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Evaluation of Species Susceptibility and Chemical Management of Phomopsis Decline

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The long-term goal of this research continues to be to provide some level of management for a disease we have called spruce decline or spruce decline. Little is known about this new and widespread disease, its fungal pathogen and its interactions with the other prominent diseases and pests of spruce. Symptoms of this disease are needle loss and branch death caused by multiple cankers located along the branches of the trees. To reach our long-term goal we set three short-term objectives: 1) Determine the susceptibility of various spruce species to *Phomopsis* strains isolated from spruce and arborvitae trees in Michigan, and 2) Determine fungicide products and application periods for best management practices. This final report provides information related to the second year of what we consider to be a 3-year project.

Objective 1: To determine the susceptibility of various spruce species to *Phomopsis* strains isolated from spruce and arborvitae trees isolated from various spruce trees in Michigan.

Our masters degree-level graduate student, Chrissy McTavish has continued to isolate fungal strains from various species of spruce in Michigan and determined that they fall into 5 groups based on ITS sequences. The 5 groups of *Phomopsis* associated with spruce decline have no association with fungal morphology, geographic region, habitat, or host species. However, they are commonly found on spruce in Michigan. Overall, *Phomopsis* isolates relating to groups 1-5 are found more than 50 percent of the time, much more frequently than any other species. Another fungal species that was found associated with cankers is *Diploidia* (about 15 percent of the time) which was not surprising as it was already reported to be a pathogen of spruce and we consider its presence to be "spill over" from the tip blight epidemic currently occurring on pines around Michigan. The low number of *Cytospora* strains recovered from cankers (less than 5 percent) was surprising as spruce decline closely mimics *Cytospora* canker and we believe that there continues to be an assumption in the plant community that current symptoms on spruce are caused by *Cytospora* canker or needle cast diseases.

To determine if *Phomopsis* strains recovered from Michigan spruce trees could actually cause disease on spruce and which strains were the most virulent (aggressive), six *Phomopsis* isolates representing the 5 genetic groups were inoculated on three spruce species: Colorado, white, and Norway. It was determined that 3 of the 5 distinct genetic groups could cause cankers on spruce and that strains in 2 groups were the most pathogenic.

Strains in he most pathogenic groups, 4 and 5 (based on disease severity and host range), were very similar differing by only a single base pair in our DNA sequence studies. Species determination of both groups remains unresolved, suggesting a novel lineage of *Phomopsis* on spruce. Group 3, the least pathogenic *Phomopsis* group was the most genetically distinct, with at least 8 base pair changes from the next closest group. Group 3 was phylogenetically determined to match existing strains of *Diaporthe eres.*

In the spring 2014, a young spruce tree inoculation trial was initiated. In the two previous years we had performed these trials to determine if the *Phomopsis* strains isolated from cankers were pathogenic on spruce. In this trial, we wanted to determine to the reaction of different species of spruce. Six species of spruce were used to test susceptibility of spruce to *Phomopsis*. Three different strains of *Phomopsis* were used, isolates 53, 82, and 15. Strains 53 and 82 were considered through earlier trials to be the more virulent strains and strain15 was considered the least virulent. Each of these strains was inoculated into 60 of each species, along with uninoculated controls. To inoculate, a small 2x2x3 mm hole was made approximately 4 cm above the soil line on the main stem of the young trees, and a fungus was inserted into the hole. The hole and plug were then wrapped with Parafilm. The trial will run for 10 weeks after inoculation.

Table 1. Spruce trees listed in their order of susceptibility to Phomopsis. Blue spruce was the most susceptible and Serbian and Meyer the least susceptible.

Common Name	Species Name	Commercial Importance	Origin	Age of Tree
Colorado blue	*Picea pungens glauca*	Yes	North America	2-1
Norway	*Picea abies*	Widely planted	Europe	2-1
White	*Picea glauca*	Xmas/ornamental	North America	2-1
Black Hills	*Picea glauca densata*	Landscape/xmas tree similar to white spruce	North America	2-2
Serbian	*Picea omorika*	Increasing popularity	SE Europe	2-1
Meyer	*Picea meyeri*	Mentioned as alternative to blue spruce	Northern China	2-2

In addition, a Colorado blue spruce seed source trial was initiated in 2014. 25 Apache, 25 Blue Wonder, 25 Wolf Creek, 25 Dolores, 50 Baby Blue, 50 Majestic, 25 San Juan, 25 Kaibab, and 25 Misty Blue spruce were included in this trial. All trees were inoculated with *Phomopsis* strain 53 and results indicated that all Blue spruce seed sources were equally susceptible (data will be shown in the Masters Thesis of Ms. Chrissy McTavish).

Objective 2: Our second short-term objective was to determine fungicide products and application for best management practices.

Fungicide Injection

On May 16, 2013, a tree injection technique was applied to these trees with the help of Arborjet. We determined at an earlier meeting that this would probably be the best method for a safe delivery of fungicides in the landscape environment. Also, since most of the damage is confined, at least for a while, to the lower branches of the tree and then spreads upward, we thought this injection method would provide the best application to the targeted branches. We injected for the second time in June 2014 and rated the previous year's injection in both May and again in June 2014. In this third year of the study, the second year injection was evaluated in June 2015 and again in August 2015.

Table 2. Highest diseased branches observed on blue spruce after injection with 3 fungicides.

Treatments	2013	2014		2015
		May	July	
Alamo	111.3cm	138.9cm	143.8cm	161.9cm
Arbotec	115.3cm	143cm	156.4cm	161.5cm
Phosphojet	125.4cm	137.2cm	139.5cm	163.3cm
Control	122.3cm	141.9cm	145.2cm	169.5cm

In general, the diseased progressed up the branches of the tree, regardless of the injected material. There were no significant differences in the height observed, but overall disease on trees with Phosophojet injections progressed the least distance after spraying.

A disease index where the first 50 cm (bottom) of the tree was evaluated and a second 50 cm to 100 cm (middle) was also evaluated was performed on each tree in the plot. The bottom and middle sections of the trees were evaluated with a rating system where 1 = no disease observed; 2 = 1–25% infection; 3 = 26–50% infection; 4 = 51–75% infection; and, 5 = 76-100% infection observed.

Table 3. Overall disease rating after injection in 2013 and 2014.

Treatments	2013	2014 May	2014 July	2015
Alamo	2.3	2.3	3.1/2.3	2.5/2.4
Arbotec	2.9	3	3.6/2.4	3.2/2.4
Phosphojet	2.4	2.9	3.4/2.5	2.9/2.3
Control	2.3	2.4	3/2.3	2.8/2.0

July of 2014 and 2015 ratings were made by dividing the tree from 0cm to 50cm and 50cm to 100cm. The number prior to the slash corresponds to 0 cm to 50 cm. The number following the slash corresponds to 50 cm to 100 cm.

Summary of Injection Trial

Alamo

1st Treatment Block

One out of four blue spruce was severely impacted by Phomopsis decline. An overall end of experiment rating of 7 out of 10 for all 4 blue spruce was recorded.
A white spruce tree was rated a 4 on a 1 – 10 score with 10 being best.

2nd Treatment Block:

Again, one out of four blue spruce was severely impacted by Phomopsis decline. The other 3 looked very good. **An overall rating of 7 out of 10 for all the blue spruce in the plot was pulled down by one very bad looking tree as three of the best looking trees were in the entire plot were in this treatment.**
White spruce **w**as rated a 9 out of 10

Arbotec

1st Treatment Block

One out of 4 blue spruce was severely impacted by Phomopsis decline, one tree was rated medium, and 2 blue spruce were improved at the end of 3 years. An overall rating of 7 out of 10 was recorded for this group of trees.
The white spruce was rated 7 out of 10.

2nd Treatment Block
Two out of four blue spruce were rated medium in terms of impact by Phomopsis decline. The group of trees garnered an overall rating of 8 out of 10.
The white spruce was rated 6 out of 10.

Phosphojet

1st Treatment Block
One out of 4 blue spruce was severely impacted by Phomopsis decline and the other three were improved at the end of 3 years.. Overall rating of the group of trees was 9 out of 10.
The white spruce tree received a rating of 9 out of 10.

2nd Treatment Block
Four out of four blue spruce looked good. An overall rating of 10 out of 10 was assigned to these trees.
The white spruce rated a 10.

Control

1st Treatment Block
All four blue spruce looked good. They received an overall rating of 10 out of 10.
The white spruce was also rated a 10 out of 10.

2nd Treatment Block
All four blue spruce looked good. Overall rating of 10 out of 10.
White **spruce** was rated a 7 out of 10,

Conclusion
Phosophojet appeared to be the best material overall, but it was difficult to separate it from the controls as the controls were just as healthy. This is unfortunate as the disease is still progressing through the landscape throughout Michigan. Four of the 32 trees in the study showed severe infection and none of these were in the control plots. This severe disease appearance parallels what is seen in the landscape as some blue spruce appear unaffected, others appear moderately affected and a minority (but significant number) are seriously affected. It could be stated that the injection itself caused this severe damage, as these trees were randomly selected for their treatments. But it is my opinion that these trees were randomly distributed in the plot and the control plot did not happen to have any trees with the ability to show severe infections. Taken as a whole, the data do not support tree injection technique to control Phomopsis decline. However, Phosophoject treated trees reduced the height of the disease on the tree, and the Alamo injections had the overall best looking trees in the plot. Only one severely infected tree skewed the Alamo treatment.

Spray trials of Spruce Christmas trees

In addition, at MSU Kellogg Forest, four fungicides were applied to a Colorado blue spruce Christmas tree plantation. The purpose of this was to determine if there are any fungicides that could improve the quality of these trees. These trees were in various states of disease and a disease index was determined based on the amount of the disease observed by applying two ratings, the height of the dead branches and the number of diseased branches within three sections of each tree. While *Phomopsis* was found on these trees, it is difficult to say that *Phomopsis* was the only cause of their overall poor condition. Materials were applied to the trees every two weeks from the end of May through the end of August. This was repeated beginning May 2014. Evaluation of the trees occurred in May 2014. Treatments currently underway in 2014 will be evaluated in June 2015.

In 2014, we re-evaluated our disease index ratings to provide a better picture of the disease incidence and severity in field grown trees. These small changes made the evaluations simpler to understand. The trees were divided into 3 sections; top, middle and bottom. Each section was rated where 1 = no disease; 2 = 1–25% disease; 3 = 26–50%; 4 = 51–75%, and 5 = 76–100% disease. The single symptom used to determine disease in the field was tip death of the branch.

Table 4. Christmas Tree spray trial summary in 2015 after 3 years of applying 4 materials.

Treatment	2013		2014		2015	
	bottom	middle	bottom	middle	bottom	middle
Alamo/Banner	4	1.1	4.4	1.8	1.5	1.5
Clearys 3336	3.8	1.5	3.9	1.9	1.2	1.3
Spectro 90	4.1	1.7	4.2	2.3	2.2	1.9
Control	4.2	1.5	4.4	1.9	1.6	1.3

Summary

Due to the improvement in the disease status of the trees in the healthy control plot there are no significant data to present. However, the trees that showed the greatest improvement and overall best appearance were trees sprayed with Cleary 3336. This is the material that has been recommended for Phomopsis control in nurseries for several years. Clearys 3336 reduced disease in both the bottom and middle portions of the trees. The active ingredient of Clearys 3336 is thiophanate methyl and this material was also in Spectro 90, but that was the poorest performing material in the study.

It is believed that all of these treatments reduced disease in the entire plot and disease pressure was reduced by the third year allowing good branch growth and extension without expressing tip death (symptom of disease), even in the controls. It could have been a change in the environmental aspects (weather) required for disease, but if anything, the wet springs and prolonged wet periods experienced during these studies do not support this idea. My suggestion is to continue Cleary 3336 as the suggested material for management of Phomopsis decline of spruce. We applied it every two weeks throughout the summer, and this is not warranted as the control plots also improved without application of materials.