Projects Title: Spruce decline in Michigan: Disease incidence, causal organisms & epidemiology

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Accomplishments during funding period:
Objective 1. What pathogens are causing spruce decline?
a. Initial survey of Spruce Decline in Michigan.
Our first work on this question was to survey spruce across the Lower-Peninsula of Michigan (Fig. 1). Samples were obtained from six sample sites within each of the four areas depicted in Fig. 1 and eight trees that were separated by at least 0.1 miles were sampled within each site. Cankers were detected by removing the top layer of bark (Fig. 2).
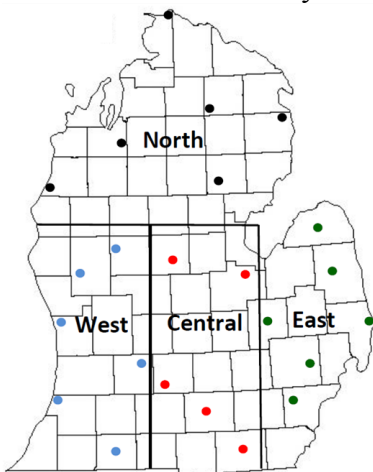

Figure 1. Regions and populations sampled in the spruce decline canker survey. Dots represent populations

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

Spruce decline symptoms were common throughout the Lower Peninsula. Isolations were made from 134 cankers, which yielded 493 isolates. A wide variety of fungi were isolated ranging from known endophytes (*Parapheaeosphaeia*), weak pathogens that are known to invade dying branches (*Alternaria, Epiicocum, Fusarium,* and *Pestalotiopsis*), to known pathogens of spruce (*Cytospora*) (Fig. 3). However, *Cytospora* comprised only 2% of our samples and is clearly not the cause of spruce decline. *Diplodia* comprised 18% of samples, but was rare in both the western and northern areas and, thus, could not account for the spruce decline found in these

area. Further, we suspect that *Diplodia* found on spruce is due to a severe epidemic of *Diplodia* tip blight on pines that is occurring in many areas of Michigan. Thus, *Diplodia* on spruce is due to the spill-over effect from the epidemic on pines. In contrast, *Phomopsis* was common across all areas of the Lower-Peninsula and preliminary experiments suggested that isolates were virulent on spruce and could cause symptoms similar to that seen with spruce decline.
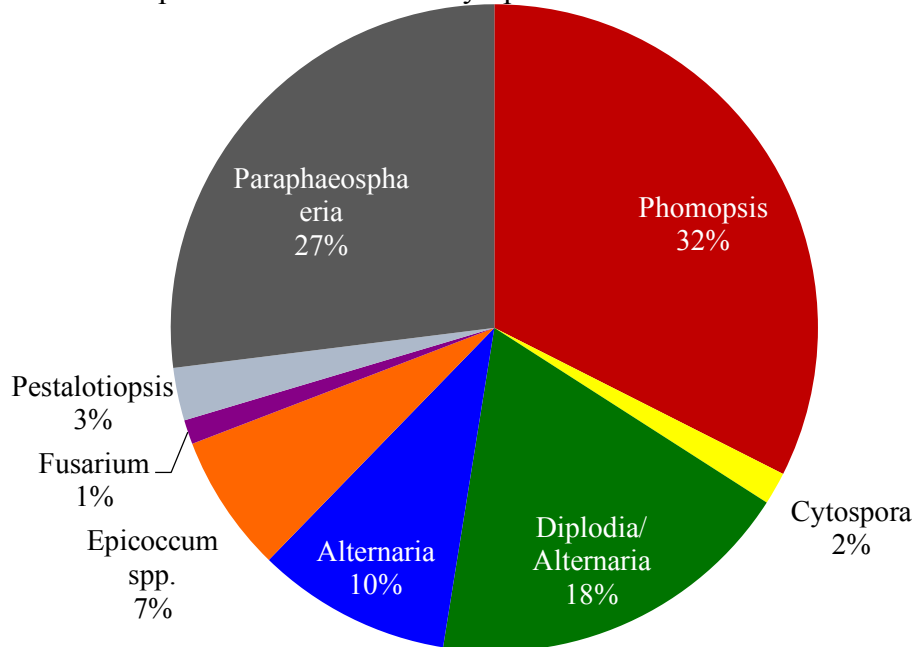

Figure 3. Identified fungal isolates from all region of the Lower Peninsula (n=493)

b. Molecular characterization of *Phomopsis* isolates
Sequences of the internal transcribed spacer (ITS) regions of the ribosomal RNA regions of our Phomopsis isolates revealed five *Phomopsis* different haplotypes could be found on declining spruce in Michigan (Fig. 4).
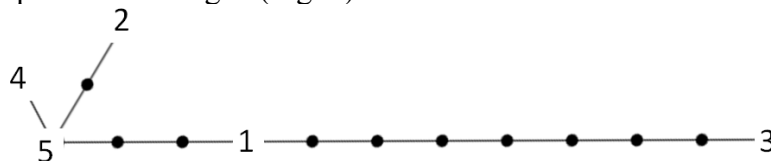

Figure 4. Minimum spanning tree representing the *Phomopsis* haplotype network. Lines between black dots are representative of necessary single base pair (bp) changes between groups.

Objective 2. Do spruce species differ in their susceptibility to spruce decline?

Two greenhouse inoculation experiments were carried out to investigate whether *Phomopsis* could cause cankers on spruce and also determine the level of susceptibility of various spruce taxa. In the first experiment, isolates from all five *Phomopsis* haplotypes (Fig. 4) were used to inoculate three-year-old Colorado blue, Norway and white spruce trees. Results from this work determined that isolates from haplotypes 2, 4 and 5 were the most virulent on spruce, while isolates from haplotypes 1 and 3 were less virulent (Fig. 5). Further, Colorado blue spruce was highly susceptible to *Phomopsis*, while Norway and white spruce were mildly susceptible.
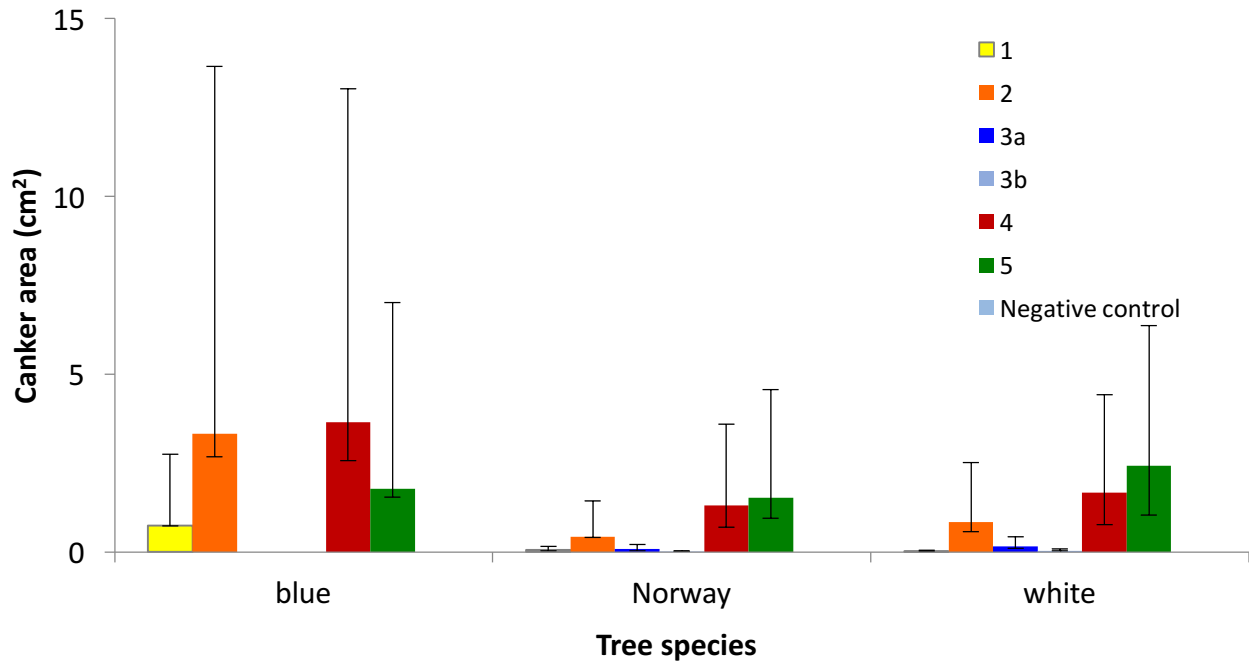
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Figure 5. Mean *Phomopsis* canker area (cm^2) of isolates on 3-year-old blue, Norway, and white nursery spruce trees. Error bars represent the 95% confidence interval.

A second experiment found that spruce taxa differed in their overall susceptibility to haplotypes 3, 4 & 5 with Colorado blue being the most susceptible, followed by Norway, then white spruce (Fig. 6). Black Hills, Serbian and Meyer spruce are only slightly susceptible to *Phomopsis* and may represent alternatives to using Colorado blue and Norway spruce in the landscape.
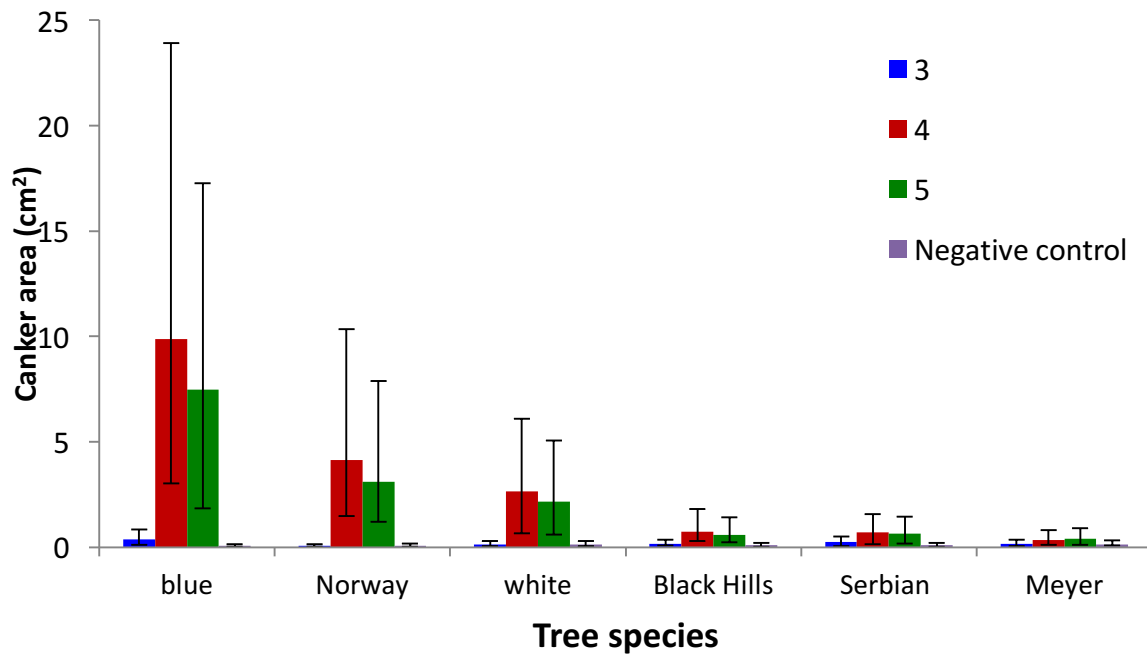

Figure 6. Mean *Phomopsis* canker area (cm^2) of isolates on 3-year-old blue, Norway, white, Black Hills, Serbian, and Meyer nursery spruce trees.

Objective 3. Identify biotic and abiotic factors associated with spruce decline.
Work on this objective is currently be addressed in year two of the project. We will be carrying out transect in eight areas of across the Lower-Peninsula of Michigan to determine which factors are associate with disease symptoms. Results from this work will assist growers and homeowners by identifying factors that pre-dispose trees to spruce decline.

Impacts:
Our work has demonstrated that *Phomopsis* is the likely cause of spruce decline, which is now found widespread across the Lower-Peninsula of Michigan. Our current work is targeted at identifying the exact species of *Phomopsis* that is causing spruce decline so we can register fungicides to manage the epidemic. Our preliminary work on species identification indicates that the five haplotypes represent two and possibly three different species of *Phomopsis*. Additional sequencing of the *Beta*-tubulin gene will resolve the species identities.

Our work has demonstrated that Colorado blue, Norway and white spruce are highly susceptible to spruce decline, but Serbian, Meyer and Black Hills spruce are only slightly susceptible and may represent alternatives to planting Colrado blue and Norway spruce in the landscape.

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Publications:
Cregg, B., C. McTavish, A Jarosz, J. McDonnell and D. Fulbright. 2015. What is spruce decline and what should you know about it? MSU extension Bulletin (http://msue.anr.msu.edu/news/what_is_spruce_decline_and_what_should_you_do_about_it)