

# Survey for asymptomatic persistence of *Sphaeropsis sapinea* on or in stems of red pine seedlings from seven Great Lakes region nurseries

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## INTRODUCTION

The potential for *Sphaeropsis sapinea* to be a latent pathogen has been proven. It can persist in the absence of gross symptoms and proliferate to rapidly cause disease upon physiological alteration of its host. Asymptomatic persistence of *S. sapinea* on or in red pine seedlings from two Wisconsin nurseries has been reported, and water stress after transplanting has been shown to allow development of the fungus in seedling stems. The resulting "collar rot" rapidly kills seedlings.

Two forms of *S. sapinea* sensu lato can be differentiated using molecular markers. Isolates of the A group are more aggressive on inoculated red pine shoots and have been obtained from asymptomatic red pine nursery seedlings and those killed by collar rot. Isolates of the B group are much less aggressive on red pine shoots. The B group has recently been named *Diplodia scrobiculata*.

Reports of red pine collar rot in young plantations have become more frequent and widespread in the Great Lakes region. This survey was undertaken to obtain current information about the frequency of asymptomatic persistence of *S. sapinea* on red pine seedlings in public nurseries.



Conidia of *Sphaeropsis sapinea* are disseminated from diseased red pine trees in nursery windbreaks (background) to seedlings in adjacent beds (foreground). In addition to causing shoot blight symptoms on some seedlings, the pathogen persists asymptotically on or in others.

## METHODS

Seven nurseries (Table 1) that produce bareroot red pine seedlings were surveyed in early spring 2002. Seedlings had arisen from seed sown in fall 1999 and had completed two seasons of growth except at Toumey, where half the surveyed seedlings were from seed sown in spring 1999 and half were from seed sown in spring 2000. Use of fungicides to prevent *Sphaeropsis* shoot blight in these nurseries varied from repeated benomyl applications (e.g., Griffith) to none (e.g. Badoura).

Red pine windbreaks including diseased trees that can serve as a source of inoculum were present at Griffith, Hayward, Wilson, and Badoura, and symptoms were common on seedlings in beds near these windbreaks. At these four nurseries, seedlings were surveyed in beds adjacent to windbreaks (high inoculum pressure) and also distant from windbreaks (low inoculum pressure). At one nursery (General Andrews) a red pine windbreak was present, but symptoms were not observed on windbreak trees and were not common in nearby beds. At Wyman and Toumey, red pines were not present (or were rare) in windbreaks, and again symptoms were not common in nearby beds. At the General Andrew, Wyman, and Toumey nurseries seedlings were surveyed both in beds adjacent to a windbreaks and distant from windbreaks, but these were not considered to represent differences in exposure to inoculum.

At the two survey locations (adjacent to or distant from a windbreak) in each nursery, seedlings were visually examined to determine the incidence of shoot blight symptoms including mortality. In each of five beds, all seedlings within 1 m of row were counted and the numbers symptomatic or asymptomatic were recorded. Then 20 arbitrarily selected asymptomatic seedlings from within each surveyed meter of row were cut near the soil line, bagged, and taken to the laboratory. In addition, up to 10 (if available) symptomatic seedlings from each nursery were bagged and later examined for the presence of *S. sapinea* pycnidia and conidia.

Each of 1400 asymptomatic seedlings was assayed for presence of *S. sapinea*. A segment 5-6 cm long of the lower stem and/or root collar of each seedling was cut, needles were removed, and it was surface-disinfested by successive immersions in 95% EtOH and 1.05% NaClO. Segments were then incubated on tannic acid agar in the dark at ambient laboratory temperatures. Transfers were made from margins of subsequently produced colonies to water agar with sterile pine needles to promote production of pycnidia and identification of *S. sapinea*.

Ten arbitrarily selected isolates from each of the five nurseries where *S. sapinea* was detected were characterized by ISSR-PCR fingerprint analysis. PCR reactions were performed using primer 5'-HYH(GT)7 and isolates were characterized as either the A or B group of *S. sapinea* by the distinctive fingerprint patterns this primer yields.

## RESULTS

Incidence of symptomatic seedlings varied widely within and between nurseries (Table 1). Symptomatic seedlings were more frequent in beds adjacent to, than distant from, windbreaks including diseased trees. Symptomatic seedlings were infrequent in the three nurseries where red pines were not present (or were rare) or appeared healthy in windbreaks.

Detection of *S. sapinea* on symptomatic seedlings, differed among the nurseries (Table 1). Characteristic pycnidia and conidia were obtained from most seedlings examined from each of the three nurseries in Wisconsin and the two nurseries in Minnesota. In contrast, signs of the pathogen were not found on the symptomatic seedlings from the two nurseries in Michigan.

Frequency of identification of *S. sapinea* from asymptomatic seedlings also varied widely within and between nurseries (Table 1). The pathogen was found more frequently from asymptomatic seedlings in beds adjacent to, than distant from, windbreaks including diseased trees. The pathogen was not identified from asymptomatic seedlings from the two nurseries in Michigan (at which red pines were not present or were rare in windbreaks), but was identified from >20% of asymptomatic seedlings from the General Andrews Nursery (where red pines in windbreaks appeared healthy and symptomatic seedlings were infrequent).

All isolates from Wilson, Griffith, Hayward, and Badoura were characterized as A group. However, seven of ten isolates from General Andrews (where red pines in windbreaks appeared healthy and symptomatic seedlings were infrequent) were B group.

Agency	Nursery	Location	Inoculum pressure <sup>a</sup>	Seedlings symptomatic (%) (dead, or alive but blighted) mean, range <sup>b</sup>	Symptomatic seedlings positive for <i>S. sapinea</i> (no. + / no. examined)	Seedlings asymptomatic but positive for <i>S. sapinea</i> (%) mean, range <sup>c</sup>
WI DNR	Wilson	Boscobel, WI	High	43, 25-59	10/10	63, 35-75
			Low	8, 0-14		8, 0-25
	Griffith	Wis. Rapids, WI	High	21, 14-34	9/10	35, 20-70
			Low	1, 0-4		3, 0-10
	Hayward	Hayward, WI	High	26, 20-31	9/10	30, 20-35
			Low	12, 5-16		24, 15-40
MN DNR	Badoura	Badoura, MN	High	58, 41-72	10/10	88, 75-95
			Low	4, 1-7		19, 5-50
	General Andrews	Willow River, MN	NA	4, 2-6	10/10	26, 5-40
MI DNR	Wyman	Manistique, MI	NA	3, 0-7	0/10	0, 0-0
			NA	1, 0-2		0, 0-0
			NA	1, 0-4		0, 0-0
USDA, For. Serv.	Toumey	Watersmeet, MI	NA	1, 0-4	0/4	0, 0-0
			NA	0, 0-1		0, 0-0

<sup>a</sup> Except at Wyman, General Andrews, and Toumey, two areas within each nursery were selected to differ in proximity to an inoculum source (red pine windbreak) and incidence of seedling symptoms. At these three nurseries, neither a difference in proximity to a primary inoculum source nor incidence of symptoms were apparent.

<sup>b</sup> Mean and range (to nearest %) for five sampled beds; approx. 1-m-long portion of an interior row inspected in each.

<sup>c</sup> Mean and range (to nearest %) for five groups of 20 asymptomatic seedlings each from an inspected 1-m-long portion of an interior row.



Red pine seedling killed by *Sphaeropsis* collar rot shortly after planting.

## CONCLUSIONS AND IMPLICATIONS

*Sphaeropsis sapinea* persists on or in many asymptomatic red pine seedlings from some nurseries. Fungicide treatment to prevent shoot blight has not prevented asymptomatic persistence. Failure to detect *S. sapinea* from seedlings from two nurseries, however, indicates that asymptomatic persistence is **NOT** inevitable.

As observed in field locations and demonstrated in greenhouse studies, occurrence of the virulent A group of *S. sapinea* on seedlings presents a risk of mortality from collar rot after transplanting.

Asymptomatic persistence can serve to widely disseminate the pathogen.

Frequent asymptomatic persistence of the B group of *S. sapinea* (now known as *Diplodia scrobiculata*) on or in red pine seedlings has not been previously reported. Although shoot inoculations have suggested that this fungus is not aggressive on red pine, the nature of its relationship with pines, and potential to cause collar rot, merits further investigation.

The previously recognized relationship between *S. sapinea* on windbreak trees and seedlings in adjacent beds is confirmed. Failure to sanitize or remove windbreaks contributes to losses from shoot blight in the nursery and collar rot after transplanting.

## REFERENCES

- Blodgett, J., et al. 2003. An effective medium for isolating *Sphaeropsis sapinea* from asymptomatic pines. For. Path. 33:395-404.
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