Effects of Moderate Water Stress on Disease Development by *Sphaeropsis sapinea* on Red Pine

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ABSTRACT

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The aggressiveness of *Sphaeropsis sapinea* isolates was compared on water-stressed and nonstressed 3-year-old red pines (*Pinus resinosa*) in greenhouse and growth chamber experiments. Water was withheld from stressed seedlings to achieve mean predawn needle water potentials (ψ_{PD}) above –1.9 MPa. The lowest mean ψ_{PD} of well-watered seedlings was maintained at or above –0.8 MPa. Young shoots were inoculated by placing colonized agar plugs on wounds made by removing a needle fascicle. Two isolates of each recognized morphotype (A and B) were used in the greenhouse experiment and two isolates of morphotype A were used in the growth chamber experiment. After 4 weeks, isolates of morphotype A caused more severe symptoms and could be recovered farther from the inoculation site on water-stressed than on nonstressed trees in both experiments. In the greenhouse experiment, isolates of morphotype A also caused more severe symptoms and could be recovered farther from the inoculation site than isolates of morphotype B, regardless of watering regime. These results indicate that water stress at levels observed typically in the field can result in increased disease development by isolates of *S. sapinea* morphotype A on red pine. The reduction of water stress of red pines in the field may reduce losses due to Sphaeropsis shoot blight.

Additional keyword: *Diplodia pinea*.

Sphaeropsis sapinea (Fr.:Fr.) Dyko & Sutton in Sutton (syn. *Diplodia pinea* (Desmaz.) J. Kickx fil.) causes a shoot blight and canker disease of various conifers worldwide (10). Field observations have related severe drought to increased losses due to *S. sapinea* (6,9,16,20). In a survey of red (*Pinus resinosa* Aiton) and jack pines (*P. banksiana* Lamb.) in Minnesota and Wisconsin, tree mortality attributed to *S. sapinea* was as high as 30% for red pine and 51% for jack pine (20). Poor sites, drought, hail, snow, and insects were indicated as possible stress factors. In Chile and New Zealand, more than 50% crown death with some mortality of radiata pine (*P. radiata* D. Don) was attributed to *S. sapinea* during prolonged drought (9). Although considerable losses by *S. sapinea* have been associated with droughts, these field observations do not separate the effects of drought from the possible effects of other environmental factors.

Generalizations of the conclusions from previous controlled studies are limited by the host species used, the extreme water stress to which they were subjected, or the lack of statistical analyses (2,9,17,33). Chou (9) reported that stems of radiata pine were only invaded by *S. sapinea* when predawn needle water potential (ψ_{PD}) fell below –2.5 MPa. By the end of that experiment, ψ_{PD} averaged –3.5 MPa and mortality of 20% of the noninoculated control plants was attributed to permanent wilting (ψ_{PD} well below –3.5 MPa). Increased severity of cankers caused by *S. sapinea* f. sp. *cupressi* on cypress (*Cupressus sempervirens* L.) was reported only at –4.5 to –5.5 MPa (17). Severe water stress (ψ_{PD} from –4.5 to –5.0 MPa) after inoculation resulted in more colonization of Scots pine by *S. sapinea* than water stress prior to inoculation (33). More moderate levels of water stress (ψ_{PD} from –1.2 to –1.5 MPa) have been suggested to enhance the coloni-

zation of *S. sapinea* on Austrian (*P. nigra* Arnold), Scots (*P. sylvestris* L.), and Japanese black (*P. thunbergiana* Franco) pines, although no statistical analysis was presented (2).

Previous studies also have not examined the potential influence of pathogen variability on colonization of water-stressed hosts by *S. sapinea*. There are two distinct morphotypes of *S. sapinea* (A and B) in the north central United States (21). Morphotypes are morphologically distinguishable groups of individuals, within a species, with unknown or no taxonomic significance (11). The morphotypes of *S. sapinea* differ in colony morphology, growth rates on potato dextrose agar, average spore sizes, and isozymes (21). Isolates of the A morphotype are more aggressive on red pine than isolates of morphotype B (7). Random amplified polymorphic DNA analysis also separates isolates of the two morphotypes into two distinct groups (28).

The objectives of studies described in this paper were to determine if shoot colonization by *S. sapinea* could be enhanced in moderately water-stressed trees (ψ_{PD} above –1.9 MPa) and to compare responses of water-stressed hosts using isolates of each morphotype. The null hypotheses tested in this study were that water stress does not affect symptom development and colonization of red pine by *S. sapinea* and that the effect on symptom development and colonization of water-stressed red pines is the same for each morphotype. The responses of water-stressed and nonstressed red pine seedlings to isolates of each morphotype were compared in a greenhouse. Isolates of the A morphotype also were used in a subsequent experiment in a growth chamber.

MATERIALS AND METHODS

Greenhouse experiment. Dormant, 2-year-old red pine nursery seedlings (Griffith Nursery, Wisconsin Rapids, WI) were potted during April 1992 into Tall One Treepots (approximately 10 × 10 × 36 cm deep; Stuewe & Sons Inc., Corvallis, OR) and grown in a greenhouse. A soil mix (vol/vol) of one-half Plainfield sand from

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a 35-year-old red pine plantation in central Wisconsin, and one-half Pro-Mix BX growing mix (Premier Brands Inc., Rivière du Loup, Québec, Canada) was used as the growth medium. Seedlings were periodically fertilized the summer before the experiment by watering to field capacity with 2.0 cc of Peters Acid Greening soluble fertilizer (17:6:6 N/P/K; Scotts Co., Allentown, PA) per liter. In October 1992, potted seedlings were placed in a cold-frame and allowed to harden before the experiment was initiated. Seedling height in late October was 28.4 cm ± 0.4 (standard error).

In December 1992, seedlings were moved into a greenhouse supplemented with artificial light to provide a 16-h photoperiod. The photon flux density of the supplemented light averaged 118 μE s^{-1} m^{-2} with a maximum recorded ambient greenhouse photon flux density of 936 μE s^{-1} m^{-2}. The average greenhouse temperature was 27°C during the day and 20°C during the night and average relative humidity was 38%. Initially, all seedlings were watered to field capacity every 3 days. After 33 days, seedlings were either watered to field capacity daily (nonstressed) or watered when the mean ψ_{PD} fell below –1.64 MPa (stressed). A pressure bomb (27) was used to measure ψ_{PD} 2 to 3 times per week. For each watering regime, readings were taken from five noninoculated seedlings that were placed randomly among the inoculated seedlings. The use of the pressure bomb required destructive sampling of individual needles. To avoid any effect of repeated needle removal, a new set of 10 noninoculated seedlings was used for the determination of ψ_{PD} during the second half of each trial.

After 2 weeks under the different watering regimes, elongating shoots were inoculated with 4-mm-diameter agar plugs cut from margins of actively growing cultures of *S. sapinea*. Inoculations involved placing a colonized 1.5% water agar plug (WA; Difco Laboratories, Detroit) fungus-side-down on a wound (approximately 3 × 1.5 mm) made by removing a needle fascicle (by a scalpel cut flush to the stem) 2 cm below the shoot apex. Shoots were wrapped with Parafilm (American National Can, Chicago) for 4 days. Two isolates of morphotype A (A1 and A2) and two of morphotype B (B1 and B2) collected from pines in Wisconsin and Minnesota were included as treatments (Table 1). Seven seedlings per watering regime and isolate treatment combination, and seven wounded and nonwounded control seedlings for each watering regime, were used in each of two separate (separated by 2 weeks) completely randomized trials. A noncolonized WA plug was applied to wounded controls.

Observations recorded 4 weeks after inoculation included presence of necrotic needles and cankers, wound color (green, tan, brown, or black), and resin flow from the wound (no resin off the wound site, resin less than 3 mm from the wound site, or resin 3 mm or farther from the wound site). Symptom severity, expressed as the distance along the stem from the inoculation site to the farthest point below it at which necrotic needles were observed, was measured at 4 weeks after inoculation. Shoot dry weight and soil moisture content also were determined at this time. Soils were weighed immediately and soils and shoots were dried at 50°C for 1 week prior to determination of the dry weight.

The distance from the site of inoculation at which *S. sapinea* could be recovered was determined at 4 weeks after inoculation. After removing needles, 22-cm-long shoots were surface-disinfected for 10 s in 95% ethanol followed by 4 min in 1.05% NaOCl solution with 2 drops of Tween 80 (Fisher Scientific Co., Toronto, Ontario, Canada) per liter. One-centimeter-long segments centered at 0, 3, 6, 9, and 12 cm from the inoculation site were cut aseptically. These segments were placed in individual slants containing 2% WA and incubated for 12 weeks at ambient laboratory temperature (approximately 24°C) and light. The presence of *S. sapinea* in incubated shoot segments was determined by examining the resulting mycelia, pycnidia, and spores.

Growth chamber experiment. A growth chamber experiment was conducted at the University of Wisconsin-Madison Biotron

(Madison, WI) to further examine the effects of water stress using two isolates of *S. sapinea* morphotype A. Isolates of morphotype B were not used in this experiment because they did not show a significant response to host water stress in the previous experiment. Dormant, 2-year-old red pine nursery seedlings were potted and fertilized as described previously during April 1993 into a soil mix (vol/vol) of one-half Plainfield sand from a 10-year-old red pine plantation in central Wisconsin, and one-half Fafard growing mix no. 2 (Conrad Fafard Inc., Inkerman, New Brunswick, Canada). In November 1993, potted seedlings were placed in a cold-frame and allowed to harden before the experiment was initiated. Seedling height in early November was 22.2 cm ± 0.4.

In February 1994, seedlings were moved into a greenhouse as in the greenhouse experiment. Artificial light supplemented natural light to provide a 16-h photoperiod. Initially, all seedlings were watered to field capacity every 3 days. After 26 days, seedlings were either watered to field capacity daily (nonstressed) or not at all (stressed) for the rest of the experiment.

After 1 week under the different watering regimes, seedlings were moved into a growth chamber. The average environmental conditions of the growth chamber were as follows: temperature (day 25°C, night 20°C), relative humidity (day 81%, night 95%), light (photon flux density of 790 μE s^{-1} m^{-2}) with a 40 min step-up and step-down of intensity on each end of a day (20 min at both 55 and 268 μE s^{-1} m^{-2}), and a day length of 14 h. The high relative humidity facilitated a prolonged drying period compared with the greenhouse experiment. Every 3 days, ψ_{PD} were measured with a pressure bomb from 20 of the inoculated seedlings. These seedlings were divided equally among watering regime and isolate treatment combinations.

After 1 week in the growth chamber, elongating shoots of the 3-year-old seedlings were inoculated with isolates A1 and A2 as previously described for the greenhouse experiment. Twelve seedlings per watering regime and isolate combination, and seven wounded controls for each watering regime, were used in each of two separate (separated by 2 weeks) completely randomized trials. A noncolonized WA plug was applied to wounded controls.

Observations recorded 4 weeks after inoculation included the presence of necrotic needles and cankers, and wound color and resin flow from the wound as described in the greenhouse experiment. Symptom severity, expressed as the distance along the stem from the inoculation site to the farthest point below it at which necrotic needles were observed, was measured at 2 and 4 weeks after inoculation. At 4 weeks after inoculation, the distance below the inoculation site at which cankers were observed also was measured. Soil moisture content and shoot dry weight were determined as described in the first experiment.

S. sapinea was recovered from shoots as described above. After removing needles, 22-cm-long shoots were surface-disinfected as described in the greenhouse experiment. One-centimeter-long segments centered at 0, 2, 4, 6, 8, and 12 cm from the inoculation site were cut aseptically and treated as described in the greenhouse experiment. The presence of *S. sapinea* was determined as described in the greenhouse experiment.

Statistical analyses. Daily ψ_{PD} from the greenhouse experiment were analyzed by one-way analyses of variance with watering regime as the factor. Daily ψ_{PD} from the growth chamber experiment were analyzed by two-factor analyses of variance with inter-

TABLE 1. Origin of *Sphaeropsis sapinea* isolates used in experiments

Isolate[a]	Isolate no.[b]	Host	Geographic origin
A1	411	*Pinus resinosa*	Clearwater Co., MN
A2	128	*P. resinosa*	Grant Co., WI
B1	124	*P. banksiana*	Jackson Co., WI
B2	215	*P. resinosa*	Douglas Co., WI

[a] Morphotype and isolate number.
[b] Culture collection numbers of M. A. Palmer.

actions. Factors used as main effects were watering regime and isolate. Other quantitative data (symptom severity, distances of recovery, shoot dry weights, and soil moisture content) were analyzed by three-factor analyses of variance with all interactions; factors used as main effects were watering regime, inoculation treatment or isolate, and trial. For the growth chamber experiment, to examine time (2 versus 4 weeks) as a fourth-factor for the response variable of symptom severity, a split plot model was used with plant as the whole plot and time of the severity measurement as a subplot. The symptom severity data were analyzed both untransformed and after $\ln(x + 1)$ transformation was applied. The P values and resulting conclusions were similar for both forms of analysis, therefore, results are reported here only for the untransformed data. If significant differences were found ($P \leq 0.05$), means were separated using Fisher's least significant difference (LSD) at $P = 0.05$. Two different LSD values were determined in the growth chamber experiment for symptom severity because of the experimental design (18). LSD*wt* was calculated to separate means within a treatment combination at different times and LSD*at* was calculated to separate means across treatment combinations. Simple linear regression analyses were used to examine relationships between quantitative variables (symptom severity versus recovery distance and distance of cankers). Chi-square goodness-of-fit analyses were used to analyze frequency data (incidence of disease and ratings of resin flow from the wound). Analyses of variance (using general linear model procedure) and linear regression analyses were performed with the Minitab for Windows program (release 10.2; Minitab Inc., State College, PA). Chi-square goodness-of-fit analyses were performed with the Statgraphics program (release 5.1; STSC Inc., Rockville, MD).

RESULTS

Greenhouse experiment. The watering regimes produced significant differences in ψ_{PD} between stressed and nonstressed

seedlings for all days except the first readings immediately after watering in both trials (Fig. 1). The mean ψ_{PD} (all days) for trials 1 and 2, respectively, were –1.20 MPa ± 0.03 (standard error) and –1.09 MPa ± 0.03 for stressed seedlings; –0.75 MPa ± 0.01 and –0.71 MPa ± 0.01 for nonstressed seedlings. The lowest daily mean ψ_{PD} of stressed seedlings were –1.90 MPa ± 0.10 for trial 1 and –1.90 MPa ± 0.12 for trial 2. Soil moisture content at the end of the experiment averaged 11.0% ± 1.0 and 35.7% ± 0.6 for the stressed and nonstressed treatments, respectively ($P < 0.001$). Inoculation treatment had no affect on soil moisture content at the end of the experiment ($P = 0.520$).

Needles produced during the current year on stressed seedlings appeared slightly chlorotic compared with needles of nonstressed seedlings after 23 days under the different watering regimes. Differences in shoot dry weights were significant for watering regime ($P < 0.001$), but not for the inoculation treatment ($P = 0.121$). The mean shoot weights were 12.7 g ± 1.0 for stressed seedlings and 15.6 g ± 1.2 for nonstressed seedlings.

Symptoms of inoculated seedlings included necrotic needles, cankers around the wound site, darkening of the wound site, and greater resin flow from the wound. Earliest symptoms (necrotic needles and resin flow from the wound) were observed after 3 days on nonstressed seedlings inoculated with isolates of morphotype A and 5 days on stressed seedlings inoculated with isolates of morphotype A. The wound sites of stems inoculated with morphotype A isolates turned black or darker than wound sites of stems inoculated with isolates of morphotype B. The wound sites of wounded controls either remained green or turned tan. Although there was no effect of watering regime on wound color, there was an effect of watering regime on the amount of resin flow from the inoculation (Table 2). Inoculated, nonstressed seedlings produced more resin from wounds than stressed seedlings. The wound sites of stems inoculated with isolates of morphotype A also had greater resin flow than wounds of stems inoculated with isolates of morphotype B. Resin flow from the wounds of inoculated stems was greater than from wounds of control seedlings. The flow of resin beyond the wound was rarely observed for control seedlings of either watering regime.

Incidence of disease (number of seedlings with necrotic needles) was influenced by morphotype, regardless of watering regime (P
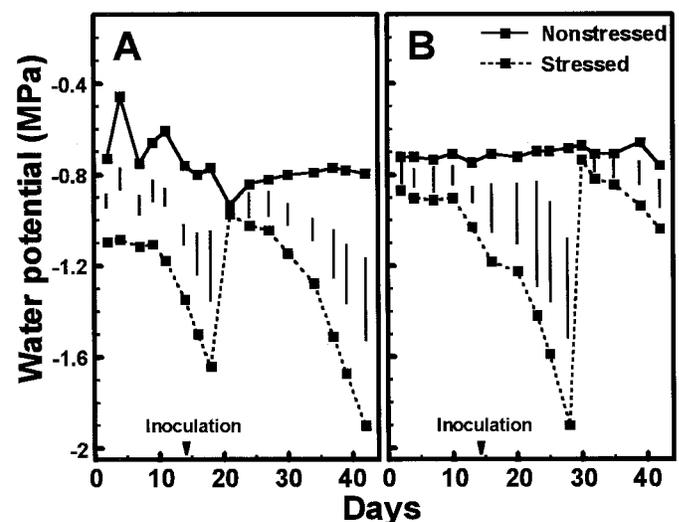
TABLE 2. Number of observations[a] in three resin flow categories[b] for red pine (*Pinus resinosa*) seedlings inoculated with *Sphaeropsis sapinea* morphotypes and either water stressed or well watered

| Watering regime[c] | Inoculation treatment | Resin flow categories | | | P[d] |
		Low	Moderate	High	
Nonstressed	A1	0	4	10	0.004
	A2	2	2	10	0.008
	B1	3	7	4	0.555
	B2	3	4	7	0.390
	Wounded control	11	3	0	<0.001
Stressed	A1	1	8	5	0.121
	A2	4	3	7	0.343
	B1	7	7	0	0.030
	B2	3	8	3	0.273
	Wounded control	9	5	0	0.008
P[d]		0.002	0.456	<0.001	

[a] Values are the total number from two greenhouse trials each having seven seedlings per treatment (watering regime and isolate combinations) with seven seedlings for controls (total 14 in a row). Young shoots were inoculated by placing colonized agar plugs on wounds made by removing a needle fascicle.

[b] Resin flow from the wound was visually estimated 4 weeks after inoculation and ranked as low (no resin off the wound), moderate (resin less than 3 mm from the wound), or high (resin 3 mm or farther from the wound).

[c] Seedlings were either watered daily (nonstressed) or watered when the mean predawn needle water potential fell below –1.64 MPa (stressed).

[d] Probability that there is no difference among resin flow categories within a row, or among watering regime and inoculation treatment combinations within a column, based on chi-square tests. The expected frequencies used were $(S \times C)/N$, in which S is the number of observations in a category for an observation, C is the total number of observations of the category of interest, and N is the total number of observations.

Fig. 1. Mean predawn needle water potentials for red pine (*Pinus resinosa*) seedlings grown in a greenhouse. On day 0, all seedlings were watered to field capacity. Seedlings were then either watered daily (nonstressed) or when the mean predawn needle water potential fell below –1.64 MPa (stressed). On day 14, seedlings were inoculated. Values are means of two separate trials (**A and B**) with five seedlings per observation in each trial. Vertical lines indicate Fisher's least significant differences for separating means for a single day at $P = 0.05$.

= 0.011 for stressed; $P < 0.001$ for nonstressed). The incidence of disease was greater among seedlings inoculated with isolates of morphotype A than among those inoculated with isolates of morphotype B. Four weeks after inoculation, isolates of morphotype A caused disease on all seedlings under both watering regimes. On average, isolates of morphotype B caused disease on 57% of seedlings under both watering regimes. The incidence of disease did not differ among seedlings subjected to different watering regimes that were inoculated with different isolates of the same morphotype ($P = 1.000$ for A isolates; $P = 0.405$ for B isolates). Necrotic needles and cankers were observed on one wounded control and on none of the nonwounded control seedlings.

Although there were no watering regime effects on the incidence of disease among seedlings inoculated with the same morphotype, there were both watering regime and inoculation treatment effects on symptom severity. Three-factor analysis of variance of symptom severity 4 weeks after inoculation indicated effects of watering regime ($P = 0.007$) and inoculation treatment ($P < 0.001$), but not of trial ($P = 0.601$). Stressed seedlings inoculated with isolates of morphotype A developed necrotic needles at greater distances from the inoculation site than nonstressed seedlings inoculated with the same isolates (Fig. 2). Based on the LSD comparisons, this effect was significant for isolate 2 of morphotype A at $P = 0.05$ in both trials and for isolate A1 at $P = 0.05$ in one trial and at $P = 0.08$ in the other. The effect of watering regime was not significant for either B isolate in either trial. The interactions between inoculation treatment and watering regime ($P = 0.061$) or trial ($P = 0.322$) were not significant.

Neither isolate nor watering regime influenced the recovery of *S. sapinea* from the point of inoculation, but these did affect the distance at which the fungus was recovered. *S. sapinea* was always recovered from the inoculation point of seedlings of both morphotype treatments and both watering regimes, except for the controls from which it was not recovered at any distance. Three-factor analysis of variance of distance at which *S. sapinea* was recovered at 4 weeks after inoculation indicated effects of the inoculation treatment used ($P < 0.001$) and watering regime ($P = 0.011$), but not of trial ($P = 0.989$). The mean distance below the inoculation site of recovery for isolates of morphotype A was 4.8 cm ± 1.1 on stressed and 2.5 cm ± 0.7 on nonstressed seedlings. Mean distance below the inoculation site of recovery for isolates of morphotype B was 0.2 cm ± 0.2 on stressed and 0.0 cm ± 0.0 on nonstressed seedlings. Symptom severity was positively correlated with distance of recovery from the inoculation point for isolates of morphotype A ($r = 0.94$, $P < 0.001$, slope = 0.95, intercept = –0.41, $n = 56$) and for isolates of morphotype B ($r = 0.80$, $P < 0.003$, slope = 0.91, intercept = 0.42, $n = 56$).

Growth chamber experiment. The watering regimes produced significant differences in ψ_{PD} between stressed and nonstressed seedlings for all days except the first reading of trial 1 and the first two readings of trial 2 (Fig. 3). The mean ψ_{PD} (all days) for trials 1 and 2, respectively, were –1.11 MPa ± 0.09 and –1.14 MPa ± 0.12 for the stressed seedlings; –0.46 MPa ± 0.01 and –0.52 MPa ± 0.01 for nonstressed seedlings. The lowest mean ψ_{PD} (last day of experiment) for stressed seedlings were –1.90 MPa ± 0.18 for trial 1 and –1.73 MPa ± 0.22 for trial 2. The effect of inoculation treatment and interactions between inoculation treatment and watering regime were not significant for any day in either trial. The mean soil moisture content at the end of the experiment was 7.9% ± 1.0 and 38.0% ± 0.2 for the stressed and nonstressed treatments respectively ($P < 0.001$). Inoculation treatment had no affect on soil moisture content at the end of the experiment ($P = 0.590$).

Needles produced during the current year on stressed seedlings appeared slightly chlorotic compared with needles of nonstressed seedlings after 28 days under the different watering regimes. Differences in shoot dry weights were significant for watering regime
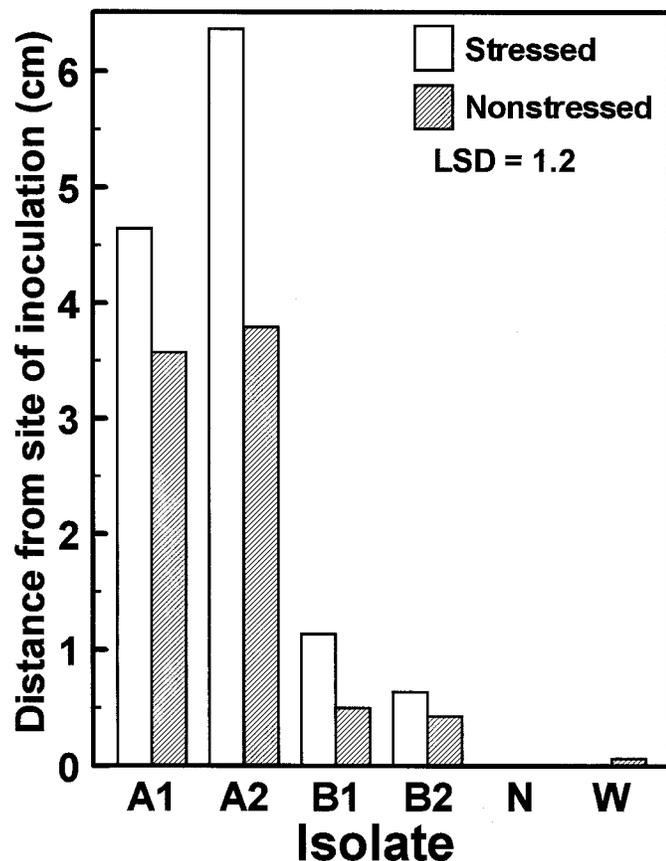

Fig. 2. Symptom severity, expressed as the mean distance below the inoculation site at which necrotic needles were observed 4 weeks after inoculation. Greenhouse-grown red pine (*Pinus resinosa*) seedlings were wounded and inoculated with agar plugs colonized by *Sphaeropsis sapinea* isolates. Treatments include two A (A1 and A2) and two B (B1 and B2) isolates and both nonwounded (N) and wounded (W) controls. Seedlings were either watered daily (nonstressed) or when the mean predawn needle water potential fell below –1.64 MPa (stressed). Values are combined means of two separate trials with seven trees per treatment combination in each trial. The LSD value is the Fisher's least significant difference for separating the means at $P = 0.05$.

TABLE 3. Number of observations[a] in three resin flow categories[b] for red pine (*Pinus resinosa*) seedlings inoculated with the A morphotype of *Sphaeropsis sapinea* and either water stressed or well watered

Watering regime[c]	Inoculation treatment	Resin flow categories			P[d]
		Low	Moderate	High	
Nonstressed	A1	1	13	10	0.002
	A2	4	9	11	0.007
	Wounded control	11	3	0	0.003
Stressed	A1	10	12	2	0.320
	A2	7	14	3	0.328
	Wounded control	11	3	0	0.003
P[d]		<0.001	0.376	<0.001	

[a] Values are the total number from two growth chamber trials each having 14 seedlings per treatment (watering regime and isolate combinations) with seven seedlings for controls (total 24 in a row for isolates and 14 in a row for controls). Young shoots were inoculated by placing colonized agar plugs on wounds made by removing a needle fascicle.

[b] Resin flow from the wound was visually estimated 4 weeks after inoculation and ranked as low (no resin off the wound), moderate (resin less than 3 mm from the wound), or high (resin 3 mm or farther from the wound).

[c] Seedlings were either watered daily (nonstressed) or never watered (stressed).

[d] Probability that there is no difference among resin flow categories within a row, or among watering regime and inoculation treatment combinations within a column, based on chi-square tests. The expected frequencies used were ($S \times C)/N$, in which S is the number of observations in a category for an observation, C is the total number of observations of the category of interest, and N is the total number of observations

($P < 0.001$), but not for the inoculation treatment ($P = 0.275$). The mean shoot weights were 14.7 g $\pm$ 1.1 for stressed seedlings and 18.9 g $\pm$ 1.7 for nonstressed seedlings.

Symptoms observed on inoculated seedlings were similar to those on seedlings inoculated with isolates of morphotype A in the greenhouse experiment. Earliest symptoms (necrotic needles and resin flow from the wound) were observed after 3 days on non-stressed seedlings and 5 days on stressed seedlings. The wound sites of inoculated stems also turned dark and the wound site of wounded controls remained green or turned tan. Although there was no effect of watering regime on wound color, watering regime influenced the amount of resin flow from the inoculation (Table 3). Inoculated, nonstressed seedlings produced more resin from wounds than stressed seedlings. Resin flow from the wounds of inoculated stems was greater than from wounds of control seedlings. The flow of resin beyond the wound was rarely observed for control seedlings of either watering regime.

Four weeks after inoculation, all inoculated seedlings had some disease. Necrotic needles and cankers were never observed on control seedlings. The incidence of disease (number of seedlings with necrotic needles) did not differ among inoculated seedlings subjected to different watering regimes ($P = 1.000$).

There were both watering regime and time effects on symptom severity. Four-factor analysis of variance of symptom severity at 2 and 4 weeks after inoculation indicated effects of watering regime ($P < 0.001$) and time ($P < 0.001$), but not of isolate ($P = 0.093$) or trial ($P = 0.840$). Inoculated, stressed seedlings developed necrotic needles at greater distances from the inoculation site than non-stressed seedlings (Fig. 4). The interaction between isolate and watering regime was not significant ($P = 0.736$), suggesting that the isolates responded consistently under the different watering regimes. At 4 weeks after inoculation, symptom severity was positively correlated with the mean distance of cankers ($r = 0.97$, $P < 0.001$, slope = 0.86, intercept = 0.31, $n = 96$).

There was an interaction between time and watering regime ($P < 0.001$), but not between time and isolate ($P = 0.136$). The significant interaction between time and watering regime indicates differences in rates of symptom development between watering regimes. The rate of disease development decreased as time after inoculation increased for both watering regimes. The rate of disease development for inoculated seedlings was 1.7 cm/week $\pm$ 0.1

for stressed seedlings and 1.0 cm/week $\pm$ 0.1 for nonstressed seedlings from 0 to 2 weeks. The rate of disease development from 2 to 4 weeks dropped to 0.9 cm/week $\pm$ 0.1 for stressed seedlings and 0.2 cm/week $\pm$ 0.1 for nonstressed seedlings.

The watering regime did not influence the recovery of *S. sapinea* from the point of inoculation, but it did affect the distance at which the fungus was recovered. *S. sapinea* was always recovered from the inoculation point of seedlings of both watering regimes, except for the controls, from which it was not recovered at any distance. Three-factor analysis of variance of distance at which *S. sapinea* was recovered at 4 weeks after inoculation indicated effects of isolate used ($P = 0.022$) and watering regime ($P < 0.001$), but not of trial ($P = 0.897$). Mean distance below the inoculation site of recovery for *S. sapinea* was 5.3 cm $\pm$ 0.7 on stressed and 2.4 cm $\pm$ 0.4 on nonstressed seedlings. Symptom severity was positively correlated with distance of recovery from the inoculation point ($r = 0.90$, $P < 0.001$, slope = 0.94, intercept = 0.14, $n = 96$).

DISCUSSION

Environmental conditions are known to influence both the incidence and severity of woody plant diseases (24,25). Water stress
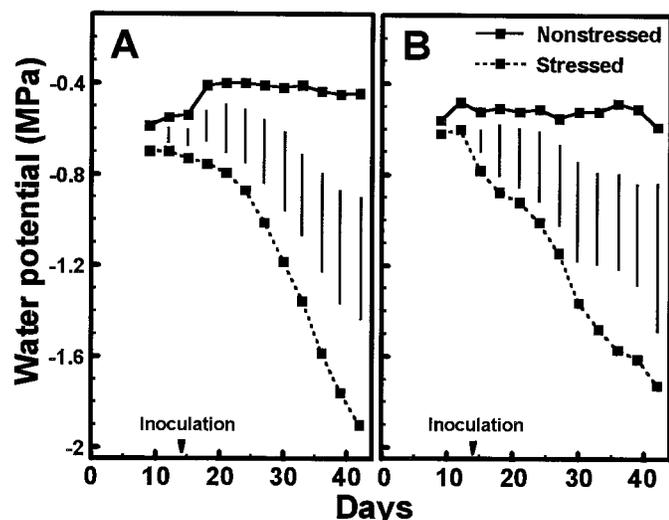
Fig. 3. Mean predawn needle water potentials for red pine (*Pinus resinosa*) seedlings grown in a growth chamber. On day 0, all seedlings were watered to field capacity. Seedlings were then either watered daily (nonstressed) or not at all (stressed). On day 14, seedlings were inoculated. Values are means of two separate trials (**A and B**) with 10 seedlings per observation in each trial. Vertical lines indicate Fisher's least significant differences for separating means for a single day at $P = 0.05$.
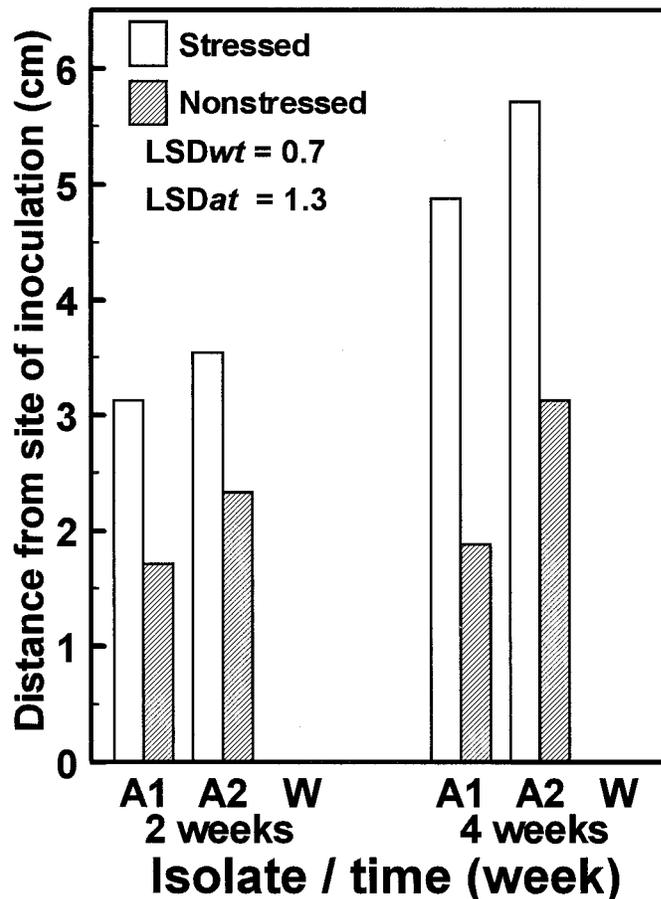
Fig. 4. Symptom severity, expressed as the mean distance below the inoculation site at which necrotic needles were observed 2 and 4 weeks after inoculation. Growth-chamber-grown red pine (*Pinus resinosa*) seedlings were wounded and inoculated with agar plugs colonized by *Sphaeropsis sapinea* isolates. Treatments include two isolates (A1 and A2) and a wounded control (W). Seedlings were either watered daily (nonstressed) or not at all (stressed). Values are combined means of two separate trials with 12 trees per treatment combination and seven trees per treatment combination for controls in each trial. The LSD values are Fisher's least significant differences for separating the means at $P = 0.05$. LSD*wt* is for separating means within a treatment combination at different times and LSD*at* is for separating means across treatment combinations.

is associated with enhancement of disease development on several tree species by many pathogens (1,3,4,22,25,26). However, water stress also may have a neutral or negative influence on the development of certain woody plant diseases (5,13,17,30,31). In this study, the results of both experiments showed that water stress of established red pine seedlings enhances disease development and colonization by isolates of *S. sapinea* morphotype A. This conclusion is consistent with field observations and experimental results obtained with other host species for *S. sapinea* isolates of unknown morphotype (2,6,9,16,17,20,33).

Although this study shows that water stress can result in more severe Sphaeropsis shoot blight and canker disease, increases in disease severity were only significant for isolates of morphotype A. In contrast, isolates of morphotype B showed no significant increases in aggressiveness when their hosts were water stressed. In a previous study, isolates of morphotype B were not aggressive on red pine (7). These isolates either do not cause severe disease to red pine or the water potentials used in this study were not low enough to stimulate such development.

The current study also showed that relatively moderate water stress, compared with previous controlled studies (9,17,33), can result in greater disease severity by isolates of *S. sapinea* morphotype A. Predawn water potentials of stressed trees were comparable with those observed in the field (29; J. T. Blodgett, *unpublished data*). Results from the current study support field observations that water stress can result in increased disease severity due to Sphaeropsis shoot blight (6,9,16,20). Extremely low host water potentials (–3.0 MPa or lower) or other environmental factors (such as poor site, temperature extremes, hail damage, defoliation, and transplanting stress) not controlled in field studies are not required for severe development of this disease on red pine.

Schoeneweiss (25) concluded that threshold levels of water stress must be exceeded before woody plants are predisposed to colonization by many nonaggressive pathogens. The stems of these hosts remained resistant to fungal attack until the threshold levels were exceeded. Consistent with this assertion, a threshold water potential was reported for the predisposition to colonization by *S. sapinea* for radiata pine to be around –2.5 MPa (9). Although there was less disease development on nonstressed seedlings in our study, seedlings inoculated with isolates of morphotype A became diseased and no threshold was apparent. Therefore, based on seedling inoculations, isolates of *S. sapinea* morphotype A do not respond as nonaggressive pathogens (25), but better resemble moderate to aggressive pathogens on red pine, for which host water deficits can enhance disease with no apparent thresholds (24).

The effect of environmental stresses on disease development is likely to be on host colonization (i.e., postinfection processes) rather than on exclusion of the pathogen (24). Pathogens can often enter resistant and susceptible plants with equal frequency (8,24,32), and a previous study has demonstrated that both morphotypes of *S. sapinea* can penetrate well-watered red pines without wounding (7). The enhancement of disease associated with water stress in the current study using wounded, inoculated seedlings also supports colonization as opposed to initial infection as the important factor of disease of water-stressed red pine.

Host water stress appears to inhibit both rapidly occurring and slowly developing responses of red pine to colonization by *S. sapinea*. Resinous lesions rapidly develop in phloem and sapwood around sites of conifer invasions by other fungi (14,19,23). In this study, water stress resulted in less resin flow from the wound. This reduced resin flow indicates reduced potential for physical and chemical inhibition of the pathogen. Moreover, water stress apparently inhibits defensive responses that require more time for expression by red pine. This was indicated by reduction in rates of colonization during the last half of the growth chamber trials. Because the rate of disease development decreased over time in seedlings of both watering regimes (decreasing more in nonstressed

than in stressed seedlings), defense mechanisms may be slowed more in stressed versus nonstressed seedlings, at the water potentials investigated in this study.

Reduction in long term defensive responses may indicate hosts that are metabolically compromised. Pines respond to drought stress by closing stomates and reducing the rate of many physiological processes (12,15,24). The reduction in shoot growth of water-stressed seedlings observed in this study suggests that these seedlings were experiencing reduced carbon assimilation. Reduced carbon reserves may decrease some defense mechanism(s) and diminish the plants' ability to restrict colonization by pathogens (24).

Currently it is not clear how water stress influences colonization of pines by *S. sapinea*. The methods used in this study provide a foundation for further comparisons of nutritional, physical, chemical, or other host differences between different watering regimes that may explain differences in host colonization. Given the distinct differences in severity of symptoms produced by red pines inoculated with the two morphotypes, this pathogen offers opportunities for more fundamental studies of host-pathogen interactions.

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