A Phomopsis Canker Associated with Branch Dieback of Colorado Blue Spruce in Michigan

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ABSTRACT

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Branch dieback is a common problem of Colorado blue spruce (*Picea pungens* f. *glauca*) in Michigan nurseries. Isolations from 100 cankers obtained from 20 seedlings exhibiting dieback symptoms all yielded *Phomopsis occulta*. Both mycelia and conidia of *P. occulta* were shown to cause branch dieback. Root pruning of spruce resulted in an increase in lesion lengths from 8.0 mm to 32.2 mm and an increase in the number of seedlings with girdling cankers from 50 to 90%. Current year's growth had longer lesions (42.9–64.3 mm) after mycelial inoculations than older tissue (9.0–32.2 mm) had. Wounding was required for infection by *P. occulta* mycelia but was not required for infection by conidia. The results show that *P. occulta* causes branch dieback in Colorado blue spruce, especially on young tissue and root-pruned seedlings.

Since 1986, Michigan nursery growers have noticed branch dieback and reported annual losses of 30% of Colorado blue spruce (*Picea pungens* Engelm. f. *glauca* (Regel) Beissn.) ranging from 30–40 cm to 2–3 m in height. One nursery reported that 80% of the crop was lost when credit claims were taken into account. Additional losses occur because many affected trees require cosmetic pruning before sale, trees must be sold at a lower price because of poor form, dead plants must be destroyed, warranteed plants must be replaced, and the grower's reputation is questioned.

The initial symptom of the disease was needle loss concentrated on the lower part of the tree. Further symptom development was expressed by lower branch dieback and in extreme cases death of the entire plant. Inconspicuous stem cankers were always associated with needle loss. When trees resumed growth in the spring, shoots curled downward, browned and died. While similar symptoms occasionally developed under field conditions, symptom development seemed exacerbated by harvesting. Symptoms of this disease appeared similar to those caused by a common canker-causing pathogen of mature Colorado blue spruce, *Cytospora kunzei* Sacc. var. *picea* Waterman, which is considered the most destructive disease of Colorado blue spruce in Michigan (4). Samples of spruce showing characteristics of decline submitted to the Michigan State University Plant and Pest Diagnostic Clinic consistently yielded cultures of *Phomopsis occulta* (Sacc.) Traverso. The identification of *P. occulta* was confirmed by Dr. Alvin Rogers of the Department of Botany and Plant Pathology, Michigan State University.

In 1986, Sanderson and Worf (5) reported that *P. occulta* caused dieback symptoms on four spruce hosts in Wisconsin: *P. abies, P. obovata, P. glauca* 'Densata,' and *P. p.* f. *glauca* after artificial inoculation with spores. Colorado blue spruce was the most susceptible of the four spruce in their research. This study was initiated to determine the role of *Phomopsis* as a factor in the decline of nursery-grown Colorado blue spruce in Michigan, confirm its presence on symptomatic spruce, demonstrate its ability to cause disease using inoculation studies, and evaluate the influence of wounding, tissue age, and root pruning on disease development.

MATERIALS AND METHODS

Isolations. Fifty cankers from Colorado blue spruce were collected each fall in 1988 and 1989 from 10 plants that exhibited dieback symptoms in nursery holding areas. Each sample was rinsed under running tap water for 3 min, surface sterilized for 10 min in 1.05% NaOCl, and then rinsed twice with sterile water. The outer bark was removed and four small sections from the margin of each canker were plated directly on potato-dextrose agar (PDA) in 90-mm diameter petri dishes. The culture dishes were incubated at room temperature for 7 days before evaluation.

Inoculum. Seven-millimeter disks of actively growing mycelium taken from cultures directly plated from cankered stems of Colorado blue spruce were grown on PDA at room temperature for 14 days under fluorescent light. Two 14-day-old cultures of an isolate of *P. occulta* were macerated for 2 min in a commercial blender with 30 ml of sterile water to produce a mycelial inoculum. Different isolates were used in each inoculation experiment. Sterile PDA was used as a control inoculum and was prepared in the same manner.

Conidial suspensions were obtained by incubating cultures of each of five isolates under continuous fluorescent light for 16 days, flooding those cultures with mature pycnidia with sterile water for 30

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min, scraping the surface using a sterile needle, and straining through four layers of cheesecloth. The concentration of alpha conidia in the aqueous suspension was adjusted to approximately 1.74×10^4 conidia per milliliter using a hemacytometer.

Pathogenicity. One-year-old Colorado blue spruce plants grown in plug trays were potted in a peat-based, soilless commercial mix (Baccto, Michigan Peat Company, Sandusky, MI) 3 mo prior to inoculation. Seedlings were placed in a greenhouse with 16 hr supplemental fluorescent lighting and day/night temperatures of 24/20 C.

Four inoculation experiments were completed in which different inoculation procedures and stresses were evaluated. At the end of each experiment three samples were taken from half of the seedlings for reisolation of the fungus using procedures as previously described.

In experiment 1, 10 trees were wounded 10 cm above the soil line by pushing a sterile pin, 1 mm in diameter, through the stem. A sterile hypodermic needle was used to inject macerated mycelial inoculum into the wound until a small amount of inoculum was seen exuding from each side of the wound. Control trees were inoculated with sterile PDA. All wounds were wrapped with a strip of Parafilm. To simulate a harvest procedure, the root mass was pruned on half of the trees in each treatment by removing the lower 6 cm of a 10-cm root plug. Treatments were arranged in a completely randomized design as a 2×2 factorial experiment with five replicate trees. After 6 mo, lesion length was measured by scraping away the outer bark and measuring the length of the necrotic region.

A second experiment was conducted using the same treatment as in experiment 1 but two wounds were made on each seedling instead of one. The first wound was located 10 cm above the soil line and the second wound was located on the terminal, in the center of the most recent flush of growth. Both wounds on one tree received the same treatment. Twenty trees were inoculated with *P. occulta,* and 20 trees were inoculated with sterile PDA as described above. Lesion length was measured 5 mo later. The experimental design was a completely randomized $2 \times 2 \times 2$ factorial experiment with 10 replicate trees per treatment. The incidence of cankers that killed terminal leaders of seedlings in each treatment was analyzed using a chi-square analysis.

In the third experiment, wounding, sealing the wound with Parafilm, and inoculating at an upper and lower location on the plant were variables evaluated. These factors composed 16 treatments in a 4-way factorial experiment with 10 replications in a completely randomized design. Lesion length was measured after 4 mo.

In the fourth experiment, two techniques of wounding were used to determine if wounds were required for conidial penetration. Twenty trees were wounded by pruning the distal 3 cm from each of five branches. Another set of 20 trees was injured by cutting needles in half on five branches per tree. Twenty control plants remained unwounded. An average tree had 15 branch tips. A conidial suspension of 1.74×10^4 alpha conidia per milliliter was sprayed with an atomizer to the point of runoff on half of the trees in each wound treatment. Control plants were sprayed with sterile water. Each tree was covered with a clear polyethylene bag for 72 hr. Plants were periodically observed for the development of symptoms and the number of cankered branches was recorded 4 mo after inoculation. The experimental design was completely randomized, with 10 replicate plants per treatment.

RESULTS

All 100 samples collected in 1989 and 1990 from symptomatic spruce yielded cultures of *P. occulta.* Pycnidia were formed in PDA cultures within 10 days when isolates were incubated at room temperature under continuous fluorescent lighting. Within 14 days, pycnidia were exuding tendrils of alpha and beta conidia typical of *Phomopsis* species.

Inoculated plants in all pathogenicity trials had significantly longer lesions or more blighted shoots (Tables 1–3). In experiment 1, necrotic tissue was present at the inoculation site after 4 mo on all 10 plants inoculated with mycelia 10 cm above the soil line (Table 1). The mean lesion lengths of inoculated plants were significantly longer than those in control plants. Root pruning resulted in significantly longer lesions on inoculated plants. After 6 mo none of the plants showed symptoms of needle loss, tip death, or death of the entire plant (Fig. 1).

Table 1. The effect of inoculation with *Phomopsis occulta* and root pruning on lesion length in 1-yr-old Colorado blue spruce seedlings after 4 mo (experiment 1) and 5 mo (experiment 2) incubation

	Mean lesion length (mm)			
	Lower wound[y]		Upper wound[y]	
Inoculum	Roots intact	Roots pruned	Roots intact	Roots pruned
Experiment 1				
Sterile PDA	3.7 c[z]	6.0 bc	...	...
Phomopsis occulta	8.2 b	14.4 a	...	...
Experiment 2				
Sterile PDA	4.3 d	4.2 d	2.8 d	4.4 d
Phomopsis occulta	8.0 cd	32.2 bc	53.3 ab	64.3 a

[y] Each tree was wounded 10 cm above soil line, identified as lower wound location, and in center of most recent flush of terminal growth, identified as upper wound location.
[z] Treatment means in rows and columns followed by same letter(s) are not significantly different according to Student's *t* test at 5% level.

Table 2. Effect of *Phomopsis occulta* inoculations and sealing wounds with Parafilm at two wound locations on 1-yr-old Colorado blue spruce, after 4 mo incubation

	Mean lesion length (mm)	
Inoculum	Parafilm	Exposed
Sterile agar		
Upper wound location[y]	3.3 e[z]	3.4 e
Lower wound location	3.8 de	4.4 d
Phomopsis occulta		
Upper wound location	42.9 a	66.2 a
Lower wound location	9.0 c	13.7 b

[y] Each of 10 plants per treatment was wounded in two locations. Each wound per plant was treated alike. Lower wound location is wound inflicted on main stem 10 cm above the soil line. Upper wound location is wound located in center of terminal leader.
[z] Treatment means in rows and columns followed by same letter(s) are not significantly different according to Student's *t* test at 5% level.

Table 3. Mean number of shoots blighted per tree on injured 1-yr-old Colorado blue spruce seedlings sprayed with 1.74×10^4 conidia per milliliter of *Phomopsis occulta*, 4 mo after inoculation

	Mean number of blighted shoots		
Inoculum	No injury	Stem injury	Needle injury
Sterile water	0.0	0.0	1.8
Phomopsis occulta	8.8 a[z]	4.7 b	7.1 a

[z] Treatment means in rows and columns followed by same letter(s) are not significantly different according to Student's *t* test at 5% level.

Fig. 1. Experiment 1. Typical symptom development of Colorado blue spruce seedling wounded in a lower location and inoculated with *P. occulta* mycelia.

Fig. 2. Experiment 2. Four treatments represented by trees from left to right: (1) inoculated, roots cut; (2) inoculated, roots intact; (3) control, roots cut; and (4) control, roots intact. All plants wounded in two locations. The first two trees had girdling cankers at upper wound location. Tips turned brown and curled downward.

All plants produced new growth.

In experiments 2 and 3, lesions that developed on inoculated terminals with the same root treatment were significantly longer than those at the lower wound location (Tables 1 and 2). The average lesion length on inoculated plants was longer for root-pruned seedlings than lesions on plants without root disturbance; however, this difference was nonsignificant (Table 1). Although the data are not presented in Table 1, nine of 10 inoculated plants with roots pruned developed girdling cankers that killed terminals leaders within 1 mo after inoculation (Fig. 2). Only five of 10 inoculated plants without root treatment developed lethal girdling cankers. This difference was significant at $P = 0.05$ according to the chi-square analysis. None of the control plants developed cankers.

In experiment 3, lesion length was significantly less when inoculated lower wounds were wrapped with Parafilm. The longest mean lesion length, 66.2 mm, was achieved by leaving the wound exposed (not wrapped with Parafilm) and by wound-inoculating the terminal. No lesions developed on surface-inoculated plants when tested at either location (data not shown in Table 2).

In experiment 4, young shoots had very small purplish cankers on the tips within 10 days after inoculation, when plants were sprayed with a 1.74×10^4 alpha conidia per milliliter suspension. After 3 wk, cankers were visible as purple-bronze streaks extending from the branch tips, and needles became chlorotic. Ten uninjured plants sprayed with the conidial inoculum developed an average of 8.8 blighted shoots per tree after 4 mo (Table 3). Cutting the tips off five branches per tree significantly reduced the number of blighted shoots compared with inoculated seedlings with no pruning.

In all experiments, *P. occulta* was successfully reisolated from all samples of discolored tissue in plants that had been originally inoculated with *P. occulta*. *P. occulta* was only rarely isolated from control plants, and isolation probably resulted from natural background of saprophytically colonized bark.

DISCUSSION

The dieback of lower branches of nursery-grown Colorado blue spruce in Michigan was similar to the symptoms expressed by trees infected with Cytospora canker. However, *C. kunzei picea* was never isolated, while *P. occulta* was consistently isolated. Subsequent inoculation experiments showed that *P. occulta* was capable of infecting and causing dieback symptoms on artificially inoculated spruce seedlings.

Until recently, *P. occulta* was considered a common saprophyte found growing on many conifers (1,2,3,9). However, many stress-related diseases are caused by organisms that usually grow as saprophytes on the host plant (6). Suspicions of the pathogenicity of *P. occulta* arose as early as 1929, when White (10) published a report in New Jersey of stressed *P. pungens* with blighted tips from which *P. occulta* was isolated. In 1986, further evidence of the potential of *P. occulta* to cause disease was reported by Sanderson and Worf (5), who artificially inoculated four species of spruce and two species of fir with conidia of *P. occulta.* The fungus was isolated from Colorado blue spruce showing blighted and necrotic new growth that were growing in nurseries and landscapes in Wisconsin. This was the first report of *P. occulta* causing disease on Colorado blue spruce in Wisconsin. Sanderson and Worf (5) described this disease as Phomopsis shoot blight. In this study, the disease is more accurately described as Phomopsis canker since cankering was the initial symptom of naturally and artificially inoculated plants. Further progression of symptoms resulted in shoot blight only under conducive environmental or cultural conditions. This is the first report of Phomopsis canker on Colorado blue spruce in Michigan and confirms the findings of Sanderson and Worf (5).

In inoculation experiments, a slow progression of canker growth was observed when a mycelial inoculum was applied to wounds 10 cm above the soil. After 6 mo callus formation appeared inhibited and necrotic tissue was observed well beyond the initial wound. Although infections were slowly developing, trees appeared healthy and continued to grow without further symptoms. Cankers resulting from wound-inoculated terminals developed rapidly and were significantly longer than those at the lower, 10-cm site, confirming that younger, succulent tissue is more susceptible than older woody tissue. Conidial infection resulted in canker formation on branch tips, while older tissue was symptomless.

Wounding was necessary for infection of shoots by mycelia but not for the infection by conidia. In fact, trees sprayed with conidia had fewer blighted tips when shoots were pruned than when shoots were left intact. Pruning shoot tips apparently removed the most susceptible tissue and lowered the potential for infection.

In nurseries, open wounds are commonly created by pruning practices. Open wounds were shown experimentally to be susceptible to infection by *P. occulta* mycelia. If Colorado blue spruce are pruned when highly susceptible new growth is present, infection by *P. occulta* mycelia growing saprophytically on the stem may occur.

Another potential contributing factor to disease severity is root pruning during harvest. Removing approximately 60% of the root mass of inoculated plants resulted in significantly increased lesion lengths and frequency of shoot death. Pathogens that cause stem cankers are mostly nonaggressive pathogens that only attack weakened or wounded hosts (6). Host vigor may be reduced by stresses such as drought, flooding, freezing, defoliation, and transplanting. Cutting plant roots reduces stored food reserves, and reduces the absorptive surface area for taking up water and nutrients (7). Transplanting will induce stress in most plants until an adequate root system can be reestablished (6). The experimental simulation of harvest stress in Colorado blue spruce in the previously discussed experiments has shown that stressed plants were more symptomatic than nonstressed plants. Drought-stressed Colorado blue spruce have also been shown to be more susceptible to Cytospora canker, caused by *C. kunzei* var. *picea,* when artificially wound-inoculated (4,8).

P. occulta was shown to be a causal agent in the dieback of Colorado blue spruce growing in Michigan nurseries. It is clear from these trials that young tissue is most susceptible to infection, therefore control measures that protect young growth are needed. Currently there are no known fungicide controls for Phomopsis canker. However, from the observations and results of this study, minimizing the root disturbance during cultivation and transplanting may reduce the expression of symptoms. Further studies are required to determine effective comprehensive control measures for this disease.

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