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Research

Water stress and *Sphaeropsis sapinea* as a latent pathogen of red pine seedlings

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Summary

- The role of water stress in the initiation of collar rot by *Sphaeropsis sapinea* in asymptomatically colonized *Pinus resinosa* seedlings is reported.
- Mortality and frequency of identification of the pathogen was quantified for seedlings subjected to different water regimes or watering regime–fungicide (benomyl) combinations in glasshouse experiments.
- In experiment 1, seedling mortality ranged from 8% of repeatedly watered seedlings to 50% of those in the driest regime; data analysis indicated a high probability that mortality was not independent of watering regime. Seedlings developed symptoms resembling those of *Sphaeropsis* collar rot, and *S. sapinea* was identified from living (42%) and dead (92%) seedlings. In experiment 2, mortality of repeatedly watered seedlings was low, irrespective of fungicide application. For nonwatered seedlings, however, mortality was greater among seedlings not treated with fungicide (61%) than among benomyl-treated seedlings (37%); data analysis indicated a high probability that mortality was not independent of fungicide treatment.
- *Sphaeropsis sapinea* can act as a latent pathogen; physiological alteration, through water stress, can effect release from the quiescent condition to result in rapid disease development.

Key words: canker, collar rot, endophyte, latent pathogen, *Pinus resinosa* (red pine), shoot blight, *Sphaeropsis sapinea*, water stress.

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Introduction

A variety of effects of abiotic environmental factors on development of plant diseases caused by fungi have long been recognized. Favourable temperature and moisture conditions, for example, are necessary for spore germination and initial infection by fungal plant pathogens. Climatic factors that alter host attributes (e.g. drought effects on woody plant physiology) can influence subsequent colonization. Enhanced colonization of water-stressed trees and shrubs following inoculation with fungal pathogens has been frequently reported (Schoeneweiss, 1981). Experimental evidence supporting the potential for a change in woody plant physiology to release a latent pathogen from quiescence and induce development of disease, however, has been scarce.

Shoot blight caused by the fungus *Sphaeropsis sapinea* (Fr.:Fr.) Dyko and Sutton (syn. *Diplodia pinea* (Desmaz.) J. Kickx fil.) is among the most common and widespread diseases of pines and other conifers (Stanosz, 1997). Infection occurs during moist periods when the fungus penetrates through stomata of elongating needles and directly into young, nonsuberized stems. Infection is quickly followed by symptom development by these succulent plant parts (Brookhouser & Peterson, 1971; Chou, 1978). Colonization following infection of fresh wounds also leads to rapid symptom development. Without repeated application of fungicides, incidence of shoot blight on red pine (*Pinus resinosa* Aiton) seedlings in Wisconsin nurseries has exceeded 30% (Palmer & Nicholls, 1985; Palmer *et al.*, 1986; Stanosz & Smith, 1996).

Epidemics caused by *S. sapinea* in young Wisconsin red pine plantations, however, have developed in the absence of shoot blight on trees that also lack apparent wounds (Stanosz & Cummings Carlson, 1996). The pathogen rapidly proliferates in lower stems and root collars to kill newly planted or established red pine seedlings and saplings. This disease, referred to as *Sphaeropsis* collar rot, has been associated with periods of lower than normal rainfall (Stanosz & Cummings Carlson, 1996).

Rapid development of collar rot in the absence of wounds and during environmental conditions that are not conducive to infection led to investigation of the potential for *S. sapinea* to persist on or in asymptomatic trees (Stanosz *et al.*, 1997). The pathogen was isolated frequently from surface-disinfested lower stem segments from asymptomatic red pine nursery seedlings. Virulence of these isolates was demonstrated by seedling inoculation. Thus, *S. sapinea* is able to maintain itself, associated with a susceptible host, without immediate or apparent disease development.

The following studies were undertaken to determine whether a change in host plant physiology could induce rapid development of *Sphaeropsis* collar rot of red pine seedlings. Two glasshouse experiments were conducted using seedlings from nurseries at which asymptomatic persistence of *S. sapinea* has been previously demonstrated (Stanosz *et al.*, 1997). In each experiment, seedlings were not artificially inoculated, but were subjected to different watering regimes to achieve host water stress with the intent of releasing *S. sapinea* from quiescence to result in development of disease.

Materials and Methods

Plant material

Dormant 2-yr-old red pine seedlings used in experiment 1 were obtained in April 1996 from the Wisconsin Department of Natural Resources (WI DNR) Wilson Nursery, Grant Co., WI, USA. Similar seedlings used in experiment 2 were obtained in May 1997 from the WI DNR Griffith Nursery, Wood Co., WI, USA. Seedlings were selected to avoid shoot blight symptoms that were evident on some seedlings at each location. Because of the chronic occurrence of *S. sapinea* on seedlings, beds of red pine at each nursery are routinely sprayed with the locally systemic fungicide benomyl (Benlate®, DuPont, Wilmington, DE, USA). This fungicide was applied by nursery staff during spring and summer at intervals of approx. 2 wk. Persistence of *S. sapinea* on or in asymptomatic shoots of red pine seedlings at each of these nurseries has been previously demonstrated (Stanosz *et al.*, 1997).

Experiment 1

Asymptomatic seedlings were transplanted into Deepot cones (conical tubes; width 6.4 cm, depth 25.4 cm; Stuewe & Sons Inc., Corvallis, OR, USA) and grown in a glasshouse. A soil mixture (1 : 1 vol : vol) of Plainfield sand (containing 89% sand and 7% silt) from a 14-yr-old red pine plantation in central Wisconsin, and Fafard growing mix no. 2 (Conrad Fafard Inc., Agawam, MA, USA) was used as the growth medium. Seedlings were watered to field capacity immediately after transplanting and again approx. 24 h after transplanting. Ambient light was supplemented with 400 W high-pressure sodium vapour lamps to provide a 16 h photoperiod. The mean photon flux density of the supplemented light was 118 μmol s^{-1} m^{-2} with a maximum recorded ambient glasshouse photon flux density of 1213 μmol s^{-1} m^{-2}. The mean glasshouse temperature was 26.6°C ± 1.2 (SE) and the mean rh was 67% ± 4.

In each of two separate trials (i.e. experimental repeats), 100 seedlings were assigned randomly to each of six watering regimes (1200 seedlings total). Regimes differed by how negative the mean predawn needle water potential (Ψ_{PD}) was allowed to become before seedlings were rewatered. A pressure bomb (Scholander *et al.*, 1965) was used to measure Ψ_{PD}. Readings were taken from five additional seedlings allotted to each watering regime, in each trial, that were placed randomly among the other seedlings. After the initial two waterings seedlings were either watered to field capacity every other day or left nonwatered until mean Ψ_{PD} decreased to approx. −1.1, −1.7, −2.0, −2.5, or −3.2 MPa. After mean Ψ_{PD} for a regime decreased to the desired level, all seedlings under that regime were watered to field capacity every other day and maintained in the glasshouse until the end of the trial. When the mean Ψ_{PD} of seedlings in the driest regime reached approx. −3.2 MPa (102 d in trial 1; 85 d in trial 2), shoots of seedlings in all regimes were collected by severing them from roots near the soil line. Trials were conducted in the same glasshouse, but starting dates were 3 wk apart.

Each shoot was examined after collection and also incubated to determine the presence of *S. sapinea*. Whether the shoot was alive or dead (at collection), the presence or absence of cankers and pycnidia on the lower stem, and presence or absence of blue to black stain on the cut surface were recorded. After removing needles, a 9-cm shoot segment (measured upward from the base) was surface disinfested for 10 s in 95% ethanol followed by 4 min in 1.05% NaOCl solution with 2 drops of Tween 80 per l. Segments were placed in slants containing approx. 10 ml of sterile 2% water agar and incubated for 11 wk at ambient laboratory temperature (approx. 24°C) and light. Pycnidia produced on or in the agar and segments were examined and *S. sapinea* was identified on the basis of conidial characteristics (Punithalingam & Waterston, 1970).

RAPD markers were analyzed to characterize 58 single conidial isolates. Each isolate was obtained from a different seedling, including some seedlings from each watering regime in each trial. Half of the isolates were collected from seedlings that had died and the others were from seedlings that were alive at the time of harvest. Materials and procedures have previously been described in detail (Smith & Stanosz, 1995). Primers DS9 and DS19 were used, each of which yield banding patterns that differentiate the two RAPD marker groups of this pathogen that are found in the north-central United States (Smith & Stanosz, 1995; Stanosz *et al.*, 1996, 1999).

Data were analyzed to determine effects of watering regime on seedling responses and identification of *S. sapinea* on or from incubated stem segments. The mean Ψ_{PD} for seedlings watered every other day and the lowest mean Ψ_{PD} for each of the other watering regimes were compared by two-way ANOVA with interactions. For seedlings watered every other day, values used to calculate the mean for this regime were means for Ψ_{PD} measured on several different days (i.e. mean Ψ_{PD} throughout the trial). For the drier seedlings, values used to calculate the mean for each regime were those obtained on a single day (just before rewatering). Factors used as main effects were watering regime and trial. Because results indicated no significant effect of trial on Ψ_{PD} or trial by treatment (watering regime) interaction, water potential and other seedling response data for the two trials were pooled for subsequent analyses. Means for Ψ_{PD} were separated using Fisher's least significant difference (LSD) at $P = 0.05$. Simple linear regression analysis was used to examine the relationship between Ψ_{PD} and seedling mortality. Mortality and identification of the pathogen were analyzed using two-way cross tabulations and the Chi-square test of independence (also known as the Chi-square test of association or interaction) (Steel & Torrie, 1960; Snedecor & Cochran, 1989; Sokal & Rohlf, 1995). For these tests, each seedling was considered an individual experimental unit that was categorized by response (e.g. living or dead, pathogen identified or not) and by watering regime. The procedure was used to test if the probabilities of seedlings being classified for one variable (e.g. living or dead) depended on classification for the other variable (watering regime). A large Chi-square value (small value of P, such as < 0.05) indicates a high probability that the two variables are not independent. All analyses were performed with the Minitab for Windows program (release 10.2; Minitab Inc., State College, PA, USA).

Experiment 2

A fungicide treatment was incorporated into this experiment to clarify whether activity of the pathogen, as opposed to drought stress alone, might be responsible for the mortality that resulted in experiment 1. Asymptomatic seedlings were transplanted, initially watered, and maintained in the same glasshouse under similar conditions as those described for experiment 1. In each of two separate trials (experimental repeats), 100 seedlings were assigned randomly to each of the following four watering regime–fungicide treatment combinations (800 seedlings total): watered every other day and treated weekly with benomyl; watered every other day and not fungicide treated; not watered, but treated weekly with benomyl; not watered and not fungicide treated. Benomyl (0.6 g active ingredient l^{-1} of water) was applied to needles and stems to the point of drip with a hand tank sprayer. A pressure bomb again was used to measure Ψ_{PD}. Readings were taken from 10 additional seedlings, allotted to each watering regime in each trial, that were placed randomly among the other seedlings. After mean Ψ_{PD} for nonwatered seedlings decreased to approx. −2.8 MPa (approx. 5 wk in each trial), all seedlings were watered to field capacity every other day for an additional 2 wk. Shoots of seedlings in all treatments were then collected by severing them from roots near the soil line. Trials were conducted in the same glasshouse, but starting dates were 2 wk apart.

Whether each seedling was alive or dead (at collection) and the presence or absence of blue to black stain on the cut surface were recorded. Lower stems of all seedlings were examined for pycnidia, and *S. sapinea* again was identified on the basis of conidial characteristics. Seedlings positive for *S. sapinea* were discarded. If *S. sapinea* was not identified on a seedling at this time, a segment of the lower stem was surface-disinfested, incubated, and examined as described for experiment 1 to determine the presence of the pathogen.

Data were analyzed to determine effects of watering regime on seedling responses and identification of *S. sapinea* on or from incubated stem segments. The mean Ψ_{PD} for seedlings watered every other day and the lowest mean Ψ_{PD} for each of the other watering regimes were compared by two-way ANOVA with interactions. For seedlings watered every other day, values used to calculate the mean for this regime were means for Ψ_{PD} measured on several different days (i.e. mean Ψ_{PD} throughout the trial). For the drier seedlings, values used to calculate the mean for each regime were those obtained on a single day (just before rewatering). Factors used as main effects were watering regime and trial. Because results indicated no significant effect of trial on Ψ_{PD} or trial by treatment (watering regime) interaction, water potential and other seedling response data for the two trials were pooled for subsequent analyses. Mortality and identification of the pathogen were analyzed using two-way cross tabulations and the Chi-square test of independence. For these tests, each seedling was considered an individual experimental unit that was categorized by response (e.g. living or dead, pathogen identified or not) and by watering regime–fungicide treatment combination. The procedure was used to test if the probabilities of seedlings being classified for one variable (e.g. living or dead) depended on classification for the other variable (watering regime–fungicide treatment combination). As for experiment 1, a large Chi-square value (small value of P, such as < 0.05) indicates a high probability that the two variables are not independent. All analyses again were performed with the Minitab for Windows program.

Results

Experiment 1

Symptoms and signs that developed resembled those of recently planted seedlings in red pine plantations during drought-associated epidemics of *Sphaeropsis* collar rot

Table 1 Predawn needle water potential (Ψ_{PD}), mortality, and identification of *Sphaeropsis sapinea* for noninoculated red pine seedlings under six watering regimes in a glasshouse

			Identification of *Sphaeropsis sapinea*[3]		
	Ψ_{PD}[1] (MPa)	Mortality[2] number (%)	from dead number (%)	from living number (%)	total number (%)
	−0.55 a	15 (8)	12 (80)	71 (38)	83 (42)
	−1.12 ab	25 (13)	22 (88)	73 (42)	95 (48)
	−1.72 bc	79 (40)	72 (91)	52 (43)	124 (62)
	−2.04 bc	81 (41)	73 (90)	50 (42)	123 (62)
	−2.54 cd	89 (45)	82 (92)	47 (42)	129 (65)
	−3.18 d	100 (50)	96 (96)	47 (47)	143 (72)
P[4] =	< 0.001	< 0.001	0.312	0.838	< 0.001

[1] Mean Ψ_{PD} for 10 seedlings per watering regime. Values followed by different letters are significantly different ($P < 0.05$) based on Fisher's LSD. [2] Number (percentage, to nearest whole percent) that died, of 200 seedlings per watering regime. [3] Number (percentage, to nearest whole percent) from which *Sphaeropsis sapinea* was identified, of 200 seedlings per watering regime. N for dead, living, and total was, respectively, 389, 811, and 1200. [4] Probability that there are no differences among values within a column based on ANOVA for $\Psi_{PD,}$ and probability of independence of values within a column from watering regime based on Chi-square tests for numbers of seedlings.

(Stanosz & Cummings Carlson, 1996). Needles turned pale grey-green or light-yellow to light-brown, and desiccated. Slightly swollen cankers were observed on the lower stems of 5% of the living seedlings and on 21% of the dead seedlings. Blue to black stain was never noted on the cut surface of living seedlings, but was seen on 29% of the seedlings that died. Pycnidia (of various fungi including *S. sapinea*) were never observed on lower stems of the living seedlings, but were noted on 33% of the dead seedlings.

Two-way ANOVA indicated an effect of watering regime on seedling Ψ_{PD} ($P < 0.001$) (Table 1). Effects of trial ($P = 0.970$) and interaction between treatment and trial ($P = 0.999$) were not significant. Pooled mean Ψ_{PD} varied from −0.55 MPa for trees watered every other day throughout the experiment to −3.18 MPa for those in the driest watering regime.

Incidence of dead seedlings increased as mean Ψ_{PD} became more negative (Table 1). Mortality ranged from 8% for seedlings watered every other day to 50% for those in the driest watering regime. The Chi-square test indicated a high probability that mortality was not independent of watering regime ($P < 0.001$) and seedling mortality was positively correlated with more negative Ψ_{PD} ($r = 0.94$, $P = 0.006$).

The identification of *S. sapinea* on or from incubated stem segments was more frequent among seedlings of the drier watering regimes compared with those of regimes in which less negative mean Ψ_{PD} was achieved (Table 1). Identification of *S. sapinea* was independent of the various watering regimes for seedlings that had survived ($P = 0.838$) and for seedlings that died ($P = 0.312$), when analyzed separately. When the total identification of the pathogen (i.e. regardless of whether the seedlings lived or died) was considered, however, the Chi-square test indicated a high probability that identification was not independent of watering regime ($P < 0.001$). The more frequent identification of the pathogen from seedlings in the drier regimes was related to the numbers of seedlings that lived or died (greater mortality in the drier regimes). For seedlings in the driest regime, *S. sapinea* was identified on or from incubated segments of 47% of the seedlings that survived, and on or from 96% of the seedlings that died, with an overall rate of 72%. The pathogen was identified after incubation on or from 95% of those segments on which any pycnidia had been noted before incubation. All of the 58 tested isolates of *S. sapinea* obtained from these seedlings produced banding patterns consistent with those expected for *S. sapinea* RAPD marker group A.

Experiment 2

Symptoms and signs that developed were similar to those in experiment 1. Slightly swollen stem cankers were observed on the lower stems of < 1% of the living seedlings and on 39% of dead seedlings. Blue to black stain was noted on < 1% of living seedlings, but was seen on 55% of the seedlings that died. Before incubation, pycnidia (confirmed as *S. sapinea*) were observed on lower stems of < 1% of living seedlings, but were noted on 72% of the dead seedlings.

Two-way ANOVA indicated an effect of watering regime on seedling Ψ_{PD} ($P < 0.001$) (Table 1). Effects of trial ($P = 0.259$) and interaction between treatment and trial ($P = 0.259$) were not significant. Pooled mean Ψ_{PD} varied from −0.65 MPa for trees watered every other day throughout the experiment to −2.84 MPa for those in the drier watering regime.

The frequency of seedling mortality differed among watering regime-fungicide treatment combinations (Table 2). Because the incidence of dead seedlings was very low (≤ 4%) and similar among well-watered trees whether or not benomyl was applied, the Chi-square test indicated a high probability of the

Table 2 Predawn needle water potential (Ψ_{PD}), mortality, and identification of *Sphaeropsis sapinea* for noninoculated glasshouse grown red pine seedlings under two watering regimes and either treated with the fungicide benomyl or not

				Identification of *Sphaeropsis sapinea* (%)[c]		
	Ψ_{PD}[a] (MPa)	Fungicide treatment	Mortality[b] number (%)	from dead number (%)	from living number (%)	total number (%)
	−0.65	benomyl	6 (3)	2 (33)	3 (2)	5 (3)
		none	7 (4)	5 (71)	29 (15)	34 (17)
	−2.84	benomyl	74 (37)	40 (54)	10 (8)	50 (25)
		none	121 (61)	112 (93)	20 (25)	132 (66)
P^d =	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001

[a] Mean Ψ_{PD} for 20 seedlings per watering regime. [b] Number (percentage, to nearest whole percent) that died, of 200 seedlings per watering regime–fungicide treatment combination. [c] Number (percentage, to nearest whole percent) from which *Sphaeropsis sapinea* was identified, of 200 seedlings per watering regime–fungicide treatment combination. *N* for dead, living, and total was, respectively, 208, 592, and 800. [d] Probability that there are no differences among values within a column based on ANOVA for Ψ_{PD}, and probability of independence of values within a column from watering regime based on Chi-square tests for numbers of seedlings.

independence of mortality and fungicide treatment for these well-watered trees ($P = 0.778$). Mortality among nonwatered trees was suppressed when benomyl was applied (37%), however, compared to when it was not (61%). Thus, the Chi-square test indicated a high probability that mortality was not independent of fungicide treatment for these trees in the drier watering regime ($P < 0.001$).

A high probability that identification of *S. sapinea* was not independent of watering regime–fungicide treatment combination was indicated by the Chi-square test (values of $P < 0.001$ for living, dead, and total). The pathogen was identified most frequently on or from the segments from nonwatered seedlings that had not been treated with benomyl. Among these, *S. sapinea* was identified on or from 25% of the seedlings that survived the experiment and on or from 93% of the seedlings that died (66% overall). Regardless of watering regime or seedling condition, *S. sapinea* was identified less frequently on or from seedlings to which benomyl had been applied during this experiment.

Discussion

Latency of a plant pathogen could help explain sudden or rapid disease development, especially under conditions that induce host stress. Secondary tissues of woody plants have the potential to accumulate and maintain a large number of diverse microorganisms, including plant pathogenic fungi. Perennial plants often grow in situations with limited opportunity for cultural amelioration, and are subject to both short-term extremes and long-term changes in the abiotic environment. Development of a clear and experimentally supported concept of latency of fungal plant pathogens, therefore, may be particularly important to understanding the long-term relationships of trees and shrubs and their pathogens. Examination of the red pine–*S. sapinea* relationship has allowed development of criteria for confirming latency as a feature of a woody plant–fungal pathogen interaction. Clarification of the factors involved in development of *Sphaeropsis* collar rot also allows formulation of a strategy to reduce its impact in red pine plantations.

The concept of latency in the interactions of fungal plant pathogens and their hosts has been variously described by different authors (Verhoeff, 1974; Mussell, 1980; Swinburne, 1983; Sinclair, 1991; Williamson, 1994). Features of latency proposed by Mussell (1980) include: relatively long periods of contact without gross damage to the host; pathogen quiescence (inactivity) imposed by some aspect of host physiology; and an eventual shift in host physiology that permits active development of the pathogen which then becomes manifest by rapid development of disease. Thus, latency can be thought of as more than merely a state in which the host is colonized but does not exhibit symptoms (Agrios, 1997; Shurtleff & Averre, 1997) or on which sporulation of the pathogen has not begun (Vanderplank, 1975). Instead, a richer concept of latency (Mussell, 1980) and characterization of a fungus as a latent pathogen would indicate a full appreciation of the critical role of altered host condition in disease development.

Use of the term latency in a manner consistent with Mussell's concept to characterize one phase of a plant–fungus interaction is predicated on knowledge of the inherent potential of the fungus to cause disease. Virulence (here referring to the ability of a strain of a pathogen to cause a disease of the particular host) therefore is prerequisite for that strain to be a latent pathogen. Latent fungal pathogens can be functionally differentiated from endophytes if, as proposed by Carroll (1986), the latter term is reserved for those nonpathogenic microorganisms that internally inhabit living plants. Likewise, latency would not be used to describe the relationship of an avirulent strain with a host species or cultivar on which it is incapable of causing disease.

Although many pathogenic species of fungi, including *S. sapinea*, have been identified among the large numbers of fungi isolated from asymptomatic stems of tree and shrub hosts (Bassett & Fenn, 1984; Fisher *et al.*, 1986; Pusey *et al.*, 1986; Petrini & Fisher, 1988; Fisher & Petrini, 1990; Kowalski & Kehr, 1992; Pusey, 1993), the ability of strains isolated from asymptomatic hosts to cause disease has remained untested. Recently, however, virulence of *S. sapinea* isolates obtained from asymptomatic red pine seedlings was investigated (Stanosz *et al.*, 1997). The seedlings from which those isolates were obtained had been grown in the same nurseries that furnished seedlings for the current study. Almost all (44 of 46) were determined to be of RAPD marker group A of *S. sapinea*, that has been proven to be aggressive on red pine (Blodgett & Stanosz, 1997). The ability of isolates of *S. sapinea* from asymptomatic seedlings to cause disease also was confirmed by inoculation, which resulted in stem cankers and shoot blight (Stanosz *et al.*, 1997).

Demonstration of the release from quiescence of a latent fungal pathogen in response to a physiological change in the host has proven difficult. Proliferation of postharvest pathogens has been associated with complex phenomena such as ripening of fruit, but evidence for involvement of specific host factors (such as sugar content or phytoalexin concentration) has been more indicative of correlation than causation (Verhoeff, 1974; Swinburne, 1983). Chapela & Boddy (1988) did provide direct experimental evidence for the influence of decreased water content in the initial development of fungi in the xylem of excised beech branches (*Fagus sylvatica*). Early colonizers of the xylem, including *Hypoxylon fragiforme*, *H. nummularium*, *Nectria coccinea*, *Coniophora puteana*, and coelomycete species were derived from previously quiescent inoculum in the healthy living branches. Chapela & Boddy (1988) further suggested that invasion of trees by a number of possible latent fungal pathogens could be considered to be similar phenomena. Examples they provided include: *Cytospora chrysosperma* on *Populus deltoides* (Butin, 1952), *Cryptodiaporthe salicina* on *Salix hookeriana* and *S. scouleriana*. (Bier, 1959), *Fusarium* on *Liriodendron tulipifera* (Parker, 1961; Dochinger & Seliskar, 1962), *Fusarium solani* on *Acer saccharum* (Bier, 1964; Skelly & Wood, 1966) and *Nectria cinnabarina* on *Gleditsia triacanthos* (Bedeker & Blanchette, 1983). They also particularly noted the proliferation (independently of wound inoculation) of the stem canker pathogens *H. atropunctatum* in mechanically girdled oak trees (*Quercus*)(Bassett & Fenn, 1984) and *H. mammatum* in drying stem sections of aspen (*Populus tremuloides*) (Bier & Rowat, 1962; Anderson & French, 1972). Chapela & Boddy (1988) concluded that it might be possible to explain these observations in terms of latency, and urged further investigation.

One possible latent pathogen–host relationship not mentioned by Chapela & Boddy (1988) is that of a fungus identified as *Botryosphaeria ribis* and apple (*Malus domestica*). Conner (1968) observed more rapid external symptom development by wounded, inoculated excised twigs held in the laboratory under dry conditions than by those with their bases in water. In another experiment, Conner (1968) observed that symptom development by inoculated trees was delayed until after imposition of water stress. The fungus apparently proliferated from lenticels, in which it could reside (in the absence of visible symptoms) for at least 8 wk.

Host water stress as a physiological 'trigger' that might permit development of *Sphaeropsis* collar rot was suggested by the previously mentioned studies, and by both anecdotal reports and results of experimentation with *S. sapinea.* Damage due to *S. sapinea* in forests and plantations has long been associated with the occurrence of severe drought (Birch, 1936; Laughton, 1937; Chou, 1987; Nicholls & Ostry, 1990). Greater disease severity following inoculation of several species of conifers has resulted from imposition of moderate to severe water stress (Bachi & Peterson, 1985; Chou, 1987; Madar *et al.*, 1989; Johnson *et al.*, 1997). Blodgett *et al.* (1997a,b) quantified colonization of wounded, inoculated red pine shoots of both potted seedlings and established plantation trees by RAPD marker group A isolates of *S. sapinea.* In repeated studies, Ψ_{PD} was manipulated by differences in frequency of watering, by removing competing vegetation, or both. Very moderate water stress (mean $\Psi_{PD} > -2.0$ MPa and > -1.4 MPa in the glasshouse and field, respectively) was sufficient to cause greater mean symptom severity compared to that of well-watered trees. As mentioned earlier, the rapid development of collar rot on newly planted or established red pines (Stanosz & Cummings Carlson, 1996) suggested that enhancement of the activity of *S. sapinea* might not be limited to merely increasing the severity of shoot blight and cankers, but might include release from quiescence. This hypothesis was supported by knowledge that the location of persistence of *S. sapinea* on or in asymptomatic red pine seedlings is the lower stem (Stanosz *et al.*, 1997), where collar rot develops (Stanosz & Cummings Carlson, 1996).

The role of *S. sapinea* in death of water stressed seedlings is supported by results of both experiments of the current study. We (Blodgett *et al.*, 1997a) have previously subjected potted red pine seedlings to intensities of water stress (mean Ψ_{PD} of −1.7 to −1.9 MPa) similar to those at which 40% (−1.7 MPa) and 41% (−2.0 MPa) mortality occurred in experiment 1. In the absence of *Sphaeropsis* collar rot in these previous studies, however, no mortality occurred. Survival of 40 and 50% of the seedlings subjected to extreme drought stress (mean $\Psi_{PD} < -2.8$ MPa) in the two current experiments (with relatively little additional mortality at mean $\Psi_{PD} < -1.72$ MPa in experimemt 1) indicates that water stress alone was insufficient to cause the observed mortality. The lower frequency of mortality among stressed, fungicide treated seedlings (compared with stressed, nontreated seedlings) in experiment 2 also is indicative of the involvement of *S. sapinea* in seedling death. Maintenance of quiescence (or suppression of proliferation) of *S. sapinea* in these seedlings may be similar to the phenomenon of delayed senescence of soybean leaves treated with benomyl (Sinclair, 1991). Sinclair (1991) ascribed this response of soybean leaves to benomyl application to a fungistatic effect on fungi present in asymptomatic leaves. Proliferation of

S. sapinea in some seedlings to which benomyl had been topically applied indicated that suppression of the pathogen was incomplete. Discovery of the location(s) of asymptomatic persistence of the pathogen, elucidation of whether the activity of this chemical (or its active breakdown product) against *S. sapinea* is fungicidal, or merely fungistatic, and determination of the degree to which benomyl penetrates and is distributed in red pine seedlings, especially in secondary tissues including those of the bark, may help explain this observation.

Sphaeropsis sapinea was associated with the noninoculated seedlings used in the current experiments more frequently than in our earlier studies. Previously, the pathogen was identified from 15 to 40% of incubated lower stem segments from asymptomatic 2–0 red pine seedlings (Stanosz *et al.*, 1997). Detection of *S. sapinea* in the current study, however, was comparatively easy and perhaps more efficient than in the past due to development of pycnidia on the lower stems and/or root collars of many of trees that died during the experiments. Slightly greater frequencies of identification from still living trees (compared with previous work) might be a consequence of the stress imposed. Moderately low Ψ_{PD} may have been sufficient to allow initiation of pathogen proliferation, but disease development might not have progressed to the extent necessary for mortality to occur by the termination of the experiment. Resumption of watering also may have arrested the proliferation of *S. sapinea* in seedlings. Johnson *et al.* (1997) recently demonstrated that expansion of cankers induced by *S. sapinea* can be suppressed by watering drought-stressed trees. Regardless of the explanation, however, the association of this pathogen with the majority of seedlings used in these experiments suggests that results of our previous study (Stanosz *et al.*, 1997) may have greatly underestimated the frequency of asymptomatic persistence of *S. sapinea* on or in red pine seedlings, and that this may be a typical phase in its interaction with some conifer hosts.

Consideration of *S. sapinea* as a latent pathogen has practical implications for management of the diseases it causes. Benomyl has been considered an effective tool for preventing *Sphaeropsis* shoot blight in nurseries (Palmer *et al.*, 1986; Stanosz *et al.*, 1996). Although routine application of benomyl prevents immediate damage, it apparently fails to prevent establishment of a relationship that results in dissemination of the pathogen on red pine seedlings and the later development of disease. Regardless of success in preventing disease in the nursery, the broader management of *S. sapinea* has been less than optimal. Fungicide application alone is not a substitute for cultural disease management practices, including elimination of sources of inoculum such as diseased windbreak trees (Palmer *et al.*, 1988; Stanosz *et al.*, 1996). More effective protective treatments, accurate and efficient methods for detection of the latent pathogen, and willingness to destroy the seedlings bearing the pathogen, would help to prevent the continued widespread dissemination of *S. sapinea* to planting sites on what might visually appear to be ‘healthy’ seedlings.

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