, Vienna, Austria, 2025) using lme4 (Bates et al. 2015) and emmeans function (Lenth 2021) packages. For excised branch assays, linear mixed-effects models were used to test for effects of inoculation treatment, branch diameter on canker area and depth with block nested in trial as a random effect. Canker area data were log transformed. Canker area and depth means were compared between *Caliciopsis* isolates using analysis of variance (ANOVA) followed by Tukey's test. Statistical significance was considered at $P = 0.05$.

For the live seedling assays, linear mixed-effects models were used to test for effects of inoculation treatment and root collar diameter on canker area and depth with block nested in greenhouse as a random factor. Canker area data were normalized using log transformation.

Canker area and depth means were compared between *Caliciopsis* isolates using ANOVA followed by Tukey's test. Statistical significance was considered at $P = 0.05$.

Results

Distribution of *Caliciopsis* in Michigan

Caliciospsis spp. ascocarps were observed between May 30 – October 26, 2018, and June 14 – August 22, 2019 at 31 of the 66 stands surveyed (Figure 3.1, Table 3.2). *Caliciopsis* spp. ascocarps collected throughout the survey seasons readily sporulated under lab conditions. We successfully sequenced DNA from 37 *Caliciopsis* spp. isolates from 20 different stands, 35 of which were identified as *C. pinea* (≥ 99% match with BLAST) (NCBI 2025). The remaining 2 isolates had a 98% match with *C. orientalis*, a pathogen previously only reported on *Tsuga canadensis* (L.) Carr. (Funk 1963), leading us to conclude that the isolates were possibly an undescribed species of *Caliciopsis* (*C.* sp. 1). We observed *Caliciopsis pinea* and *C.* sp. 1 co-occurring in one stand, but on different trees.

Pathogenicity to *P. strobus* excised branches

Following both excised branch trials, necrotic lesions were observed near inoculation points once the bark was removed (Figure 3.2). Lesions were dark reddish-brown in color. On branch segments inoculated with *Caliciopsis* spp., lesions grew outward from the inoculation point in all directions forming cankers that were slightly oblong or round in shape. The cankers also grew inward, resulting in a darkening of sapwood that extended deeper than the inoculation wounds. Canker development was not observed on any control branch segments. Two branch segments in the first trial had cankers that predated inoculation but were not visible until the bark was removed at the end of the trial. Three branch segment halves desiccated over the course of the second trial. These branch segments and half segments were excluded from analyses because

of confounding variables. We completed Koch's postulates by successfully reisolating fungi with colony morphology resembling *Caliciopsis* spp. from at least one margin of a canker resulting from each *C.* spp. inoculation. Re-isolation rate of *Caliciopsis* spp. from Caliciopsis canker margins was 7%. *Caliciopsis* spp. were not reisolated from controls.

Branch diameter affected canker depth ($X^2_{1,175}$ = 4.81, P = 0.03) but not canker area ($X^2_{1,175}$ = 3.20, P = 0.07). Inoculation treatment affected both canker area ($X^2_{4,171}$ = 166.14, P < 0.01) and depth ($X^2_{4,171}$ = 162.65, P < 0.01). Isolates were analyzed separately, even if from the same species. Branch segments inoculated with *C.* sp. 1 isolates MIFCC_1000 and MIFCC_1033 had larger and deeper (P < 0.01 and P < 0.01) cankers than the control segments (Table 3.3, Figure 3.3). Branch segments inoculated with *C. pinea* isolates MIFCC_1002 and MIFCC_1034 had cankers larger in area than those inoculated with *C.* sp. 1. All *C.* spp. isolates produced deeper cankers than the control. Between the *C.* spp. isolates, MIFCC_1002 produced deeper cankers than MIFCC_1033 (P = 0.03), but other pairwise depth comparisons were insignificant. Canker area (P = 0.83) and depth (P = 0.64) were similar for the two *C.* sp. 1 isolates. The two *C. pinea* isolates produced cankers that were similar to each other in area (P = 0.87) and depth (P = 1.00).

Pathogenicity to *P. strobus* seedlings

At the end of the seedling trials, sunken, necrotic, and reddish areas were visible around the inoculation points following *C. pinea* and *C.* sp. 1 treatments (Figure 3.4). These resulting cankers spread outwards through the cambial tissue and inwards through the sapwood on seedlings inoculated with *C. pinea*. Cankers on seedlings inoculated with *C.* sp. 1, however, did not spread outward beyond the inoculation point, but did grow deeper into the sapwood (Figure 3.5). Girdling cankers, cankers that extended the entire circumference of the stem, occurred on

58% of seedlings inoculated with *C. pinea* isolate MIFCC_1002 and on 21% of seedlings inoculated with *C. pinea* isolate MIFCC_1034. Four seedlings inoculated with *C. pinea* (three with isolate MIFCC_1002 and one with isolate MIFCC_1034) appeared to have patches of immature spermagonia developing on the center of their cankers (Figure 3.4). Root collar diameter did not affect canker area ($X^2_{1,83}$ = 1.53, P = 0.22) or depth ($X^2_{1,83}$ = 0.08, P = 0.77). Inoculation treatment affected both canker area ($X^2_{4,79}$ = 200.27, P < 0.01) and depth ($X^2_{4,79}$ = 101.85, P < 0.01).

Cankers produced by *C.* sp. 1 isolates were not larger than those produced by the control (P = 0.85 for isolate MIFCC_1033 and P = 0.68 for isolate MIFCC_1000; Table 3.3; Figure 3.3), but they were deeper than the control (P < 0.01 for isolate MIFCC_1033 and P < 0.01 for isolate MIFCC_1000). Cankers produced by the *C.* sp. 1 isolates were similar in area (P = 1.00) and depth (P = 1.00). Both *C. pinea* isolates produced cankers that were larger in area and deeper than the control (P < 0.01 for all pairwise comparisons). The *C. pinea* isolates also produced cankers larger in area than the *C.* sp. 1 isolates (P < 0.01 for all pairwise comparisons). *Caliciopsis pinea* isolate MIFCC_1034 produced deeper cankers than both *C.* sp. 1 isolates (P = 0.04 for isolate MIFCC_1033 and P = 0.01 for isolate MIFCC_1000). *Caliciopsis pinea* isolate MIFCC_1002 produced cankers that were deeper than *C.* sp. 1 isolate MIFCC_1000 (P = 0.02). Cankers from MIFCC_1002 had similar depths to those from MIFCC_1033 (P = 0.06). *Caliciopsis pinea* isolate MIFCC_1002 produced cankers larger in area than *C. pinea* isolate MIFCC_1034 (P < 0.01), but canker depth was similar for the two isolates (P = 1.00). Koch's postulates were completed when we reisolated fungi with culture morphology resembling *Caliciopsis* spp. from canker margins (Figure 3.6). Re-isolation rate of *Caliciopsis* spp. from Caliciopsis canker margins was 44%. *Caliciopsis* spp. were not reisolated from controls.

Five seedlings died before the end of the trial and were excluded from analyses. At the time of harvest, seedlings exhibited symptoms that differed to those we expected from Caliciopsis canker disease. These included cankers extending from the root crown (3% of seedlings, excluded from analyses), symptomatic roots (i.e., water soaked or casings sloughing off; 38% of seedlings), signs of needle cast disease (80% of seedlings) and needle tip chlorosis and necrosis (96% of seedlings). Diagnostic tests revealed that *Phytophthora* spp. were present in 95% of subsampled seedlings (9/10 asymptomatic and 10/10 symptomatic seedlings). *Mycosphaerella pini* Rostr. was identified as the needle cast pathogen and there were no fruiting bodies or other disease signs associated with the chlorotic and necrotic foliage, suggesting an abiotic cause to those symptoms. There was no association between these signs or symptoms and Caliciopis canker area or depth. There were, however, interactions between inoculation treatment and three of these symptoms (symptomatic roots, discoloration, and needle cast) on Caliciopsis canker area but not on canker depth (Table 3.4). Subsequent preliminary analyses included symptomatic roots, rate of needle cast, and needle tip discoloration as main effects along with root collar diameter and inoculation treatment with block nested in greenhouse as a random effect. Their inclusion did not change the significance of any of the pairwise comparisons of inoculation treatment on canker area or depth, so they were excluded from the final analyses presented in Chapter 3. For more information, see Appendix.

Discussion

We identified two species of Caliciopsis (*Caliciopsis pinea* and *C.* sp. 1) on *P. strobus* exhibiting cankers, resin streaks, and branch flagging. *Caliciopsis pinea* appears to be more common than the rarely encountered *C.* sp. 1. Pathogenicity trials confirmed *C. pinea* as a pathogen and found that it was more virulent than *C.* sp. 1. *Caliciopsis* sp. 1 was more effective

in colonizing down into the sapwood rather than the living cambium suggesting it is more of a weak pathogen or a saprotroph rather than a primary disease agent. Knowledge of the distribution and potential impact of a native pathogen that is associated with widespread sapling mortality in New England and southern Appalachians will facilitate forest health monitoring targets and locations.

Caliciopsis canker historically caused negligible damage but has become a threat to *P. strobus* in the last 30 years (Peck 1880; Costanza et al. 2018). Caliciopsis canker disease is much more prevalent in New England where it is also impacting a larger proportion of pole-sized and sawtimber *P. strobus* (Munck et al. 2016). Since 2018, Caliciopsis canker disease has been reported causing branch and stem cankers on young *P. strobus* in 21 counties in Wisconsin and at least one location in Minnesota, but the impacts are not well understood (WI DNR 2024; MN DNR 2020). In Michigan, Caliciopsis canker disease is widespread but only a few localized areas had severe symptoms (Chapter 2).

Caliciopsis canker is a disease caused by at least one native pathogen, leading to questions about why the disease is now emerging as a threat to eastern forests. Studies in New England found increased Caliciopsis canker severity was associated with poorer soils and overstocked stands (Munck et al. 2015, Munck et al. 2016). Silviculture guidelines call for high *P. strobus* regeneration density to manage for white pine weevil and white pine blister rust (Ostry et al. 2010), but increased planting density can favor other diseases (Munck et al. 2023). Recent studies have begun to elucidate some of the epidemiology of Caliciopsis canker. Cram and Fraedrich (2022) found *C. pinea* cannot infect through intact bark and Schulz et al. (2018b) found close associations between Caliciopsis canker and *Matsucoccus macrocicatrices*, a native bast scale that creates feeding wounds in *P. strobus* that likely create entry ports for *C. pinea*.

However, the association on *M. macrocicatrices* with Caliciopsis canker is recent (Whitney et al. 2018). In 2016, *M. macrocicatrices* was found in a stand in Michigan near a canker with *Caliciopsis* spp. ascocarps, but it was rarely encountered during our surveys (personal observations). Warmer winters and wetter springs are generally beneficial to fungal pathogens and, in combination with increased heat and/or drought stress on host trees, can contribute to increased disease (Simler-Williamson et al. 2019). In recent decades, Michigan's climate has become wetter and warmer overall but most of the additional precipitation falls from autumn to spring, creating moisture deficits during the hotter summer months (Dai et al. 2016). These climate factors may be contributing to the emergence of Caliciopsis canker disease when it previously was not observed.

During our survey, two species of *Caliciopsis* were found on *P. strobus*, with *C. pinea* more commonly encountered. This is the first time a *Caliciopsis* sp. other than *C. pinea* has been reported on *P. strobus*. It is possible that there are other undescribed *Caliciopsis* spp. in US forests. *Caliciopsis* spp. have been reported on other conifer species (Funk 1963). Genetically, *Caliciopsis* sp. 1 appears to be closely related to *C. orientalis*. This species was first described as a pathogen of *Tsuga canadensis* (eastern hemlock) in 1963 in Ontario, Canada (Funk 1963). *Tsuga canadensis* and *P. strobus* commonly co-occur in Michigan forests, therefore *C.* sp. 1 may have recently evolved due to a host jump event i.e., an ancestral *C. orientalis* moving from *T. canadensis* to *P. strobus*. More extensive phylogenetic studies, genomic studies, and pathogenicity trials are required to more fully elucidate the evolution of *C.* sp. 1 and risk it poses to coniferous hosts.

Caliciopsis pinea and *C.* sp. 1 were both associated with cankers, resin streaks, and branch flagging on *P. strobus* in forested areas and both produced similar looking cankers on

excised *P. strobus* branches. *Caliciopsis pinea* was confirmed as a pathogen consistent with other studies (Schulz et al. 2018b; Cram and Fraedrich 2022). *Caliciopsis pinea* produced cankers significantly larger in area than the control and *C.* sp. 1 in both the excised branch and seedling trials. Both *Caliciopsis* species formed cankers significantly deeper than the control in both trials. *Caliciopsis pinea* isolates generally produced deeper cankers than *C.* sp. 1 isolates, but not consistently. We found that although excised stems provided faster results and were less resource intensive they could not be used as a proxy for live plant trials as *C.* sp. 1 produced cankers significantly larger in area than the control in the excised stem trials but failed to do so in the seedling trials. *Caliciopsis* sp. 1 was able to colonize significantly deeper into the sapwood than the controls in both the excised branch and seedling trials suggesting that it is a weak pathogen or a saprophyte, rather than a primary disease agent.

As *C.* sp. 1 was isolated from ascocarps growing out of cankers on live *P. strobus* stems, it is also possible that environmental or seedling conditions were not conducive to disease development during our trial. *Caliciopsis* sp. 1 was able to colonize sapwood, so a longer inoculation period or multiple inoculation points may have allowed the species to colonize the cambium as well (Sakalidis et al. 2021). Many members of the Botryosphaeriaceae family, notably *Diplodia pinea,* are latent pathogens that can exist on a host as endophytes until a stress event occurs that allows them to enter a pathogenic phase (Slippers and Wingfield 2007; Stanosz et al. 2001). Subsequent studies examining dual inoculations of both *Caliciopsis* species, or trials in poor soils or drought conditions may illuminate the roll that additive stressors play in the emergence of native diseases.

Isolates of *Caliciopsis pinea* varied in virulence. While the two isolates of *C. pinea* produced cankers similar in area in excised branches, isolate MIFCC_1002 produced larger

cankers than MIFCC_1034 in seedlings. The seedling trial lasted much longer than the excised branch trial, though, so the difference in relative virulence could have been a result of growing time rather than host condition. Other *C. pinea* pathogenicity trials ran for longer than our trials and in some cases these trials reported more severe symptoms such as girdling cankers. Schultz et al. (2018b) found that *C. pinea* caused girdling cankers on 3-year-old *P. strobus* seedlings 19 weeks after inoculation. Cram and Fraedrich (2022) also found *C. pinea* formed cankers on 3-year-old *P. strobus* seedlings, but the cankers were not girdling the seedlings 55 weeks after inoculation. Whether that was because they conducted their trials in a lath house with partial shade where the seedlings were exposed to more natural conditions or because the isolates used were less virulent is unknown. Our intent was to run the trial until *Caliciopsis* spp. caused severe symptoms or mortality in the seedlings. However, due to the observations of signs and symptoms of other diseases and disorders (i.e., Phytophthora root rot, needle cast and branch tip cholorosis), a decision was made to end the trial after 13-14 weeks.

Future research is needed to explore the effects of other pests and pathogens, silvicultural practices, and environmental factors (i.e., soil type, water availability, and climate) have on the epidemiology of Caliciopsis canker disease and to more fully understand the risk that Caliciopsis canker poses to forests in eastern North America.

Tables

Table 3.1: PCR primers of the internal transcribed spacer region (ITSrDNA) used in this study to identify *Caliciopsis* species.

Primer	Primer sequence (5' - 3')	Size (bp)	Annealing temperature (°C)
ITS1F[1]	CTTGGTCATTTAGAGGAAGTAA	593	56
ITS4[2]	TCCTCCGCTTATTGATATGC	593	56

[1]Reference: White et al. 1990

[2]Reference: Gardes and Bruns 1993

Table 3.2: *Caliciopsis* species used in this study. All isolates were collected from *Pinus strobus* in Michigan, USA and identified to species level using BLAST (NCBI 2025) with the ITSrDNA gene region sequences.

Species	Isolate code	Year	Collected by	County
Caliciopsis pinea	MIFCC_1001	2018	K. Minnix	Cheboygan
	MIFCC_1002	2018	K. Minnix	Cheboygan
	MIFCC_1003	2018	K. Minnix	Cheboygan
	MIFCC_1004	2018	K. Minnix	Otsego
	MIFCC_1005	2018	K. Minnix	Montmorency
	MIFCC_1006	2018	K. Minnix	Montmorency
	MIFCC_1007	2018	K. Minnix	Montmorency
	MIFCC_1008	2018	K. Minnix	Cheboygan
	MIFCC_1009	2018	K. Minnix	Cheboygan
	MIFCC_1010	2018	K. Minnix	Iosco
	MIFCC_1011	2018	K. Minnix	Iosco
	MIFCC_1012	2018	K. Minnix	Iosco
	MIFCC_1013	2018	K. Minnix	Iosco
	MIFCC_1014	2018	K. Minnix	Iosco
	MIFCC_1015	2018	K. Minnix	Iosco
	MIFCC_1016	2018	K. Minnix	Alcona
	MIFCC_1017	2018	K. Minnix	Alger
	MIFCC_1018	2018	K. Minnix	Alger
	MIFCC_1019	2018	K. Minnix	Alger
	MIFCC_1020	2018	K. Minnix	Luce
	MIFCC_1021	2018	K. Minnix	Delta
	MIFCC_1022	2018	K. Minnix	Schoolcraft
	MIFCC_1023	2018	K. Minnix	Alger
	MIFCC_1024	2018	K. Minnix	Alger
	MIFCC_1025	2019	K. Minnix	Lake
	MIFCC_1026	2019	K. Minnix	Clare
	MIFCC_1027	2019	K. Minnix	Clare
	MIFCC_1028	2019	K. Minnix	Clare
	MIFCC_1029	2019	K. Minnix	Crawford
	MIFCC_1030	2019	K. Minnix	Crawford
	MIFCC_1031	2019	K. Minnix	Oscoda
	MIFCC_1032	2019	K. Minnix	Antrim
	MIFCC_1034	2019	S. Wright	Mackinac
	MIFCC_1035	2019	S. Wright	Mackinac
	MIFCC_1036	2020	R. Harkness	Crawford
Caliciopsis sp. 1	MIFCC_1000	2017	K. Minnix	Crawford
	MIFCC_1033	2019	S. Wright	Chippewa

Table 3.3: Mean canker area (mm^2) and depth (mm) following pathogenicity trials.

Trial	Treatment	Area[1] (mean ± SE)	Depth[2] (mean ± SE)
Excised	Control	65 ± 3^{A}	0.12 ± 0.04^{A}
	MIFCC_1033	136 ± 10^{B}	1.07 ± 0.10^{B}
	MIFCC_1000	170 ± 19^{B}	1.23 ± 0.08^{BC}
	MIFCC_1034	248 ± 18^{C}	1.36 ± 0.10^{BC}
	MIFCC_1002	230 ± 28^{C}	1.41 ± 0.11^{C}
Seedling	Control	78 ± 8^{A}	0.62 ± 0.08^{A}
	MIFCC_1033	90 ± 8^{A}	2.03 ± 0.19^{BC}
	MIFCC_1000	94 ± 9^{A}	1.94 ± 0.18^{B}
	MIFCC_1034	316 ± 56^{B}	2.68 ± 0.16^{D}
	MIFCC_1002	566 ± 66^{C}	2.64 ± 0.14^{CD}

[1] Values within the same trial denoted by the same letter are not significantly different ($P \geq 0.05$)
[2] Values within the same trial denoted by the same letter are not significantly different ($P \geq 0.05$)

Table 3.4: Chi squared test results for interactive effects between *Caliciopsis* spp. inoculation treatment and unexpected disease signs and symptoms on canker area and depth.

		DF			
	Sign/Symptom	Num	Den	X^2	P
Area	Roots	4	79	13.42	0.01
	Discoloration	4	79	12.95	0.01
	Needle Cast	8	74	18.93	0.02
Depth	Roots	4	79	1.60	0.81
	Discoloration	4	79	7.39	0.12
	Needle Cast	8	74	10.12	0.26

Figures

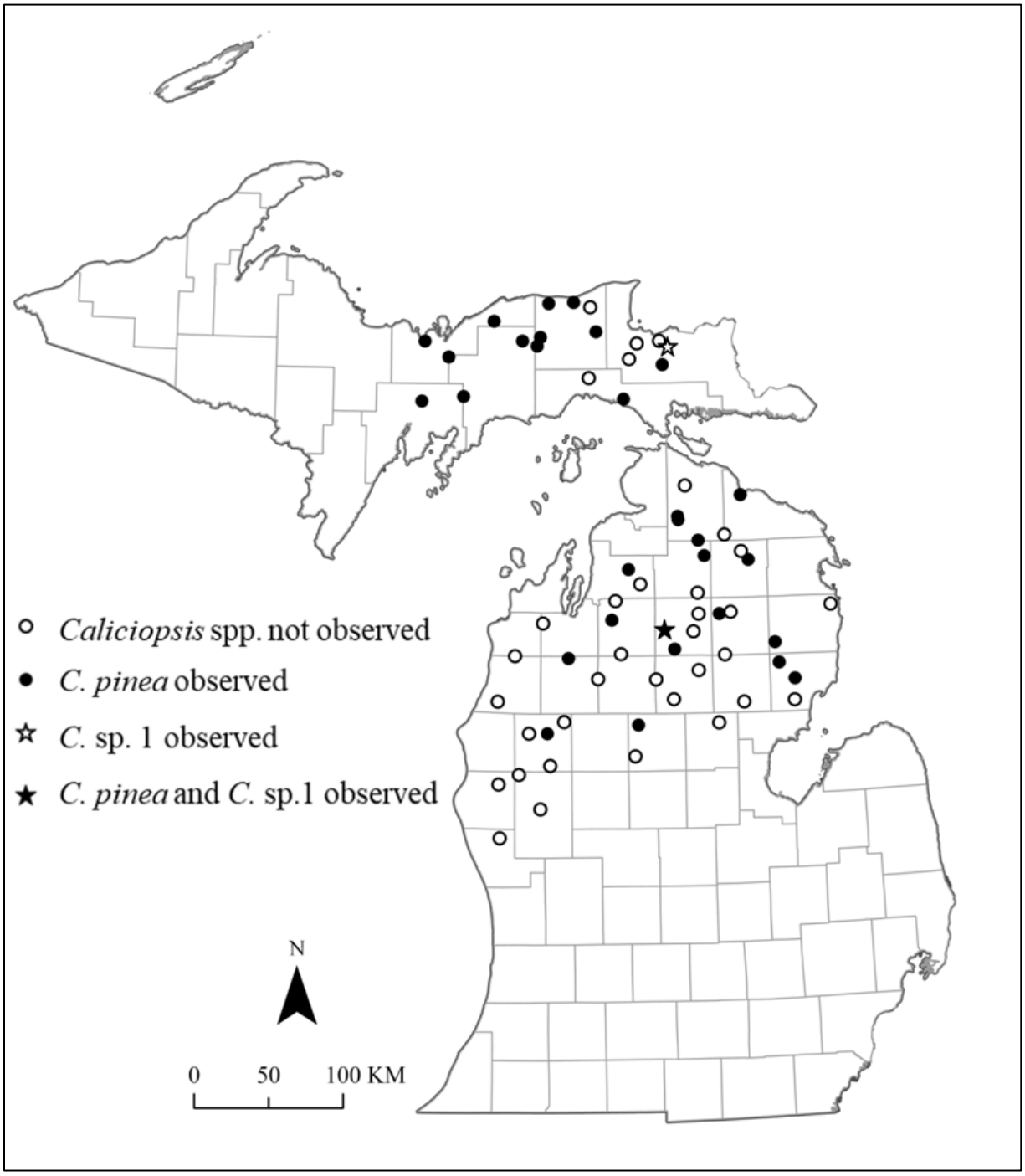

Figure 3.1: *Pinus strobus* stands surveyed in 2018-2019 for Caliciopsis canker disease. *Caliciopsis* spp. ascocarps were collected and used to isolate pure cultures. Isolates were identified to species level using BLAST (NCBI 2025) with the ITSrDNA gene region sequences.

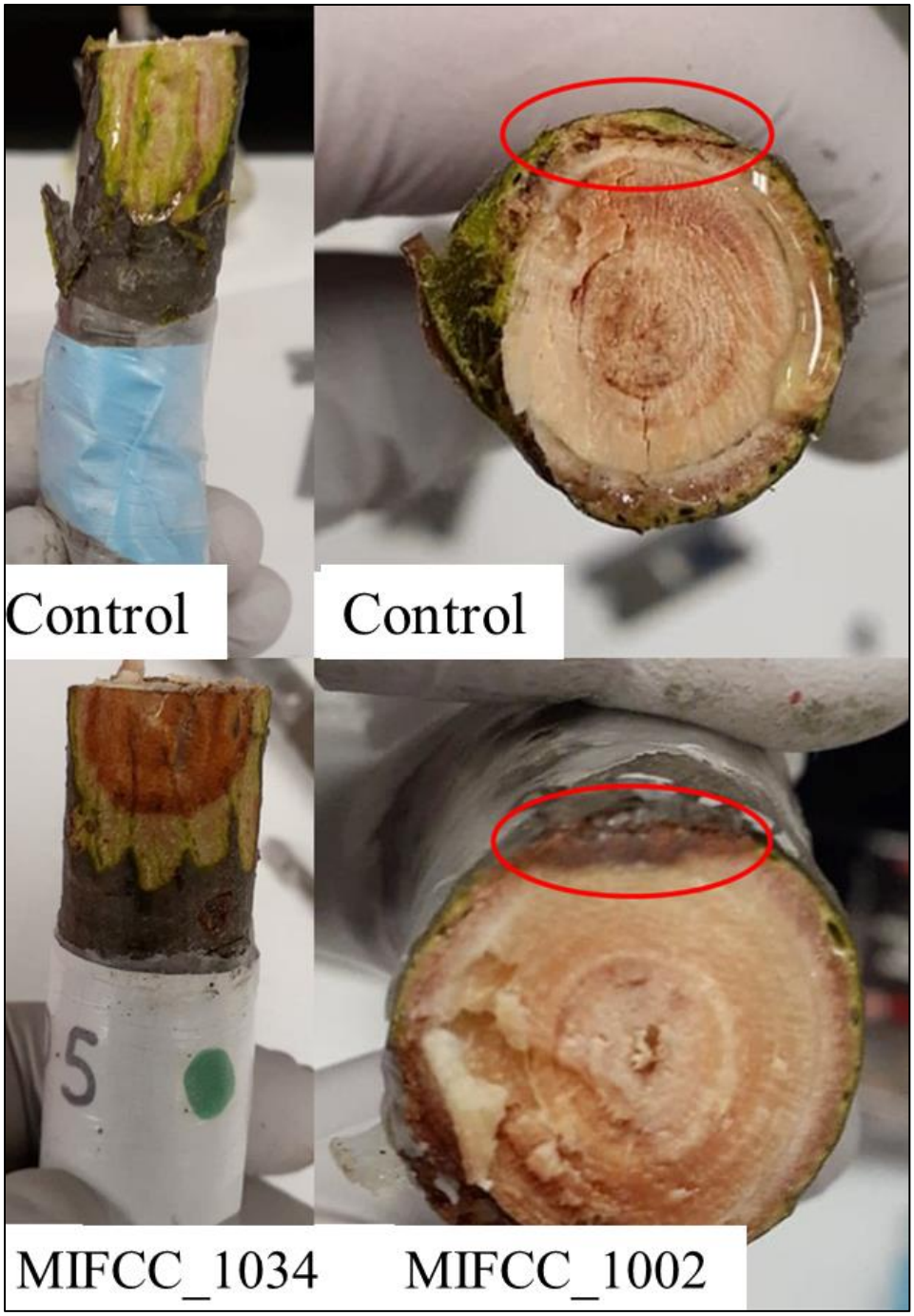

Figure 3.2: Cankers and canker cross-sections following pathogenicity trial on excised branches inoculated with sterile media (Control) and *Caliciopsis pinea* (MIFCC_1002, MIFCC_1034).

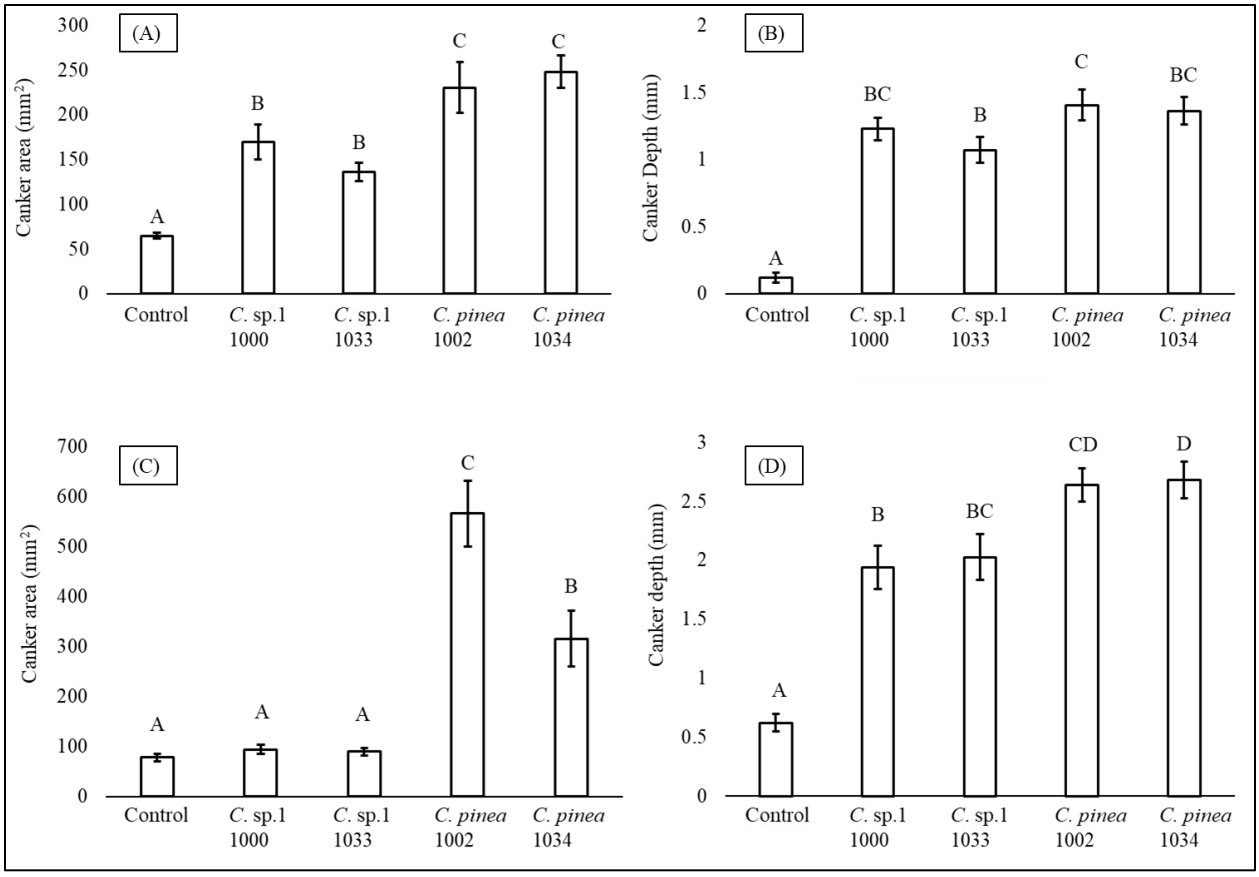

Figure 3.3: Mean lesion areas and depths of *Pinus strobus* excised branches and seedlings inoculated with *Caliciopsis* sp. 1 (isolates MIFCC_1033 and MIFCC_1000) and *C. pinea* (isolates MIFCC_1034 and MIFCC_1002). Mean canker areas on excised branches 22 days after inoculation (A); Mean canker depths on excised branches 22 days after inoculation (B); Mean canker areas on seedlings 13-14 weeks after inoculation (C); Mean canker depths on seedlings 13-14 weeks after inoculation (D). Bars denote the standard error of the mean. Bars labeled with the same letter are not significantly different at $P < 0.05$ via Tukey's honestly significance difference test.

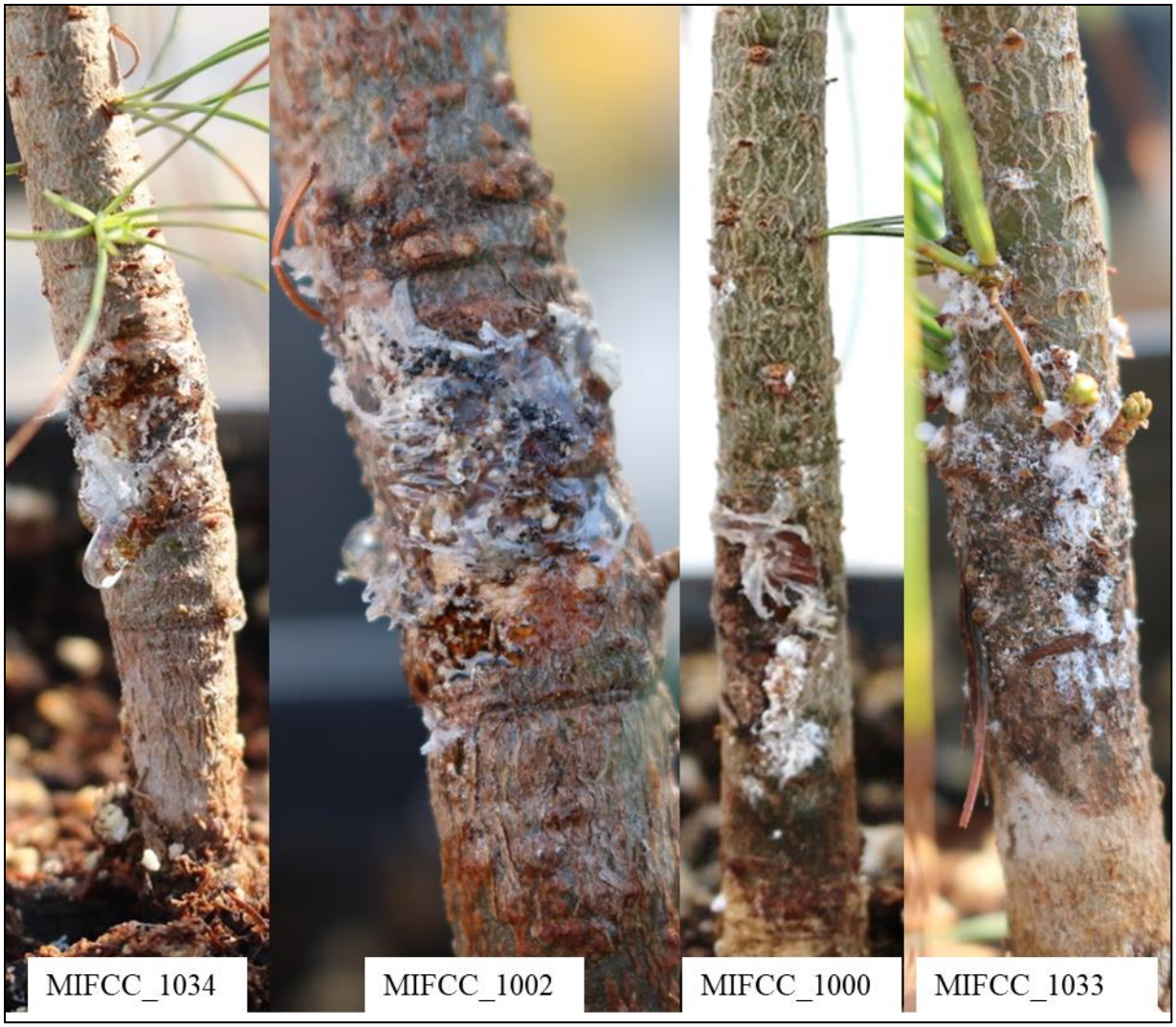

Figure 3.4: Seedlings following pathogenicity trial in which they were inoculated with *Caliciopsis pinea* (MIFCC_1002, MIFCC_1034) and *C.* sp. 1 (MIFCC_1033 and MIFCC_1000). *Caliciopsis pinea* isolates occasionally formed girdling cankers and immature spermagonia (shown beneath parafilm on MIFCC_1002).

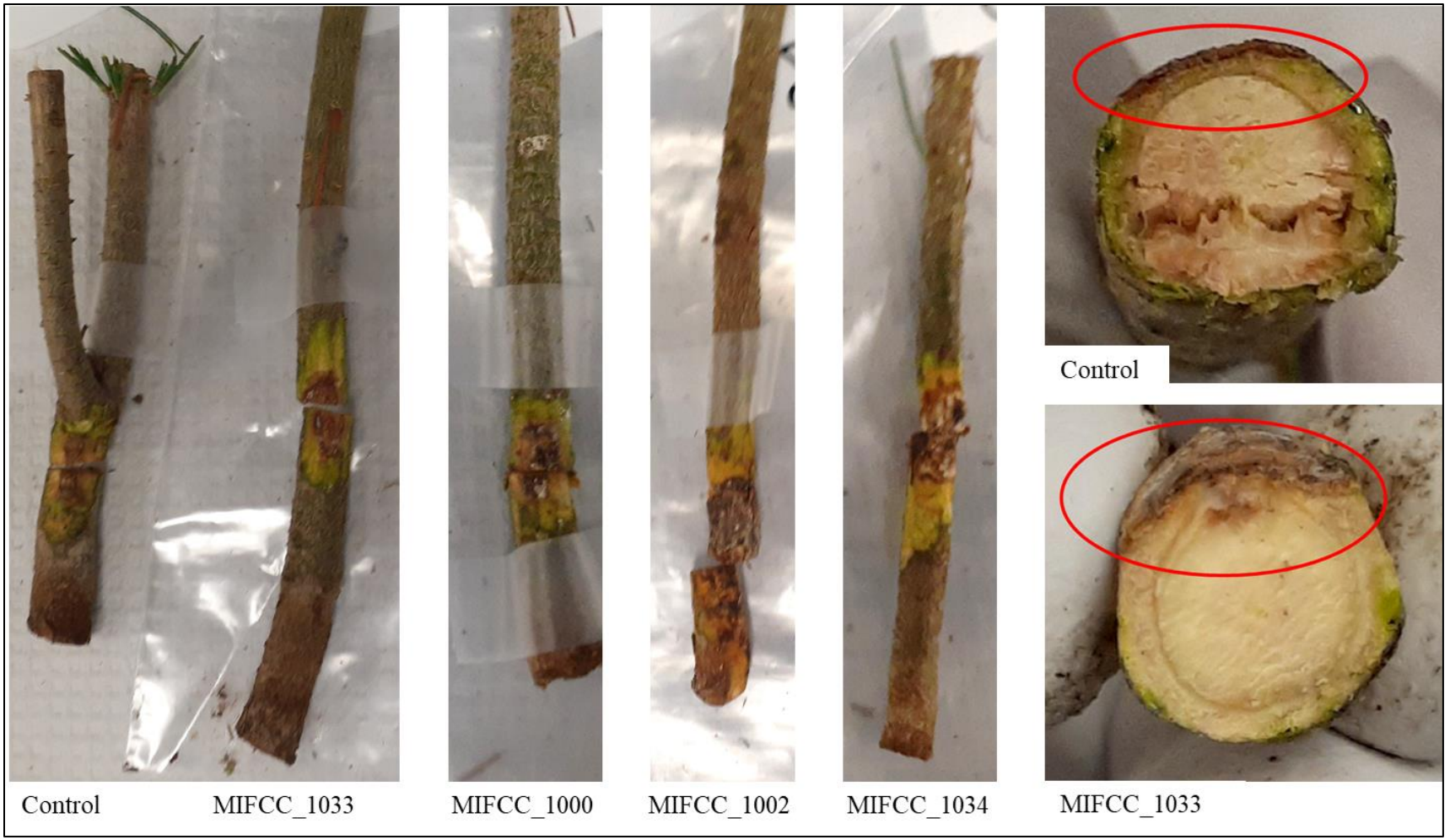

Figure 3.5: Cankers and canker cross-sections following pathogenicity trial on seedlings inoculated with sterile media (Control), *Caliciopsis* sp. 1 (MIFCC_1033 and MIFCC_1000), and *C. pinea* (MIFCC_1002, MIFCC_1034).

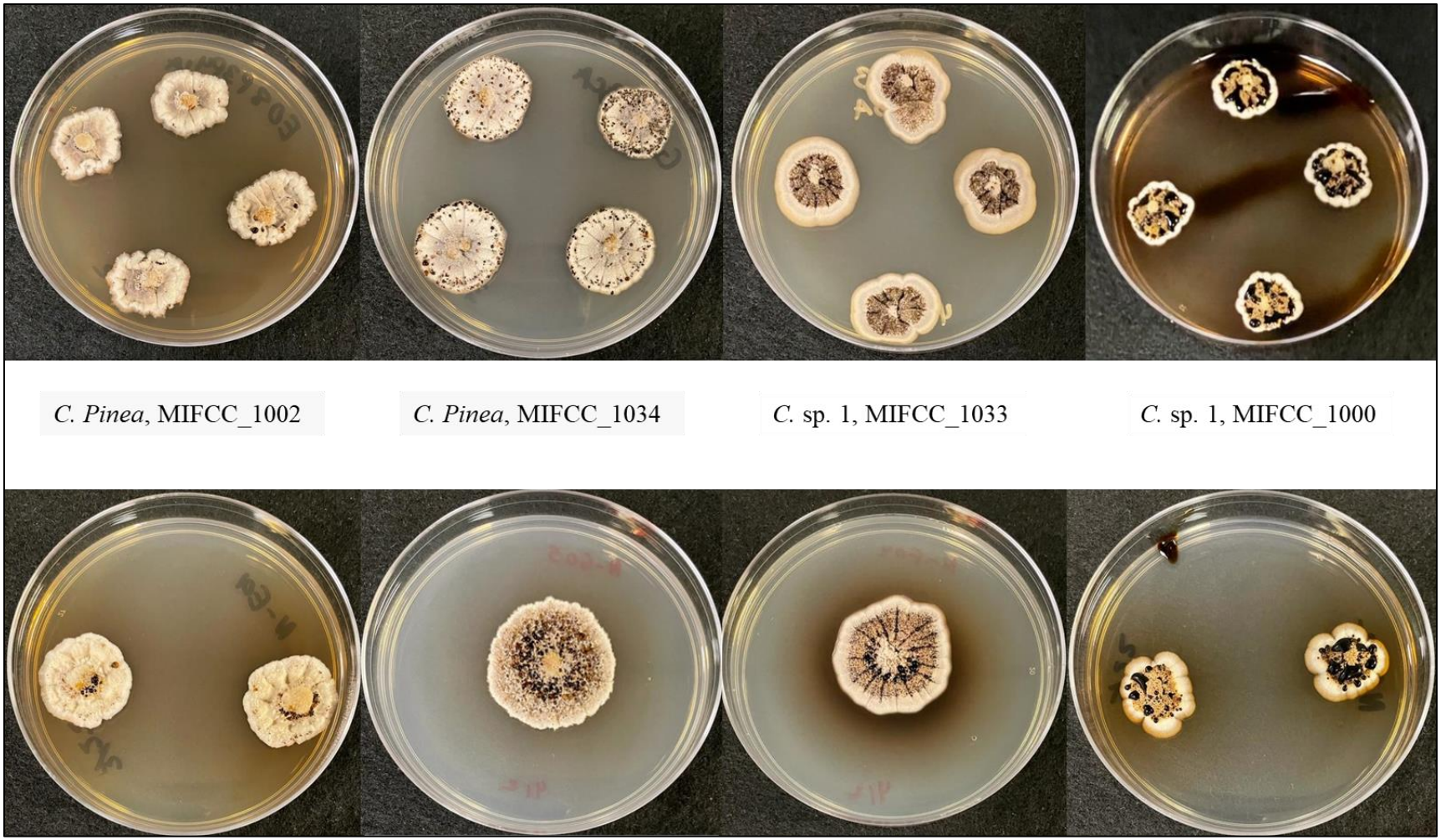

Figure 3.6: *Caliciopsis* spp. isolates used to inoculate seedlings in pathogenicity trial (top row) and cultures re-isolated from *Pinus strobus* seedlings following pathogenicity trial.

CONCLUSION

This dissertation focused on two different native damage agents on *Pinus strobus*: white pine weevil (*Pissodes strobi*), an insect that causes economic injury and that has greatly influenced *P. strobus* management for over a century, and Caliciopsis canker disease, an emerging disease that is causing dieback in New England and the southern Appalachians but whose effects are not well understood in the Great Lakes region. In the previous chapters, I explored different factors that might predict the damage caused by these two agents with the goal of identifying mitigation strategies.

In Chapter 1, I revisited *P. strobus* stands that had been planted under different regeneration strategies with the goal of quantifying the effects of competition on growth and *P. strobi* damage. In the monoculture, *P. strobus* planted in the low density plots had higher radial growth but were more likely to have severe defects than those planted in the intermediate and high density plots. Trees planted in the intermediate density plots were 10% larger in DBH and had a similar likelihood of bole defects than those planted in the high density plots.

The oak shelterwood was effective at preventing *P. strobi* injury, even at the lowest canopy cover of 46%, as only 6% of the *P. strobus* in the stand had bole defects 18 years after planting. The canopy cover, however, suppressed growth as the underplanted *P. strobus* were less than one-third the diameter and less than two-thirds the height of *P. strobus* growing in the monoculture.

There were two even-aged, mixed-species stands. In one, *P. strobus* planting density was varied and in the other herbicide was used on half of the stand to reduce hardwood regeneration. Planting density did not affect *P. strobus* DBH, total height, or likelihood of bole defects in the even-aged mixed-species stand. *Pinus strobus* in this stand competed well with hardwood

regeneration, with many reaching the overstory. At the mixed-species stand with partial herbicide control, *P. strobus* suffered up to 90% mortality, either as a result of hardwood competition, heavy levels of browse, or both.

Pinus strobus can be successfully regenerated in monocultures, shelterwoods, and even-aged, mixed-species stands, giving managers options to grow *P. strobus* while considering additional management needs. Each of these regeneration techniques, however, require additional investments to minimize *P. strobi* injury while facilitating growth. Higher density plantings will likely require pre-commercial thinning to release *P. strobus* from intraspecific competition. Overstories eventually need to be removed from shelterwoods to release *P. strobus* in the understory. Control measures may be needed in even-aged, mixed-species stands to prevent hardwood basal sprouts from overtopping *P. strobus.* Pre-commercial thinning may also be needed in even-aged, mixed-species stands 10-15 years after planting to release *P. strobus*.

In Chapter 2, I surveyed *P. strobus* stands across northern Michigan to determine the distribution and severity of Caliciopis canker disease and bole defects consistent with *P. strobi* injury. While both damage agents were widely distributed across the survey area, only a small proportion of evaluated *P. strobus* were impacted. While low levels of *P. strobi* damage in the basal log could be indicative of successful forest management, low levels of Caliciopsis canker disease may represent early warning signs of an emerging disease. This is especially true for the few stands that had *P. strobus* with severe symptoms. *Caliciopsis pinea* is a native species (McCormack 1936; Ray 1936) but reports of *P. strobus* dieback and regeneration mortality are relatively recent (O'Brien 2007). Due to changing climatic conditions, emerging diseases caused by native pathogens, such as Caliciopsis canker, are globally expected to cause an unprecedented increase of epidemics (Burgess et al. 2022). This survey describes a baseline for the distribution

and severity of Caliciopsis canker, which currently appears to be acting as a “thin from below” agent in northern Michigan. Monitoring for Caliciopsis canker over time is needed to know the impacts this disease will have on Michigan forests.

In Chapter 3, I identified isolates of *Caliciopsis* to the species level and tested their pathogenicity and relative virulence on *P. strobus* excised branches and live seedlings. *Caliciopsis pinea* and *C.* sp. 1 were both associated with cankers, resin streaks, and branch flagging on *P. strobus* in forested areas and both produced similar looking cankers on excised *P. strobus* branches. *Caliciopsis pinea* was confirmed as a pathogen consistent with other studies (Schulz et al. 2018b; Cram and Fraedrich 2022). *Caliciopsis* sp. 1 was able to colonize the sapwood in both the excised branch and seedling trials but was only able to produce cankers larger than the control in the excised stem trials. This suggests *C.* sp.1 is a weak pathogen or saprophyte rather than a primary disease agent. More extensive phylogenetic and genomic studies are required to confirm *C.* sp. 1 as a new species.

Caliciopsis canker disease is currently less prevalent and less severe in Michigan than it is in New England (Munck et al. 2015; Munck et al. 2016). Future research is needed to explore the effects silvicultural practices and environmental conditions (i.e., soil type, water availability, and climate) have on the epidemiology of Caliciopsis canker disease and to more fully understand the risk that Caliciopsis canker poses to forests in eastern North America.

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APPENDIX

At the time of harvest, seedlings exhibited symptoms that differed to those we expected from Caliciopsis canker disease. Each is described in detail below along with the methods used in preliminary analyses. None were included in the final analyses presented in Chapter 3.

Phytophthora spp. Testing

Methods: At the time of harvest, we noticed cankers extending from the root crown that appeared to be associated with Phytophthora root rot on three seedlings. We examined the roots of all seedlings and recorded whether they were asymptomatic (i.e., new growth visible at tips) or symptomatic (i.e., water soaked or casings sloughing off). We used ANOVA to look for symptomatic root disease effects on canker area (log transformed), canker depth, and interactions with treatment effects. Root and soil samples were collected in clean ziplock bags from one asymptomatic and one symptomatic seedling from each treatment and each greenhouse (a total of 20 samples) and stored in a cold room (approximately 4^{o}C). Root and soil samples were placed into DNA extraction bags (Agdia Inc, Elkhart, IN, USA) with General Extraction Buffer 2 (Agdia Inc.) and masticated with a paddle blender. Contents were then cut into 5 mm segments and placed into 2 ml screw cap tubes with 1-3 mm ceramic beads. Tubes were cyclically frozen in liquid nitrogen and thawed in a heat block at 40^{o}C three times. Samples were then disrupted for 3 min at a frequency of 30 Hz using a mixer mill grinder (TissueLyser, Qiagen). DNA was extracted from the disrupted tissue using the DNeasy® Power Soil kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Extracted DNA was amplified and detected using qPCR multiplex assay as described by Bilodeau et al. (2014) to test for the presence of *Phytophthora* spp.

Results: Symptomatic roots were observed in 38% of seedlings. Symptomatic roots had

no effect on Caliciopsis canker area ($X^2_{1,79}$ = 0.33, P = 0.57) or depth ($X^2_{1,79}$ = 0.23, P = 0.63). There were interactions between inoculation treatment and symptomatic roots on Caliciopsis canker area ($X^2_{4,79}$ = 13.42, P = 0.01) but not on canker depth ($X^2_{4,79}$ = 1.60, P = 0.81). *Phytophthora* species were detected in 19 of the 20 root samples, nine of which came from asymptomatic roots and actively growing seedlings.

Needle cast disease

Methods: At the time of harvest, signs of a needle cast disease were present in 80% of seedlings from all inoculation treatment groups and in both greenhouses. Affected needles had red bands with clear delineations. Samples of foliage containing the needle cast signs were placed in sterile petri dish with sterile water until the fruiting bodies began to swell. Using a microscope to observe the spores emerging from the fruiting bodies, we determined the needle cast was caused by *Mycosphaerella pini* Rostr., a disease agent commonly found on pines. We classified the severity of needle cast for each seedling in the greenhouse trials on a scale based on the percentage of foliage that contained *M. pini* fruiting bodies: low (0-10%), medium (>10 and <50%), or high (>50%). Low *M. pini* severity accounted for 28% of all seedlings, medium for 59%, and high for 13%. We used ANOVA to look for needle cast effects on canker area (log transformed), canker depth, and interactions with treatment effects.

Results: The severity of needle cast did not affect canker area ($X^2_{2,74}$= 1.70, P = 0.43) or depth ($X^2_{2,74}$= 0.19, P = 0.91). There were interactions between needle cast severity and inoculation treatment effects on canker area ($X^2_{8,74}$= 18.93, P = 0.02) but not canker depth ($X^2_{8,74}$= 10.12, P = 0.26).

Foliar chlorosis and necrosis

Methods: Widespread chlorosis and necrosis were observed in needle tips on 96%

seedlings across all inoculation treatment groups and in both greenhouses. There were no fruiting bodies or other disease signs associated with the chlorotic and necrotic foliage. At the time of seedling harvest, we ranked each seedling on a scale of 1-4 based on the percentage of foliage with chlorotic or necrotic tips. Seedlings were ranked as 1 (0% necrotic tipped foliage and ≤ 10% chlorotic tipped foliage; 26% of seedlings), 2 (< 50% necrotic tipped foliage; 4% of seedlings), 3 (0% necrotic foliage and ≥ 50% chlorotic foliage; 24% of seedlings), or 4 (≥ 50% necrotic foliage; 46% of seedlings). We used ANOVA to look for needle damage effects on canker area (log transformed), canker depth, and interactions with treatment effects.

Results: The severity of needle tip discoloration did not affect canker area ($X^2_{4,79} = 0.24$, $P = 0.63$) or depth ($X^2_{4,79} = 0.53$, $P = 0.47$). There were interactive effects between discoloration severity and inoculation treatment on canker area ($X^2_{4,79} = 12.95$, $P = 0.01$) but not depth ($X^2_{4,79} = 7.39$, $P = 0.12$).

Conclusions

Because symptomatic roots, needle cast severity, and needle tip discoloration all had interactive effects with inoculation treatment on canker area, they were included as main effects along with root collar diameter, inoculation treatment and block in preliminary analyses. Their inclusion, however, did not change the significance of any of the pairwise comparisons of inoculation treatment on canker area or depth, suggesting the interactive effects on canker area were much smaller than the main effects of inoculation treatment. Phytophthora root rot, needle cast, and needle tip discoloration were excluded from the analyses included in the methods and results described in Chapter 3.