Isolate Types of *Sphaeropsis sapinea* Associated with Main Stem Cankers and Top-Kill of *Pinus resinosa* in Minnesota and Wisconsin

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ABSTRACT

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Isolate types of *Sphaeropsis sapinea* associated with main stem cankers and top-kill of *Pinus resinosa* were identified as type A and type B. *S. sapinea* was cultured from recovering, dying, and dead trees of different ages and with different disease histories from five plantations and one natural stand in Minnesota and Wisconsin. The occurrence of a particular isolate type was related to plantation or stand location but not to symptoms, tree condition, isolation location, or canker size. In general, *S. sapinea* was isolated more frequently from recovered trees that became diseased in 1985 than from recovered trees that were diseased earlier. In all locations, trees were predisposed to disease by drought, hail, or frost. Cambium was killed only during the year of infection, and *S. sapinea* colonized only the wood extant when infection occurred. Both type A and type B isolates were facultative pathogens causing an annual canker of *P. resinosa*.

Sphaeropsis sapinea (Fr.:Fr.) Dyko & Sutton in Sutton (syn. *Diplodia pinea* (Desmaz.) J. Kickx fil.) affects most species of conifers throughout the world. Symptoms include shoot blight, branch dieback, blue stain of cut timber, top-kill, and main stem cankers (4,14). Infection is usually favored by drought, poor site quality, hail wounds, and insect attack (2,6,7,16).

In Minnesota and Wisconsin, shoot blight and branch dieback of native and exotic conifers are the most common symptoms. The disease is associated with two different isolate types of *S. sapinea*, which differ in cultural characteristics and virulence. Type A isolates are characterized by fluffy white to gray-green mycelia in culture, and they infect both wounded and unwounded shoots. Type B isolates produce white to black mycelia closely appressed to the agar surface, and they infect only wounds; this type is associated with trees attacked by insects or other pathogens (13).

Main stem cankers and top-kill occur periodically in young plantations of red pine (*Pinus resinosa* Aiton) and Jack pine (*P. banksiana* Lamb.) in Minnesota and Wisconsin after drought or hail damage has occurred. Mortality can be extensive, but many trees recover from the disease (9,11). The cause of tree recovery is unknown. Recovered trees may be resistant to *S. sapinea*, or canker development may be arrested by vigorous tree growth. The existence of isolate types that differ in virulence, however, suggests that cankers of recovered trees may be formed by a less aggressive strain of the pathogen, as in the *Cryphonectria parasitica*/*Castanea dentata* pathosystem (5). Isolate types of *S. sapinea* associated with naturally infected cankered or top-killed trees have not been identified; however, stem inoculations of *P. resinosa, P. banksiana*, and *P. strobus* demonstrated that both type A and type B isolates can cause cankers in wounded stems (13). The objective of this research was to identify the isolate types associated with top-kill and main stem cankers of naturally infected *P. resinosa* and to determine if isolate type was related to tree recovery.

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MATERIALS AND METHODS

P. resinosa with main stem cankers or top-kill was collected during summer and autumn in 1986 and 1987 from five plantations and one natural stand in five locations in Minnesota and Wisconsin (Table 1). The trees were 12–32 yr old, and the disease had become apparent in each plantation at different times from 1975 to 1985. Trees in the natural stand were about 18 yr old when the collections were made; the disease was initiated in these trees in 1982. In Wadena County, Minnesota, and plantation I in Douglas County, Wisconsin, where top-kill and cankers were initiated in 1985, four to seven trees were collected in each of three categories: recovering from stem infection, dying, and dead. In all other locations, only recovering and dead trees were collected, because they were the only types present. Trees with callus tissue enclosing a nonexpanding canker or with branches assuming dominance for the dead leader were considered recovering. Trees with less than 10% live branches were considered dying.

Cones and diseased or dead branches and foliage were also collected from trees in adjacent stands or the overstory of the most recently diseased plantations (1982–1985). About 20–30 samples were collected at each location. In most locations, the adjacent stand or overstory was composed primarily of *P. banksiana* with some scattered *P. resinosa*. In the Douglas County plantations, however, only *P. banksiana* was present, and in Hubbard County, Minnesota, only *P. resinosa* was present. Samples were transported to the laboratory and stored at 3 C until examinations and isolations were made.

For each tree, the year the canker was initiated and the proportion of the stem circumference killed at the widest point of the canker were recorded. The presence or absence of branch stubs or openings such as hail wounds or frost cracks associated with the canker center was also noted. Then, four transverse cuts were made in the cankered portion of the tree, dividing the canker into top, middle, and bottom sections. Top-killed trees were treated as trees with stem cankers; the lower end of the top-killed area was considered the top section of the canker. A diagram of the affected tissues was made for each cross section.

Isolations and isolate type identification. On recovering trees, bark samples (approximately 0.5 cm^2) were removed from eight equidistant locations around the circumference of the canker and two locations on the canker face. If bark was not present on the canker face, samples were taken from the exposed wood. The discolored wood associated with the inside of the canker was sampled in 12 locations. Pyramid-shaped wedges of wood (about 0.3 cm on a side) were removed from the pith and from two locations at the margin of the healthy and diseased wood in four of the surfaces exposed by the transverse cuts. The top and bottom sections and each end of the midsection of the canker were sampled. In a similar manner, samples were taken from dying and dead trees, except that the bark was sampled at 10 random locations, and the three samples in each cross section were taken at random locations, because there was no margin between healthy and diseased tissues. Tissue samples were immersed in 0.10%

Table 1. Isolate types of *Sphaeropsis sapinea* associated with main stem cankers and top-kill of *Pinus resinosa* in Minnesota and Wisconsin

Plantation location and tree condition	Year plantation established[a]	Number of trees sampled[b]	Number (percentage) of trees with *S. sapinea*				Occurrence of *S. sapinea* in overstory and adjacent stand[c]	
			In canker face		In discolored wood			
			Type A	Type B	Type A	Type B	Type A	Type B
Douglas Co., Wisconsin, plantation I	1978							
Recovering		7	0	6 (86)	0	7 (100)	No	Yes
Dying		7	0	7 (100)	0	7 (100)		
Dead		5	0	4 (80)	0	4 (80)		
Wadena Co., Minnesota	1977							
Recovering		7	1 (14)	5 (71)	1 (14)	1 (100)	Yes	Yes
Dying		4	1 (25)	3 (75)	1 (25)	4 (100)		
Dead		5	2 (40)	5 (100)	2 (40)	5 (100)		
Clearwater Co., Minnesota	1970[d]							
Recovering		4	4 (100)	0	3 (75)	0	Yes	Yes
Hubbard Co., Minnesota	1972							
Recovering		10	10 (100)	0	5 (50)	1 (10)	Yes	No
Dead		5	5 (100)	1 (20)	5 (100)	1 (20)		
Jackson Co., Wisconsin	1957							
Recovering		5	0	2 (40)	0	1 (20)	...[e]	...
Douglas Co., Wisconsin, plantation II								
Recovering	1963	2	0	2 (100)	0	0	...	...

[a] Plantations established with 3-yr-old bare-root seedlings.
[b] Data from cankered and top-killed trees combined.
[c] Based on isolations from 20–30 diseased cones and shoots from each plantation.
[d] Natural stand. Approximate year in which tree growth began.
[e] No samples collected.

Fig. 1. Main stem canker of *Pinus resinosa* caused by *Sphaeropsis sapinea*. Bark is removed.

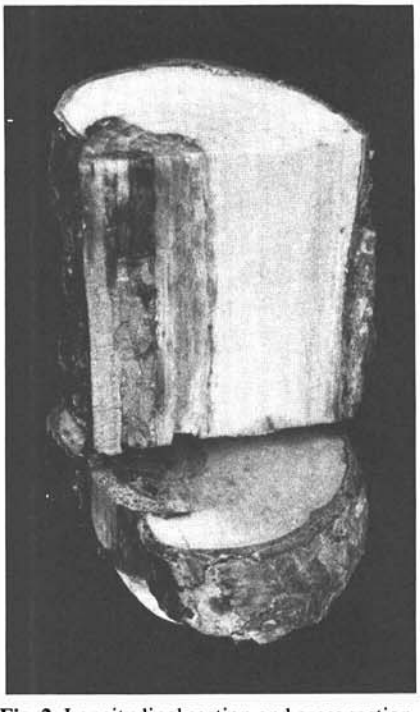

Fig. 2. Longitudinal section and cross section of a 17-yr-old *Pinus resinosa* with a compartmentalized main stem canker caused by *Sphaeropsis sapinea*.

NaOCl for 1 min, blotted dry, and then placed in petri plates containing potato-dextrose agar (Difco) amended with 0.015% streptomycin sulfate. The plates were incubated in the dark at 20 C for 5–7 days. Conidia of *S. sapinea* from pycnidia on trees in the overstory and the adjacent forest stand were cultured to determine the associated isolate types.

The isolate types were identified primarily by colony morphology. To verify the identifications, isozyme analysis was performed on 20 isolates that represented the range in culture morphology of the isolates obtained. Isolates A123 and B124 (13) were used as the controls. Isozyme analysis was performed as described previously (13), except that the fungus was grown on glucose-yeast extract media (8), and the enzymes tested were alcohol dehydrogenase, malic dehydrogenase, β-glucosidase, fluorescent esterase, and sorbitol dehydrogenase.

Data were summarized by symptom (top-kill or canker), plantation location, isolation location, canker size, and tree condition (recovering, dying, or dead) for each isolate type of *S. sapinea*.

RESULTS

Stem cankers were elliptical areas of dead cambium ranging from 3.5 to 150 cm in length (Fig. 1). Cambium was killed only during the year of infection. *S. sapinea* colonized only wood extant when infection occurred, and colonization did not extend beyond the lateral limits of the dead cambium (Fig. 2). The bark still adhered tightly to the surface of cankers initiated in 1985 (in trees in Wadena County and plantation I in Douglas County), and pycnidia of *S. sapinea* were abundant in the bark over the canker face. In plantations where cankers were initiated earlier, however, the bark had been shed. Cankered wood was decayed in older trees (in Jackson County, Wisconsin, and plantation II in Douglas County). At the widest point of the canker, 10–40% of the stem circumference was dead in trees with main stem cankers, and 30–100% of the stem circumference was dead in top-killed trees. Recovering top-killed trees had callus growth enclosing the cankered portion of the tree, and a lateral branch had replaced the dead leader. Dying trees were top-killed; dead tissue extended down to the first or second branch whorl, and callus growth was not present.

Pycnidia of *S. sapinea* were abundant in the bark of the dead portion of top-

killed or dead trees. Dead wood was blue-stained and colonized by *S. sapinea*.

Drought and hail damage occurred in the same year in which the trees were infected by *S. sapinea* in Jackson County and plantation II in Douglas County (11). Diseased trees in Wadena County and plantation I in Douglas County had one or more bark fissures (presumably due to frost), and trees in Clearwater and Hubbard counties, Minnesota, had been injured by hail. A branch stub or bark fissure occurred in the center of most stem cankers. The presence of wounds associated with top-killed trees could not be determined in many cases, because of the deteriorated condition of the dead wood above the cankered portion.

Isolations and isolate type identification. Both type A and type B isolates of *S. sapinea* were associated with diseased trees (Table 1). Of the 20 representative isolates, identified as type A or type B on the basis of culture morphology, all had isozyme patterns identical to those of isolate A123 or isolate B124, respectively.

The occurrence of a particular isolate type was related to the geographic location of the plantation or stand, but not to symptoms, tree condition, or isolation location (Table 1). Isolate type was not related to canker size, so data for top-killed and cankered trees were combined. The same isolate types that were recovered from diseased stems were recovered from trees in the overstory and adjacent stands (Table 1). Type B was not recovered from trees in Clearwater County in this study. In a preliminary set of isolations from trees in Clearwater and Hubbard counties in 1985, however, type B was recovered from the discolored wood of cankers in Clearwater County, but only type A was isolated from trees in Hubbard County (*unpublished data*).

Type B was recovered more frequently than type A in plantation I in Douglas County (Table 1), where only one type A isolate was recovered. Only type B was recovered in Jackson County and in plantation II in Douglas County. Both types were recovered in Wadena County, where type B was associated with more trees than type A was. Type A was recovered from almost all of the diseased trees in Clearwater and Hubbard counties, where type B was isolated from only one recovering and one dead tree. Usually, the same isolate type was recovered from the bark on the canker face and from the wood inside the canker. In general, *S. sapinea* was isolated more frequently from recovering trees in which the disease was initiated in 1985 than from recovering trees in which it began earlier (Table 2). *S. sapinea* was recovered more frequently from the canker face than from discolored wood associated with the canker.

DISCUSSION

Both type A and type B isolates of *S. sapinea* were associated with main stem cankers and top-kill of red pine in Minnesota and Wisconsin. No evidence suggested that the less aggressive type B was related to tree recovery. Both isolate types were associated with recovered trees. The isolate type associated with diseased trees in a plantation apparently reflected the occurrence of that isolate type in the overstory or the adjacent stand. These trees most likely served as the inoculum source for the disease outbreak (12). The association of type B exclusively with locations where *P. banksiana* was in the adjacent stand or overstory and with Jack pine seedlings and forest trees in a previous study (13) suggests that *P. banksiana* is the preferred host of type B isolates.

The response of red pine to infection by *S. sapinea* was similar to the host response in pathosystems of other facultative parasites causing annual cankers, such as *Botryosphaeria dothidea* (3), *Nectria cinnabarina* (1), and *Fusarium solani* (10). In these systems, a predisposing factor (e.g., wounding, drought, or freezing injury) favors infection by the nonaggressive pathogen, but once the stress is removed, a callus barrier is formed. Compartmentalization around the canker prevents expansion by the pathogen, which is eventually replaced by other saprophytes. If callus formation and compartmentalization are delayed, however, a diffuse canker develops, which may rapidly girdle the tree (1,15). All trees in this and other studies of this disease (9,11,16) were predisposed to disease by such factors as frost injury, hail, or drought. Unlike a perennial canker fungus, *S. sapinea* was confined to the wood present at the time of infection, which indicates that it was not able to break through the host defenses in subsequent years. The pathogen was readily recovered from discolored wood of trees in which the disease was initiated in 1985, but it was recovered infrequently from trees that became diseased earlier. This suggests that the competitive saprophytic abilities of *S. sapinea* on cankered tissues decline over time. In this study, both type A and type B isolates were facultative pathogens causing an annual canker of *P. resinosa*.

The comparative virulence and role of each isolate type in initiating cankers or top-kill could not be determined from this study. Inoculations of *P. resinosa* with type A or type B isolates demonstrated, however, that there were no differences between isolate types in their ability to initiate cankers in wounded trees (13). If a particular isolate type was solely responsible for causing cankers, substantial evidence of type A or type B should have been detected in Douglas County and Hubbard County, respectively.

Reducing stress is likely an important factor in managing top-kill and canker caused by *S. sapinea*, as it is in diseases caused by other facultative pathogens. Minimizing wounds resulting from cultural practices and not planting on poor sites have been suggested as possible management strategies (11). In addition, inoculum sanitation, i.e., removing diseased trees or trees with cones colonized by *S. sapinea* from the overstory and adjacent stands could help to reduce the incidence of top-kill and stem cankers caused by this fungus.

Table 2. Occurrence of *Sphaeropsis sapinea* in cankers of recovered *Pinus resinosa* in Minnesota and Wisconsin

Plantation location	Number of trees sampled	Year disease began	Occurrence of *S. sapinea*[a,b]			
			In canker face		In discolored wood	
			Isolations (no.)	Recovery (%)	Isolations (no.)	Recovery (%)
Douglas Co., Wisconsin, plantation I	7	1985	70	70	78	38
Wadena Co., Minnesota	7	1985	68	74	84	60
Clearwater Co., Minnesota	4	1982	40	25	48	8
Hubbard Co., Minnesota	10	1982	100	82	117	10
Jackson Co., Wisconsin	5	1975	50	4	60	3
Douglas Co., Wisconsin, plantation II	2	1975	20	50	24	0

[a] Type A and type B.

[b] Based on approximately 10 isolations per tree from the canker face and 12 isolations per tree from the discolored wood. In some locations, the size or configuration of the canker on one or more trees precluded making the specified number of isolations.

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