Spruce decline in Michigan: Disease Incidence, causal organism and epidemiology
MDARD Hort Fund (791N5500426) – Final report
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Project Justification:
Spruce trees are common throughout Michigan. In fact, spruce rank second in abundance and make up 12% of all urban and community trees in Midwestern cities. Michigan is a major nursery of landscape trees with annual sales exceeding $250 million. Conifers, including spruce, make up well over half of the nursery production acreage in our state. Spruce, especially Colorado blue spruce (*Picea pungens*), have succumbed to a new disease characterized by needle loss and branch mortality that is first evident on the lowest branches and over time progresses up the main stem. We have named this syndrome spruce decline. Generally, tree declines are slow, complicated diseases involving environmental and biotic stressors that allow a pathogen to cause disease. Initial work on the spruce decline suggested that 1) The pathogen causing decline most likely caused cankers on the tree and 2) It was a new pathogen because known pathogens did not cause symptoms similar to that found on declining trees. To assist the state's nursery industry, this work endeavored to identify the pathogen(s) responsible for spruce decline.

Objectives:
The work associated with this funding concentrated on the pathogen causing spruce decline and has addressed four questions:

1. Where is spruce decline found in Michigan?
2. What taxon is the most likely pathogen causing spruce decline?
3. How variable is the pathogen, and what is the species name?
4. How virulent is the pathogen, and are all spruce species susceptible to the disease?

Accomplishments:
As detailed below, this work documented that spruce decline is common across Michigan's Lower Peninsula. The pathogen causing cankers on dying branches associated with spruce decline was one or more *Diaporthe* species. Five "haplotypes" (= genetically distinct forms) of *Diaporthe* were isolated from cankers and the haplotypes differed in their ability to cause cankers on spruce. Colorado blue spruce was most susceptible to the virulent *Diaporthe* haplotypes, with Norway and white spruce displaying lower susceptibility. Meyer, Black Hills and Serbian spruce were resistant to all *Diaporthe* haplotypes and should be considered as potential replacements for Colorado blue spruce in landscape plantings.

To address questions one and two above, a survey of spruce in the Lower Peninsula was carried out by sampling eight trees with spruce decline in six locations in four areas of the Lower Peninsula (See Fig. 1 for sampling locations). A single branch was obtained from each sampled tree, checked for cankers, and fungi were isolated from two cankers per branch. Declining trees were always found within 0.2 miles of a chosen sampling location, with the exception of Mackinaw City where spruce decline was largely absent in the city proper. Thus, we concluded

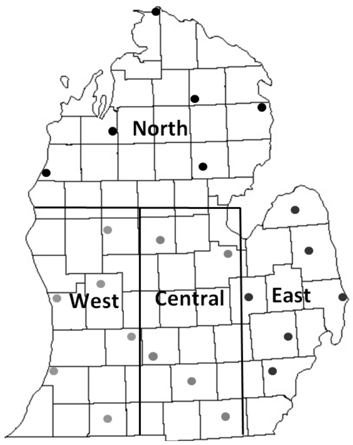


Figure 1. Sampling locations within each of the four areas of the Lower Peninsula of Michigan.

Figure 2. A segment of the top layer of bark was removed to reveal a brown canker, with uninfected plant tissue appearing white.

that spruce decline is widespread and common throughout the Lower Peninsula. Brown cankers, which could be located only after scrapping off the outer layer of bark, were found on all sampled trees (Fig. 2).

Numerous fungi were isolated from the cankers (Fig. 3). This was expected since the sampled branches were dying and, thus, were prone to attack by weak pathogens and saprophytes that can infect the branch as plant defenses diminish as it dies back. The six most commonly isolated fungi are listed in Figure 3, along with known spruce pathogens, *Cytospora* and *Fusarium*. To be considered a major cause of spruce decline, a potential fungal pathogen must be common in all areas where the disease is found. Since spruce decline is widespread, *Diaporthe* and *Paraconiothyrium* were the two most likely genera to be considered as the pathogen causing spruce decline. The remaining genera were either absent in some areas where spruce decline was common, or were too rare in one or more area. Initial inoculation studies found that *Paraconiothyrium* was not able to cause disease on spruce (unpublished data), while *Diaporthe* could cause cankers (Detailed below).

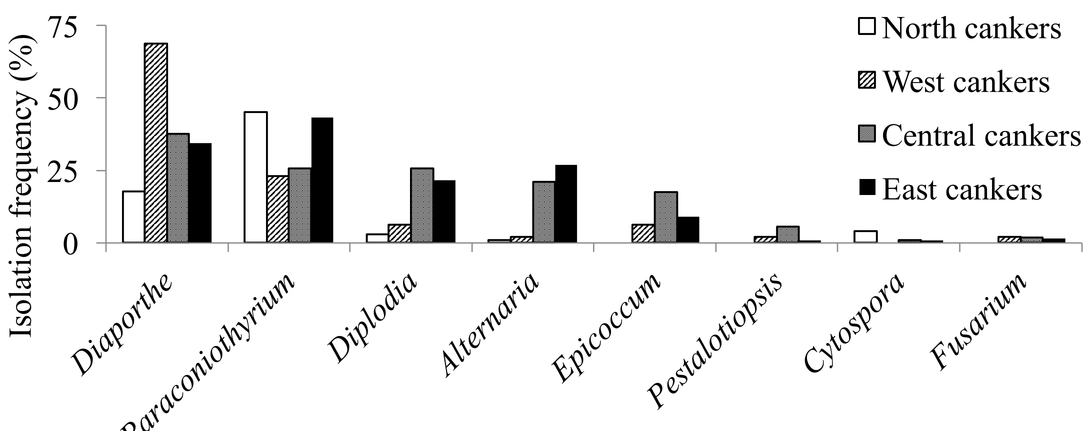


Figure 3. Frequency of isolation of fungal genera from cankers collected as part of the survey. Area total percentages are based upon number of cankers that contained the genus.

The Internal Transcribed Spacer (ITS) region was sequenced for 54 *Diaporthe* isolates collected during the survey. Five total haplotypes (Note: haplotype is equivalent to a genotype in this context) were detected among the isolates, which differed from each other by one to seven base-pair changes (Fig. 4). All five haplotypes were collected from Colorado blue spruce, a common landscape tree, which also appears to be very susceptible to decline (see below for details).

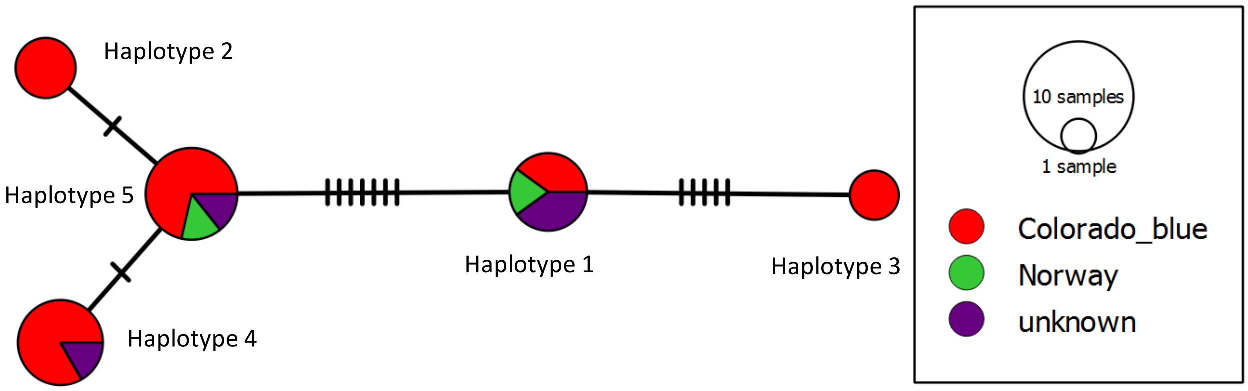
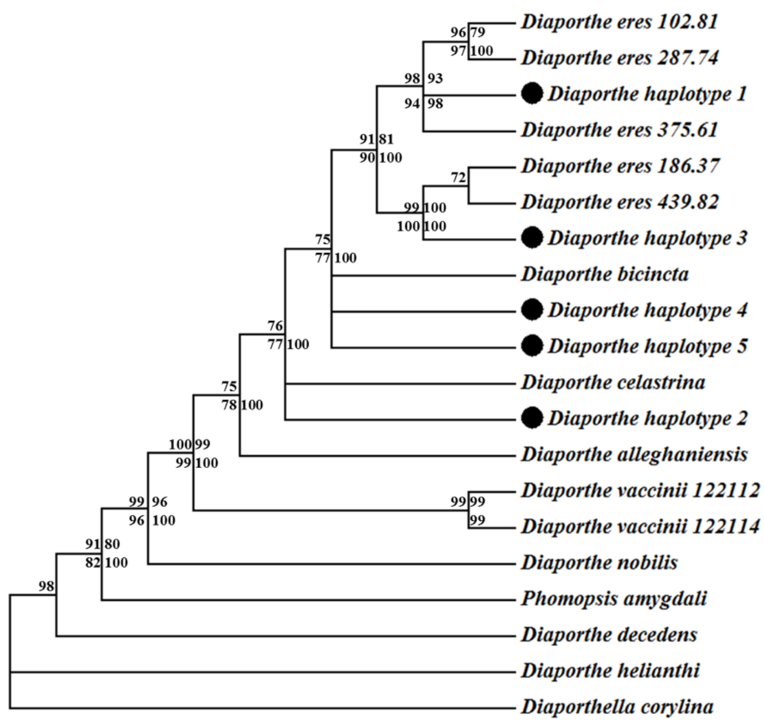


Figure 4. Relationships among *Diaporthe* haplotypes collected in this study, with host of isolates within each haplotype represented as pie charts. Tick marks represent base-pair changes between haplotypes.

The β-tubulin gene was also sequenced for 23 of the 54 isolates previously sequenced for the ITS region. This additional sequencing allowed us to make a phylogenetic analysis for the purpose of identifying the specific *Diaporthe* species that is causing spruce decline. Unfortunately, the five haplotypes fall into a poorly resolved portion of the tree, with haplotypes 1 and 3 being part of the species *Diaporthe eres* and haplotypes 2, 4 and 5 falling into a poorly resolved part of the tree that includes *Diaporthe bicinta* and *D. celastrina* (Fig. 5).

Figure 5. Phylogenetic tree based on analysis of ITS and β-tubulin gene sequence data. A black circle symbol indicates the taxon was a haplotype group involved in spruce decline



Each haplotype was also evaluated for their ability to cause disease on spruce by performing greenhouse inoculations of three and four-year-old spruce. In the initial experiment we evaluated each haplotype's ability to cause cankers on three spruce species, Colorado blue and Norway spruce, which are commonly planted as landscape trees in Michigan, and white spruce, which is both native and planted in Michigan. Our results indicated that the five haplotypes differed in their virulence on spruce with haplotypes 2, 4 and 5 having the highest virulence on spruce species, while haplotype 3 caused little or no disease on spruce (Fig. 6). Haplotype 1 caused some disease on Colorado blue spruce and almost no disease on Norway and white spruce. **Most importantly, the pattern of virulence is closely tied to the pattern of haplotype relatedness** (Fig. 4) with haplotypes 2, 4 and 5 forming a genetically related and highly virulent group of isolates, and haplotype 3, which is genetically distinct from haplotypes 2, 4 & 5, was largely avirulent. Further, haplotypes 2, 4 & 5 are also evolutionarily distinct from haplotypes 1 & 3 (Fig. 5).

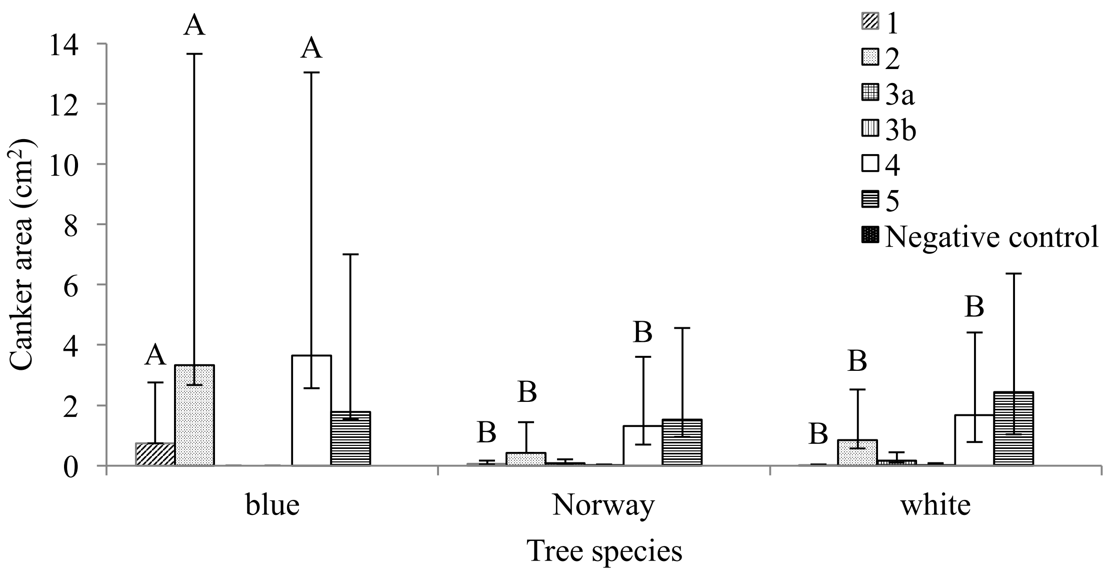


Figure 6. Canker area (cm^2) caused by *Diaporthe* haplotypes on blue, Norway, and white spruce. Error bars represent 95% confidence interval. Capital letters above the bars indicate differences among species in their susceptibility to a particular *Diaporthe* haplotype. Note: The spruce species did not differ in their response to the haplotype 3 isolates, 3a and 3b.

Results from the first experiment suggested that spruce species differed in their susceptibility to the *Diaporthe* haplotypes. Therefore, a second experiment was performed by inoculating six spruce using two virulent (4 & 5) and one avirulent (3) haplotypes. Spruce species differed in their susceptibility to *Diaporthe* haplotypes 4 and 5, with Colorado blue spruce being most susceptible, followed by Norway and white spruce, with Black Hills, Serbian and Meyer spruce being resistant (Fig. 7). All six species were resistant to haplotype 3, which confirmed the results from the first experiment. These data suggest that other species of spruce (i.e., Black Hills, Serbian and Meyer) could be considered as a substitute for the commonly planted but highly susceptible Colorado blue spruce. Two other inoculation experiments were carried as a part of this work. One experiment evaluated different seed sources of Colorado blue spruce. Results from this work suggest that there is variability among individuals, but, in aggregate, all nine seed sources evaluated were susceptible to haplotype 4 (Data not shown). A final experiment was meant to evaluate fungicides for their potential to control spruce decline, but problems with fungicide phytotoxicity made any useful evaluation impossible (Data not shown).

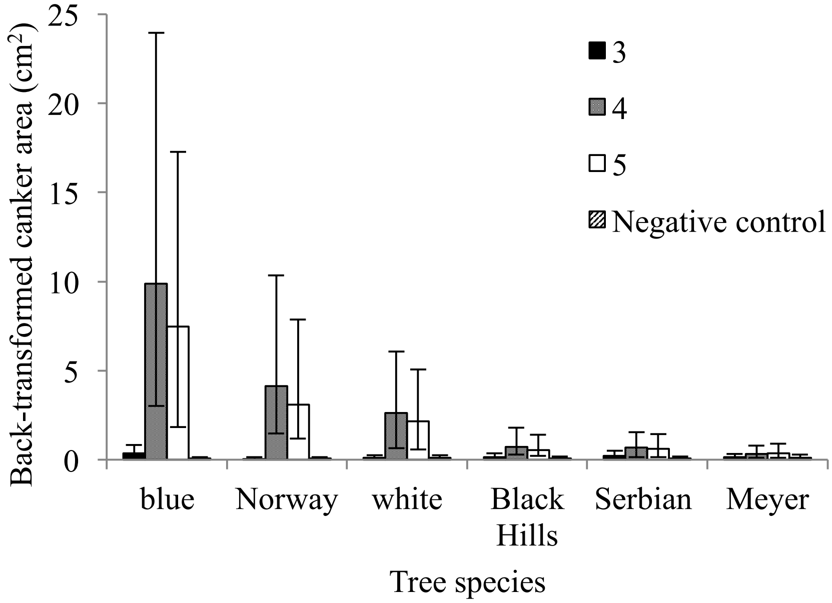


Figure 7. Mean canker area (cm^2) of isolates on three-year-old blue, Norway, white, Black Hills, Serbian, and Meyer nursery spruce trees. Error bars represent the 95% confidence interval.

Impacts:
We documented that spruce decline is caused by canker causing fungi in the *Diaporthe* genus. Colorado blue spruce is especially susceptible to the pathogen, and Norway and white spruce are moderately susceptible. We are now in a position to explore options for dealing with the spruce decline epidemic. One option is to explore possibility of using resistant spruce such as Meyer, Serbian and Black Hills spruce instead of Colorado blue spruce in landscape plantings. In addition, fungicide testing can also be initiated to control the pathogen. Finally, continuing work is attempting to determine which environmental stressors may be weakening trees and allowing spruce decline to progress and spread.

Summary statement:
Spruce decline, which is common throughout Michigan's Lower Peninsula, is caused by a fungal pathogen in the genus *Diaporthe*. Colorado blue spruce is highly susceptible to *Diaporthe*, Norway and white spruce are moderately susceptible and Meyer, Serbian, and Black Hills spruce are resistant.

Funding Partnerships:
This work was also funded by MSU GREEEN at $76,250.