

## Sources of Inoculum of *Sphaeropsis sapinea* in Forest Tree Nurseries

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### ABSTRACT

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Inoculum potential of tissues colonized by *Sphaeropsis sapinea* was determined by studying inoculum potential and spore dispersal from cones, seedlings, and litter of red pine. Cones from red pine in nursery windbreaks, cones from beneath windbreaks, and diseased nursery seedlings had the greatest inoculum potential. Conidia of *S. sapinea* were dispersed throughout the growing season from all sources, but most spores were disseminated from April through June, when second- and third-year seedlings were most susceptible to infection. The number of spores

produced by individual cones differed among and within windbreaks, and the period of peak spore production by individual cones differed among cones. Disease gradients in seedbeds of first-year red pine at the Wilson Nursery, WI, indicated that most inoculum originated from windbreaks bordering the west end of the seedbeds. The inoculum potential of cones and the pattern of disease distribution in the seedbeds suggest that cones on windbreak trees are the primary inoculum source in seedbeds of first-year red pine.

*Additional keywords:* *Diplodia pinea*, disease gradients, *Pinus resinosa*.

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Shoot blight of seedlings of red pine (*Pinus resinosa* Ait.) caused by *Sphaeropsis sapinea* (Fr.) Dyko & Sutton (*Diplodia pinea* (Desm.) Kickx) is a periodic problem in forest tree nurseries in the

north central United States. The disease was first observed in 1975 at Griffith State Nursery, Wood County, WI (12), but only recently have losses become severe (7).

*S. sapinea* has extensively damaged native and exotic conifers in plantations, ornamental plantings, and shelterbelts throughout the world (5, 10). However, there are few reports of *S. sapinea* causing a disease of nursery seedlings (2, 3, 16), and, consequently, no

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guidelines have been recommended for managing this disease in forest tree nurseries. Fungicide application programs that have been developed to protect conifers in shelterbelts and ornamental plantings (9,15), and inoculum sanitation could be easily integrated into a nursery management program. However, the life cycle and primary source of *S. sapinea* in forest tree nurseries need to be determined before we can fully understand how to control this disease.

In the north central United States, infection of different ages of seedlings occurs throughout the growing season (7). This suggests that inoculum is produced continuously in the nurseries. Second- and third-year seedlings are susceptible in early spring during budbreak and shoot elongation. Fall-seeded first-year seedlings, which emerge in late May, are probably infected by *S. sapinea* sometime before August, when the first symptoms are observed (7). Cones colonized by *S. sapinea* were found on red pine in windbreaks at Wilson Nursery, Grant County, WI, where disease incidence has reached 35% (13).

The importance of local sources of inoculum has been demonstrated in epidemics of several plant diseases (6). In forest tree nurseries, cones colonized by *S. sapinea* in windbreaks were suggested as the primary source of this fungus (9). However, because *S. sapinea* is a facultative parasite (17), infected red pine needles from adjacent windbreaks likely serve as additional inoculum sources. Diseased seedlings remaining in the seedbeds may serve as inoculum sources in subsequent years.

The objective of this research was to determine if cones on windbreak trees are the primary source of *S. sapinea* in forest tree nurseries. To accomplish this, spore dispersal and inoculum potential of several tissues colonized by *S. sapinea* were compared and disease gradients in nursery seedbeds were studied. Fungicidal controls for this disease were developed in a concurrent study (8).

## MATERIALS AND METHODS

**Conidial dispersal in nurseries.** Dispersal of conidia from seedlings, cones, and windbreak trees of *P. resinosa* was studied in 1981 and 1982 at the Griffith and Wilson State nurseries. Windbreaks at both nurseries contained three rows of 20–30-yr-old trees. At Griffith Nursery, windbreaks were composed of a mixture of conifer and hardwood species; at Wilson Nursery the windbreaks were almost exclusively red pine. Cones with pycnidia of *S. sapinea* were abundant on red pine windbreak trees. Shoot blight and collar rot caused by *S. sapinea* have been reported at both nurseries (7). Second-year seedlings, infected in 1980, and cones from windbreaks that border seedbeds containing seedlings infected by *S. sapinea* were used to determine timing and relative amount of conidia dispersed. Cones or bare-root seedlings were arranged in a single layer, wrapped in nylon net fabric, and secured between two 0.5 m<sup>2</sup> plastic-coated wire screens. Screens were mounted on four aluminum posts 43 cm above ground. Each inoculum source plot was replicated twice at a 1-m spacing in small clearings in windbreaks at the nursery where tissues were collected. The highest incidence of shoot blight occurs in beds of first-year seedlings and in beds of second-year seedlings where severe disease occurred in the first year (8). Thus, we hypothesized that most inoculum originated from existing cones or from seedlings infected the previous year. To simulate these conditions, diseased seedlings were replaced in 1982 with seedlings infected in 1981, whereas cones remained in screens for 2 yr.

Dispersal of conidia was monitored with Vaseline-coated microscope slides mounted horizontally in wire holders. One spore trap was placed 27 cm above ground beneath each screen. Spore traps were also placed beneath two red pine in each windbreak. Slides were changed weekly from 20 April to 5 October 1981 and from 5 April to 1 November 1982. The relative number of spores trapped on each slide was determined by counting the number of conidia of *S. sapinea* in three transects across a slide at 430 $\times$ . The counts for each slide were added together for a total inoculum source spore count.

In both nurseries, rain was collected and measured weekly in a rain gauge located near the plots.

**Inoculum potential of tissues colonized by *S. sapinea*.** Dispersal of conidia and seedling infection from four types of red pine tissues colonized by *S. sapinea* were studied in 1982 and 1983 at the Jackson County Forest, WI. Cones, seedlings, and litter were collected from Griffith Nursery, located 72 km east of the Jackson County Forest study site. Cones were removed from red pine in windbreaks that bordered seedbeds containing seedlings infected by *S. sapinea*. Cones and litter (mostly dead red pine needles) were collected from the ground in the same windbreak or in the adjacent seedbeds. Second-year seedlings, infected in 1981, were also collected. Tissues were placed in plastic bags, kept cool, and transported to the study area. On 28 April 1982, each type of tissue was secured between two 1.0  $\times$  0.5-m screens as described previously. Because of the short latent period of *S. sapinea* (1), two layers of nylon net were used as the control instead of disease-free material. Use of net only also allowed us to monitor numbers of naturally occurring conidia and/or conidia from adjacent plots. Screens were mounted over similar-size plots of 100 healthy second-year red pine seedlings. A randomized block design was used with five replications of each treatment. Plots were spaced 1 m apart within replicates and 1.5 m apart between replicates. Screens and inoculum remained in place during the study. Seedlings showing symptoms of shoot blight were collected on 22 July 1982 and 13 July 1983 and examined in the laboratory to verify the presence of *S. sapinea*. An analysis of variance was performed each year on the arcsine transformation (4) of the number of diseased trees per plot. Control plots were not included in the analysis because no diseased seedlings were observed in these plots. Comparisons among treatments were made with the Student-Newman-Keuls' test.

Dispersal of conidia was monitored as described previously using two traps beneath each inoculum source. Slides were changed weekly from 28 April to 1 November 1982. In 1983, spore dispersal was monitored with one spore trap beneath each screen in only two of the replicates. Slides were changed weekly from 11 April to 31 October 1983. Spore counts for each plot were summarized as described for conidial dispersal. To relate disease incidence to phenological development of seedlings, the growth of five randomly located seedlings was monitored in both years as described previously (7).

**Dispersal of conidia from individual red pine cones.** In 1982, 50 cones, 2 yr or older, of *P. resinosa*, were collected from windbreaks at Griffith Nursery. Ten cones were collected from each of five windbreaks. Five cones that macroscopically appeared to have "few" and five cones that had "many" pycnidia of *S. sapinea* were obtained. In each of these categories, one cone was selected from each of three different heights in the tree (1.4, 3, 4.6 m); and two cones, characterized as "recently fallen" or "decaying," were collected from the ground. Conidiomata from selected cones were examined microscopically to verify the presence of *S. sapinea*.

Individual cones were arranged in a completely randomized design in wire screens as described above. To minimize the chance of lateral movement of conidia, cones were placed 17 cm apart on the screen and a spore trap was placed approximately 1.5 cm directly beneath each cone. Slides were changed at approximately 21-day intervals from 29 May to 22 July.

In 1983 the experiment was modified. Forty cones were collected from two windbreaks at Griffith Nursery. In each windbreak, five cones were collected at 3 m above ground in each of three trees, and five cones were collected from the ground. Cones were placed in screens in a completely randomized design. Spore trap slides were changed weekly from 11 April to 20 June. In both 1982 and 1983, the relative number of spores trapped from each cone was determined as described above.

**Disease gradients in nursery seedbeds.** Disease gradients were studied in beds of first-year red pine in 1981 and 1982 at Wilson Nursery. Each study area contained nine essentially level 1.2  $\times$  164-m seedbeds that were bordered on the east and west by red pine windbreaks. The distance from each edge of the seedbeds to the windbreaks was approximately 10 m. Each year, the number of diseased and healthy seedlings was counted in 10 randomly selected plots (0.2  $\times$  1.2 m) in each of two randomly selected

seedbeds in each section for a total of 60 plots. Gradients were modeled using nonlinear least squares regression to estimate model parameters.

## RESULTS

**Conidial dispersal in nurseries.** Seasonal periodicity of spore dispersal was similar in 1981 and 1982. Dispersal of conidia and amount of rainfall in 1982 at the Griffith and at the Wilson nurseries are shown in Figure 1. In both years, conidia were trapped at both nurseries throughout the growing season, although most spores were trapped from April through June. More conidia were trapped from seedlings and cones than from beneath windbreak trees. Conidia were trapped only in weeks when rain fell.

**Inoculum potential of tissues colonized by *S. sapinea*.** In both 1982 and 1983, significantly ( $P = 0.01$ ) more seedlings were diseased and more conidia were trapped from seedlings, cones from trees, or cones from the ground than from litter. Only a few

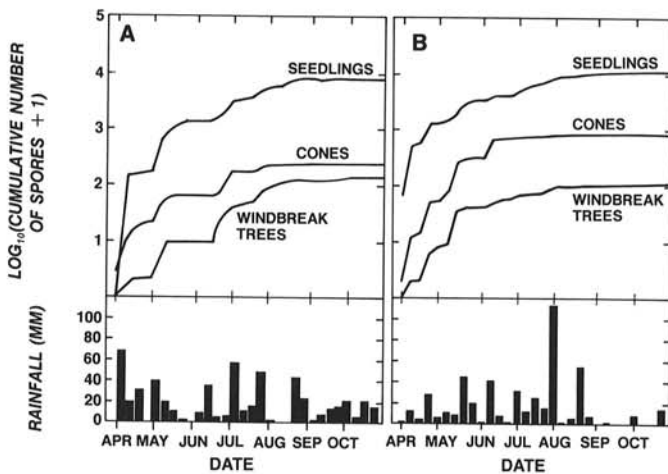


Fig. 1. Seasonal periodicity of conidial dispersal of *Sphaeropsis sapinea* from three red pine inoculum sources and weekly rainfall amounts in 1982. A, Griffith Nursery, WI, 1982. B, Wilson Nursery, WI, 1982.

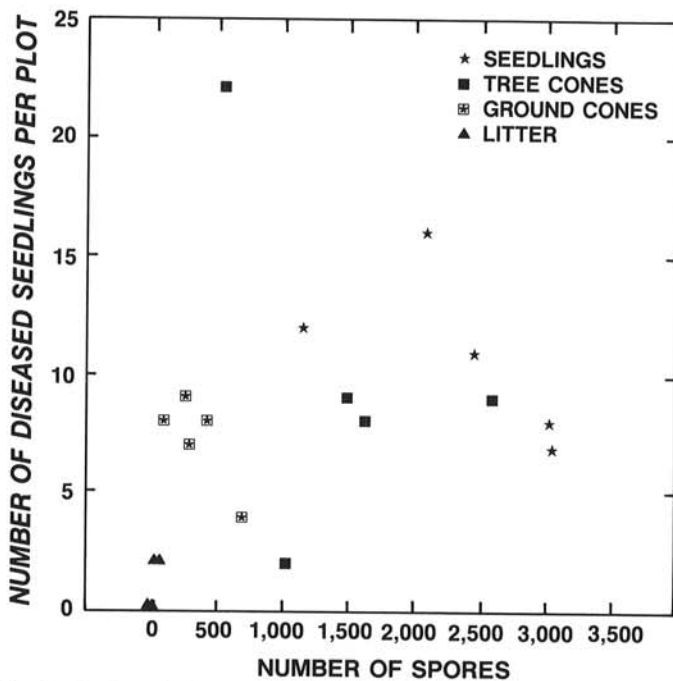


Fig. 2. Number of diseased red pine seedlings in relation to number of conidia of *Sphaeropsis sapinea* disseminated 28 April-21 June from four inoculum sources. Jackson County Forest, WI, 1982.

spores were trapped, and no trees were diseased in the control plots. The number of spores trapped differed among replicates of the same inoculum source. However, in general, the greatest number of seedlings were diseased under screens holding tissues that disseminated the most spores during shoot elongation (28 April-21 June) (Fig. 2). Seasonal periodicity of spore dispersal from the different tissues (Fig. 3) was similar to dispersal patterns observed in the nurseries: Conidia were disseminated throughout the 1982 and 1983 growing seasons, but most conidia were trapped in May and June. Conidia were trapped only in weeks when it rained.

**Dispersal of conidia from individual red pine cones.** The relative number of conidia disseminated by individual cones in 1982 and 1983 differed among and within windbreaks. There was no apparent relation between abundance of pycnidia, collection height, or windbreak location and the number of spores trapped. In 1983, more spores were trapped from one windbreak than from the other, but this difference was not consistent among trees in the windbreak. Spores were trapped throughout the study period, although peak periods of spore dispersal differed among cones from an individual tree. Weekly spore dispersal from an individual representative tree is shown in Figure 4.

**Disease gradients in nursery seedbeds.** Shoot blight gradients in 1981 and 1982 are shown in Figure 5. In both years, disease incidence generally decreased from the west to the east end of the seedbed.

Empirical models were used to describe the disease distribution, and tests of hypotheses were conducted to determine if both west and east windbreaks contributed to the gradients. The initial model for the disease gradient provided for only one windbreak as an inoculum source:

$$E(Y) = b_1 \exp(b_2 X), \quad (1)$$

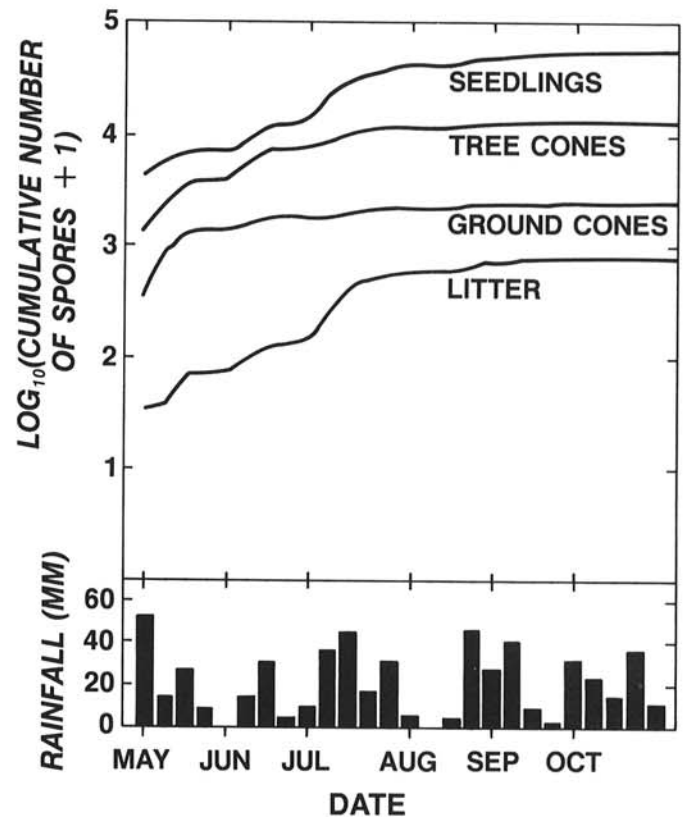


Fig. 3. Seasonal periodicity of conidial dispersal of *Sphaeropsis sapinea* from four red pine inoculum sources and weekly rainfall amounts. Dispersal from control plots is not shown because few or no conidia were trapped each week. Conidial dispersal 28 April-4 May is considered as the first week in May. Jackson County Forest, WI, 1982.

where  $Y$  is percent diseased seedlings,  $X$  is the distance from the west windbreak, and  $b_1$  and  $b_2$  are parameters to be estimated. A second model provided for contributions to the gradient by both windbreaks:

$$E(Y) = b_1 \exp(b_2 X) + b_3 \exp[b_4(184 - X)], \quad (2)$$

where  $Y$  and  $X$  are as in the model in equation 1, 184 is the distance in meters between the windbreaks, and  $b_1$ ,  $b_2$ ,  $b_3$ , and  $b_4$  are parameters to be estimated. Inferences concerning the contribution of the second windbreak were made by comparing the residual sums of squares error as outlined by Ratkowsky (11). The  $R^2$  and  $s_{res}$  values for each model and year are given in Table 1. In both years, the model described by equation 1 was statistically significant (1981:  $P < 0.01$ ; 1982:  $P < 0.01$ ). In 1982, the model described by equation 2 was significantly better than that described by equation 1 ( $P = 0.06$ ).

## DISCUSSION

Conidia of *S. sapinea* were disseminated from several red pine tissues throughout the growing season. Conidia were trapped only when rain fell, as reported previously (1). Diseased tissues differed in their inoculum potential and consequently in their ability to act as inoculum sources for seedlings.

Primary sources of inoculum in several disease epidemics have been identified by tracing disease gradients to the source (6). Analysis of disease gradients indicated that there was a relation between disease incidence and distance from the windbreaks and that the east windbreak contributed to the gradient (at least in 1982). This pattern of disease in nursery seedbeds and the inoculum potential of cones from trees suggest that red pine cones in windbreaks are the primary source of inoculum in beds of first-year seedlings.

The shoot blight gradients, particularly in 1981, were flatter than those described by Gregory (6). Schmidt et al (14) also observed a flatter disease distribution pattern in gradients of fusiform rust (*Cronartium quercuum* f. sp. *fusiforme*). They suggested that the rust gradients were flatter because they had been established over

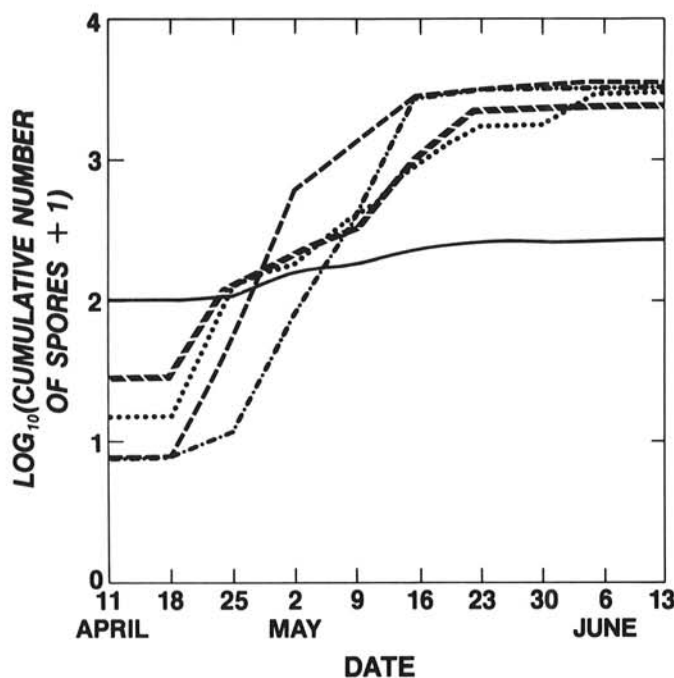


Fig. 4. Number of conidia of *Sphaeropsis sapinea* trapped each week 11 April–20 June from five red pine cones collected at 3 m in a single tree at Griffith Nursery, WI, 1983. Each date indicates the beginning of a new spore trapping period.

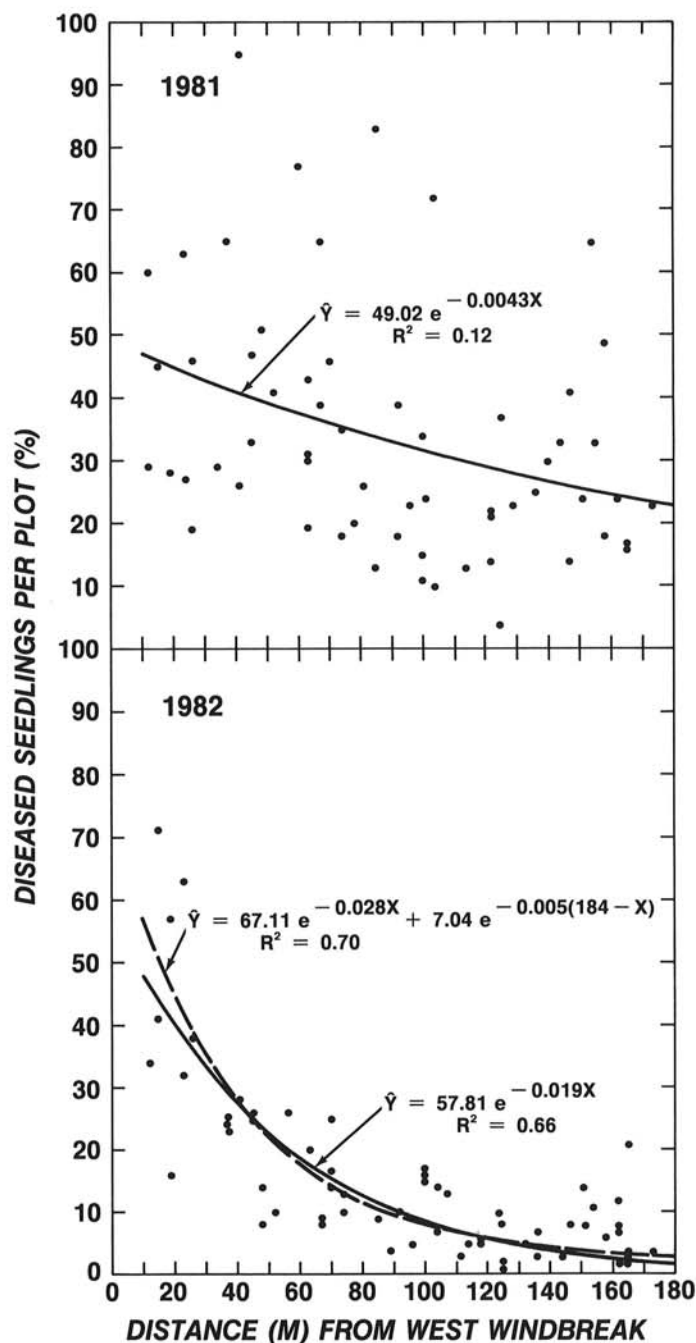


Fig. 5. Incidence of shoot blight caused by *Sphaeropsis sapinea* of first-year red pine in 1981 and 1982 at increasing distances from the windbreak bordering the west edge of the seedbeds, Wilson Nursery, WI. Each point represents the proportion of diseased seedlings ( $\times 100$ ) in a single  $0.2 \times 1.2$ -m plot or the weighted average of two or more plots at the same distance from the west windbreak.

TABLE 1.  $R^2$  and  $s_{res}$  values for two models of disease gradients of *Sphaeropsis sapinea* at Wilson Nursery, WI

Model	Year			
	1981		1982	
	$R^2$	$s_{res}$	$R^2$	$s_{res}$
1 <sup>a</sup>	0.12	0.18	0.66	0.09
2	0.12	0.19	0.70	0.08

<sup>a</sup> Model 1 =  $E(Y) = b_1 \exp(b_2 X)$ ; 2 =  $E(Y) = b_1 \exp(b_2 X) + b_3 \exp[b_4(184 - X)]$ , where  $Y$  is percent diseased;  $X$  is the distance from the west windbreak; 184 is the distance in meters between windbreaks; and  $b_1$ ,  $b_2$ ,  $b_3$ , and  $b_4$  are parameters to be estimated.

an extended time and were the result of varying atmospheric and environmental conditions. In addition, aeciospore inoculum was produced by a continuous volume source rather than a point source. The shoot blight gradients were probably the result of several spore deposition gradients of *S. sapinea* originating from cones of varying inoculum potential and elevations. Spore dispersal occurred throughout the seedling susceptibility period. Perhaps in 1981 a greater number of successful spore deposition events occurred from both west and east windbreaks than in 1982, thus explaining the higher disease incidence and more variable distribution observed in this year.

The disease gradients also indicated that most inoculum originated from the windbreaks bordering the west end of the seedbeds. Still, the contribution of the east windbreak to the disease gradient should not be ignored. In 1982, the significantly better fit achieved by the model providing for a contribution by a second windbreak indicates that the secondary source still made an important contribution. In 1981, there was decreased probability of detecting statistical evidence in support of the east windbreak as an inoculum source because of the greater variability in the data. Thus, it is unlikely that we would have detected even minimal evidence in support of the east windbreak as an inoculum source. However, we also cannot infer that the east windbreak did not contribute to the gradient. Based on the 1982 data, which support the east windbreak as a second source, and our knowledge that summer rainfall accompanied by southeasterly winds are not uncommon in this region, we advocate further investigation of the contributions of individual and multiple windbreaks to shoot blight gradients. Unfortunately, information on wind direction was not obtained during this study, so it is not known whether the disease gradients were primarily influenced by consistent westerly winds, by a substantial difference in the amount of inoculum between windbreaks, or by microclimatic conditions more favorable for infection in the west end of the seedbeds. If the amount of disease differs because of wind direction, the rapid decline of shoot blight from the west to the east end of the seedbeds would indicate that conidia of *S. sapinea* can travel only a limited distance.

The results of this study demonstrate that local sources of inoculum are important in epidemics of this disease. We suggest that inoculum sanitation is an important tool for managing outbreaks of *S. sapinea* in forest tree nurseries. Diseased seedlings and cones collected from trees had the greatest inoculum potential in our study, indicating that sanitation efforts should be directed at

eliminating these sources. Trees infected by *S. sapinea* should be removed and windbreaks should be converted to nonhost species. Inoculum in seedbeds can be reduced by removing diseased seedlings or by protecting seedlings with a fungicide (8).

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