Overwintering and Survival of *Phytophthora cinnamomi* in Fraser Fir and Cover Cropped Nursery Beds in North Carolina

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ABSTRACT

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Phytophthora cinnamomi survived extended periods of soil temperatures at or below 0 C for two consecutive winters as chlamydospores free in the soil, in pieces of organic matter, or in roots of symptomatic and asymptomatic Fraser fir (*Abies fraseri*) seedlings. Inoculum densities decreased markedly in December and January as soil temperatures fell below 0 C. Decreasing inoculum densities were found to be highly correlated ($P = 0.01$) with decreasing soil temperatures. Soil matric potential (when >-0.45 bars), soil texture, initial inoculum density, or seedling age were not correlated with decreasing inoculum densities.

Propagules of *P. cinnamomi* were recovered at every sampling date (October to June) from nonrhizosphere soil in plots with Fraser fir, crimson clover, rye, or left fallow and from rhizosphere soil of crimson clover and rye plants. Chlamydospores were the most frequent form of *P. cinnamomi* isolated from the soil or root tissue during the winter and spring months. Survival of *P. cinnamomi* in the roots of asymptomatic seedlings demonstrated that foliar symptom expression is not a reliable indicator of the incidence of infection within a nursery bed.

Phytophthora root rot of Fraser fir [*Abies fraseri* (Pursh) Poir.], incited by *Phytophthora cinnamomi* Rands, is a destructive disease in nursery beds and plantations. When environmental conditions are favorable for disease development, seedling mortality can be extensive in nursery beds where the seedlings remain for 3 yr and the plant density is high. Disease foci in several Fraser fir nurseries in North Carolina were observed to increase in size over a 3-yr period, suggesting that a portion of the pathogen population was able to survive winter conditions. The results of previous studies (2,11), however, have indicated that soil temperatures <0 C inactivate the pathogen.

Recently, in an attempt to increase the organic matter content and reduce the inoculum density of *P. cinnamomi* in nursery beds, nursery managers sowed a winter cover crop. The effects of nonhost cover crops on inoculum density of *P. cinnamomi* are unknown.

The objectives of this study were to determine how propagules of *P. cinnamomi* overwinter, to investigate the effect of soil temperature and moisture on propagule survival in Fraser fir nursery beds, and to determine the effect of two cover crops, rye and crimson clover (*Secale cereale* L. and *Trifolium incarnatum* L., respectively), on the survival of *P. cinnamomi*.

MATERIALS AND METHODS

Fraser fir nursery beds. Survival of propagules of *P. cinnamomi* in nursery beds with dead and dying seedlings was monitored during two consecutive winters at two locations at the Linville River Nursery, Crossnore, NC. Four plots (3.8 × 1.2 m) were established in each of four nursery beds at both locations during the two winters. Monthly soil samples were taken from beds with 5- and 3-yr-old seedlings from 8 October 1980 to 16 June 1981 and from 1 September 1981 to 18 June 1982, respectively.

Three nonrhizosphere soil cores (approximately 1.27 cm diameter × 12 cm deep) were removed from each of five points along a W-sampling pattern in each Fraser fir plot. Nonrhizosphere soil in this study is defined as that soil containing no root pieces and collected away from the immediate vicinity of a tap root. The three cores taken from each point were bulked to assay for *P. cinnamomi*. Three symptomatic and three asymptomatic seedlings were randomly removed from each plot at each sampling to assay for *P. cinnamomi* in root tissue. All samples were stored at 13 C in the dark and assayed within 2 days.

Soil texture (1) and a drying curve of a soil moisture characteristic (7) were determined for the soils from the two locations. Soil thermograph probes (Weathertronics Incorporated, West Sacramento, CA 95691) were buried at depths of 7.5 and 15 cm to continuously record soil temperature.

Cover crops and fallow nursery beds. On 18 August 1981 four blocks (5.6 × 1.2 m) were established in the nursery beds where the previous overwintering study (1980–1981) had been conducted. Soil (0.4 m³ per block), naturally infested with propagules of *P. cinnamomi* (average of 7.5 propagules per gram of dry soil) obtained from another nursery bed, was uniformly distributed over the surface of each block and incorporated by disking four times to a depth of 15 cm. The blocks were then subdivided into three plots (1.2 × 1.2 m) with a buffer of 0.5 m between each plot. Two weeks later the plots were planted to rye or crimson clover or left fallow. Soil samples were collected monthly from each of the 12 plots between 1 September 1981 and 18 June 1982 in the same manner as previously described. Starting with the October sampling date, 10–20 crimson clover and rye plants each were removed from their respective plots per block and used as a source of rhizosphere soil. Rhizosphere soil in this study is defined as that soil adhering to the root surface after plants had been carefully removed from the soil and gently shaken. Soil temperature was measured at depths of 7.5 and 15 cm.

Assay procedures. Roots of Fraser fir seedlings from the field were assayed for *P. cinnamomi* by thoroughly washing seedlings in tap water for 30–40 min. After washing, the entire root systems were submerged in 95% ethyl alcohol for 30 sec, rinsed in sterile distilled water, blotted dry, and cut into approximately 2-cm

segments. These surface disinfested root segments were placed onto an agar medium (PCH)(10) selective for *P. cinnamomi*. Plates were incubated in the dark at 20 C for 48–72 hr, then examined for *P. cinnamomi*. In addition, root sections were cleared, stained (3), and examined directly under a microscope for chlamydospores of *P. cinnamomi*.

Nonrhizosphere soil samples from all the plots were processed by sieving through a 2-mm screen to remove rocks and roots and to mix the sample thoroughly. A 5–25 g (fresh weight) subsample was weighed and placed in a beaker containing 120–150 ml of deionized water with 5 μg of Pimaricin (Delvocid Instant 50%, Enzyme Development Corp., NY 10001) and 10 μg of penicillin per milliliter of water. The weight of each subsample used was dependent upon the number of propagules expected to be recovered. As subsample weight increased, a corresponding increase in suspension water was necessary. The suspension was stirred for 30–60 sec to break apart any clumps of soil and mix the suspension. The entire suspension was dispensed as uniformly as possible onto a series of PCH plates. The number of plates required was dependent on the weight of the subsample (1 to 1.25 g of fresh weight of soil per PCH plate). Plates were incubated in the dark at 20 C for 48–56 hr, then gently rinsed under running tap water to remove the soil layer. Colonies of *P. cinnamomi* growing into the agar from various propagules on the agar surface were counted macroscopically and expressed as propagules per gram of oven-dried soil (inoculum density). Colony origin was determined at ×100 microscopically for up to 100 colonies from each sampling period.

In the second year of this study, a bioassay was conducted when no propagules of *P. cinnamomi* were detected by the soil suspension assay. Three 2-yr-old Fraser fir seedlings were transplanted into clay pots (6 cm in diameter × 10 cm deep) containing the soil sample. The soil containing the seedlings was then flooded for 24 hr, allowed to drain for 2 days, and watered daily thereafter. After 4 wk, root systems of seedlings were assayed on PCH for *P. cinnamomi* as described previously.

Rhizosphere soil of crimson clover and rye plants was processed in the following manner. Root systems with adhering rhizosphere soil were excised from the plants and placed into 300–600 ml of deionized water containing 5 μg of Pimaricin and 10 μg of penicillin per milliliter. After being washed for 30 min on a magnetic stirrer, the root systems were removed.

Three 5-ml aliquots were drawn from the wash water and oven dried at 103 C to determine the weight of the rhizosphere soil removed during washing. The entire remaining wash water containing the rhizosphere soil was dispensed onto PCH plates. The root systems of the crimson clover and rye plants removed from the wash water were surface disinfested by dipping in 95% ethyl alcohol for 30 sec, rinsed with sterile distilled water, blotted dry, and plated onto PCH to assay for *P. cinnamomi*.

Laboratory cold inactivation. The survival of *P. cinnamomi* in root segments of Fraser fir seedlings were examined in a factorial experiment. Three soil textures (sandy loam, loam, and loamy sand, based on particle size analysis [1]) collected from Fraser fir nursery beds were used throughout the experiment. Soil matric potential of the soils was maintained at two levels (−0.3 and −2.0 bars) for the duration of the experiment (6). There were four replications for each treatment.

Superimposed on the factorial design was a stepwise reduction in incubation temperatures: 20, 12, 3, and −2 C; ±1 C. All treatments were maintained for 2 wk at each temperature. Maximum viability of the pathogen in root segments was estimated by maintaining an additional set of all treatments at 20 C for the duration of the experiment (8 wk). The survival of *P. cinnamomi* in the root segments after each 2-wk period was treated separately in an analysis of variance.

The three soils were infested with root segments in the following manner. Two-yr-old fir seedlings were inoculated by burying five disks (5 mm diameter) removed from cornmeal agar cultures of *P. cinnamomi* to a depth of 4 cm around the base of each seedling. The culture of *P. cinnamomi* used was isolated from a dead Fraser fir seedling from the Linville River Nursery. After 5 wk, roots of inoculated seedlings exhibiting Phytophthora root rot symptoms were cut into segments 1.5 cm long. An initial sample consisting of 200 randomly selected root segments was taken prior to infesting the soil types to determine the percentage of root pieces infected. A total of 100–150 root segments was uniformly mixed in 200-g samples of each soil type at the two moisture levels and placed into sterile bottles (13.5 × 5 × 5 cm) plugged with sterile cotton. Each bottle was weighed initially, and weighed every 3 days so sterile distilled water could be added as needed to maintain the designated

TABLE 1. Origin of colonies of *Phytophthora cinnamomi* on a selective agar medium and percent infection of symptomatic and asymptomatic Fraser fir seedlings during two consecutive winters at the Linville River nursery

Sampling date	Colony origin[a] (%)			Root infection (%)	
	Chlamydospores	Organic matter	Unknown	Symptomatic seedlings	Asymptomatic seedlings
1980–1981[b]					
8 October 1980	47	51	2	100	75
5 November 1980	49	48	3	100	75
25 November 1980	77	21	2	100	75
30 December 1980	80	17	3	100	50
25 January 1981	83	16	1	50	25
24 February 1981	81	18	1	75	25
9 March 1981	79	18	3	75	25
14 April 1981	82	17	1	75	50
13 May 1981	67	31	2	100	50
16 June 1981	14	83	3	100	50
1981–1982[c]					
1 September 1981	65	21	14	100	75
8 October 1981	68	20	13	100	75
3 November 1981	72	14	14	100	75
8 December 1981	80	14	10	92	67
12 January 1982	78	15	7	67	42
18 February 1982	79	16	5	67	25
18 March 1982	81	13	6	83	42
22 April 1982	76	18	6	100	42
27 May 1982	79	14	7	83	58
18 June 1982	64	32	4	100	67

[a] Determined by examining a maximum of 100 colonies on the selective agar medium.
[b] Root isolations from 5-yr-old seedlings.
[c] Root isolations from 3-yr-old seedlings.

moisture level.

Ten root segments (40 segments per treatment) were collected from each bottle at the end of each 2-wk period and assayed for survival of *P. cinnamomi*. Root segments were rinsed in sterile distilled water, blotted dry, and placed onto PCH plates. The entire experiment was repeated once.

RESULTS

Survival of *P. cinnamomi* in Fraser fir beds. Propagules of *P. cinnamomi* survived in soil, organic matter, and roots of Fraser fir seedlings during two consecutive winters during which there were extended periods of soil temperatures at or below 0 C (Table 1, Figs. 1, 2A and D). The sharp decrease in inoculum density during both winters was highly correlated ($P = 0.01$, product-moment method for the coefficient of linear correlation) with a decrease in soil temperature (Figs. 1, 2A and D). During the 1980–1981 winter there were 50 consecutive days (3 January–21 February) at 7.5 cm and 33 consecutive days (3 January–4 February) at 15 cm during which the maximum soil temperature was at or below 0 C. In 1981–1982 there were 37 consecutive days (28 December–2 February) at 7.5 cm and 48 consecutive days (23 December–8 February) at 15 cm when soil temperatures were at or below 0 C (Figs. 1 and 2D). Inoculum density did not increase in the spring until the last sampling date in June in both years at both locations. The inoculum density was 0.05 p/g and 0.08 p/g in May and increased to 0.77 p/g and 0.16 p/g in June for 1981 and 1982, respectively.

Percent moisture and the corresponding matric potential were determined for each sampling date for the two locations. Soil matric potential was greater than −0.35 bars except at one sampling date (Fig. 3) and was not correlated with inoculum density. Despite differences between seedling age (3 and 5 yr old), soil texture (loam and loamy sand), and initial inoculum density (4.26 p/g in 1980 and 22.84 p/g in 1981), the pattern of decreasing inoculum density was similar for both locations in both winters. Generally, when the direct plating of soil suspensions did not detect propagules, neither did the bioassay except on 2 of 302 attempts.

Chlamydospores, free in the soil (Table 1), were the predominant propagule which served as origins of colonies on PCH plates in soil samples plated between November and May during both winters.
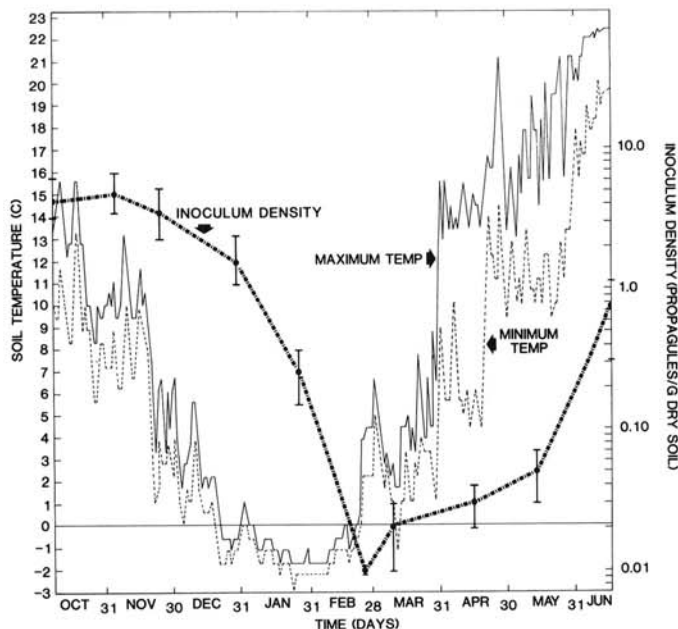

Fig. 1. Survival of propagules of *Phytophthora cinnamomi* in the nonrhizosphere of a loam soil from plots containing 5-yr-old Fraser fir seedlings during the winter of 1980–1981 at the Linville River Nursery, Crossnore, NC. Inoculum density represents an average of four plots with five samples per plot. Maximum and minimum soil temperatures at the 7.5-cm depth. Bars represent standard deviation of the mean.
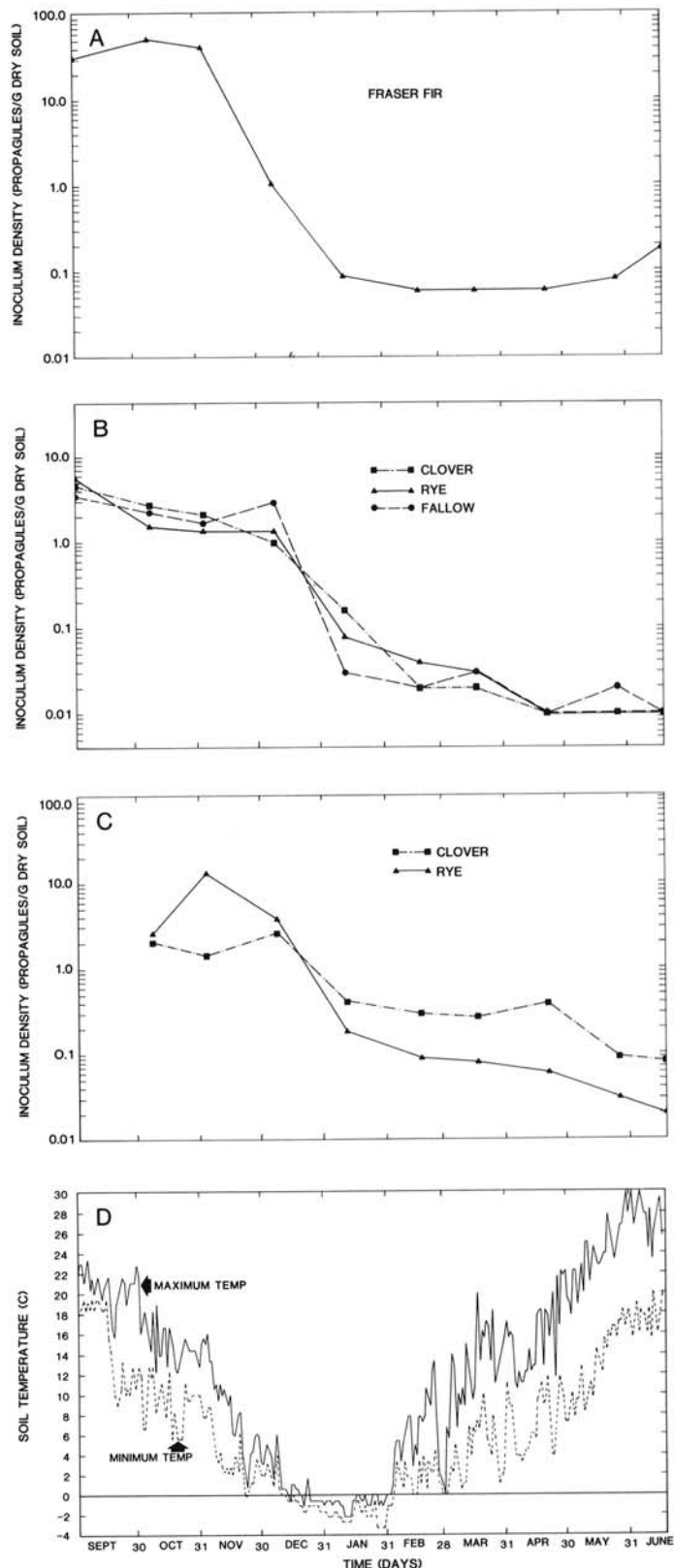

Fig. 2. Survival of propagules of *Phytophthora cinnamomi* in two naturally infested soils with different cropping systems at the Linville River Nursery, Crossnore, NC, during the winter of 1981–1982. **A,** Counts of propagules in the nonrhizosphere of a sandy loam soil from plots planted to 3-yr-old Fraser fir seedlings had coefficients of variation ranging 31–50%. **B,** Counts of propagules in the nonrhizosphere of a loam soil from plots either planted to rye or crimson clover or fallowed had coefficients of variation 42–50, 36–50, and 32–50%, respectively. **C,** Counts of propagules in loam soil from the rhizosphere of the roots of crimson clover or rye plants had coefficients of variation 32–52 and 28–48%, respectively. **D,** Maximum and minimum soil temperatures at the 7.5-cm depth. Inoculum density represents an average of four plots with five samples per plot.

There was an increase in the percentage of colonies originating from pieces of organic matter in June following both winters. This increase was attributed to an increase in root decomposition accompanying the rise in soil temperatures.

P. cinnamomi was isolated from the roots of symptomatic and asymptomatic seedlings at each sampling date (Table 1). Chlamydospores were found within the root tissue that was cleared and stained. *P. cinnamomi* was isolated more frequently from symptomatic than asymptomatic seedlings during both winters (Table 1).

Survival of *P. cinnamomi* in soils under cover crops or fallow. Inoculum densities in the nonrhizosphere soil from all the plots steadily declined between the months of September and January except for an increase in the fallow plots at the December sampling date (Fig. 2B). Once soil temperatures dropped below 0 C for an extended period of time (Fig. 2D), inoculum densities were less than 0.10 p/g for the remainder of the sampling period. No increase in inoculum density was detected in soil from these plots at the last sampling date (18 June). The inoculum density on 18 June was 0.01 p/g from nonrhizosphere soil in plots with crimson clover, rye, or left fallow. Soil matric potential values were very similar to those recorded for the loam soil in 1980–1981 and were not correlated with changes in inoculum density.

Decreases occurred in the inoculum densities of *P. cinnamomi* in the rhizosphere soil of crimson clover and rye and also were highly correlated ($P = 0.01$) with soil temperature (Fig. 2C). The inoculum density was greater ($P = 0.05$, analyzed by *t*-test) in the soil from crimson clover than the rye rhizosphere for the January, February, and June sampling dates. For the November, January, and April sampling dates, the inoculum density was greater ($P = 0.05$) in the rhizosphere than the nonrhizosphere soil from the rye plots. Greater inoculum densities ($P = 0.05$) were detected in the rhizosphere soil than the nonrhizosphere soil from crimson clover plots for January, February, April, and June. *P. cinnamomi* was not recovered from roots of rye or crimson clover plants on PCH nor were chlamydospores observed within or on the surfaces of washed roots examined microscopically.

Laboratory cold inactivation. Initially, an average of 54% of the root pieces were infected with *P. cinnamomi*. Survival of *P. cinnamomi* in root pieces in the loam soil was significantly lower ($P = 0.01$) than in the loamy sand and sandy loam soil at 20 C. Survival of the pathogen was significantly greater in the loamy sand soil than in the loam or sandy loam soil at 12 C. As temperature decreased below 12 C, the effect of soil texture was not significant. There was no significant effects of moisture or texture-moisture interaction on survival of *P. cinnamomi* at any temperature. *P. cinnamomi* was

not isolated from the root sections when the temperature was lowered to −2 C. The pathogen was isolated from every soil texture-matric potential combination maintained at 20 C for the 8-wk period of the experiment.

DISCUSSION

Low soil temperatures had a dramatic effect on the survival of *P. cinnamomi*. Inoculum densities were 0.01–0.0002 less during winter and spring months than in the later summer. The propagules of *P. cinnamomi* that survived soil temperatures at or below 0 C did so as chlamydospores free in the soil, in pieces of organic matter, or in the roots of symptomatic and asymptomatic Fraser fir seedlings. Decreasing inoculum densities were highly correlated ($P = 0.01$) with decreasing soil temperatures. Australian researchers also reported that decreases in population density of *P. cinnamomi* were highly correlated with soil temperatures < 10 C during months when soil moisture was relatively high and constant (5,12).

Benson (2) demonstrated that soil temperatures at or below 0 C inactivated propagules of *P. cinnamomi* in naturally infested soil. The ability to recover propagules of *P. cinnamomi* in nursery beds at the Linville River Nursery locations may have been due to the size of the sample assayed. By increasing the sample size, the sensitivity of the assay increases. The sample size used by Benson (2) could only have detected inoculum densities greater than 0.6 propagules per gram of dry soil compared to 0.01 in this study.

The propagules surviving the winter soil conditions, even though very low in number, are sufficient to initiate disease the following summer. Shew (9) found that 0.01 chlamydospores per gram of soil resulted in a 3% infection incidence of Fraser fir seedlings at soil temperatures between 16 and 25 C in greenhouse studies. The minimum level of inoculum required in soil for infection was determined to be less than 0.01 chlamydospores per gram of soil (9). Inoculum densities of 0.02 have been recorded in Fraser fir nursery beds in May and resulted in infection and mortality of Fraser fir seedlings (C. M. Kenerley, *unpublished*).

Chlamydospores were the most persistent form of *P. cinnamomi* isolated during the winter and spring months. The importance of chlamydospores in survival of *P. cinnamomi* has been reported (4,13,14). The drop in the proportion of colonies arising from organic matter during winter months may indicate that the pathogen in root debris is in a form that does not survive low temperatures. Laboratory experiments confirmed that soil temperatures below 0 C inactivated the fungus in root pieces. Benson (2) found similar results when he buried infected root segments (1 cm) or oat grains in a sandy loam or clay soil. Shea (8) also reported that mycelium of *P. cinnamomi* in organic matter was short-lived and that the fungus survived on freely-drained sites in large roots and stumps of susceptible species. Although no measurements of root diameter were made, the roots of symptomatic and asymptomatic Fraser fir seedlings sectioned for isolation were generally larger than those used by Benson (2) or in the laboratory study reported here. Chlamydospores were found in the roots of seedlings sampled from the field, but root segments used for the laboratory experiment were not examined for chlamydospores. Whether root size, association with an intact host, or the ability to form chlamydospores in the root tissue is the critical factor for survival in root tissue or debris remains to be determined.

The survival of the pathogen in the roots of asymptomatic seedlings presents several epidemiological considerations. Transplanting of infected, asymptomatic seedlings could serve as a source of inoculum for infesting beds within the same nursery, other nurseries, or plantations. This may account for the spread of the pathogen to previously uninfested nurseries and the random pattern of mortality seen in transplant nursery beds. Survival of the pathogen in asymptomatic seedlings indicates that foliar symptom expression is not a reliable indicator of disease progression or the incidence of infection within a nursery bed. Other methods, such as changes in photosynthetic rates, need to be developed to determine infection of seedlings without destructively sampling seedlings. The *A. fraseri-P. cinnamomi* pathosystem could be useful in studying
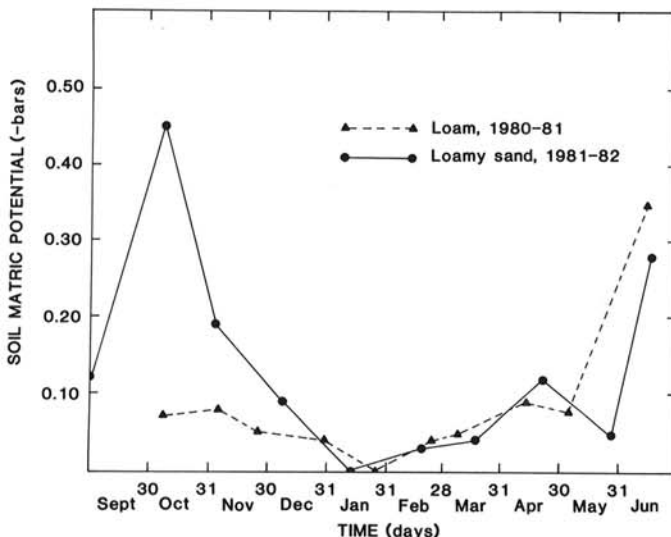

Fig. 3. Soil matric potential of a loam and loamy sand soil in Fraser fir nursery beds at the Linville River Nursery, Crossnore, NC, during 1980–1981 and 1981–1982, respectively. Values illustrated for January in both years may not reflect accurate matric potential values because the soils were frozen during those months.

the relationship among number of root infections, location of infections within the root system, and the role of the soil environment in symptom expression in hosts of soilborne pathogens.

Factors other than decreasing soil temperature were not correlated with decreasing inoculum densities. Laboratory and field studies indicated that soil matric potential values >-2 bars were not correlated with propagule survival. Weste and Rupin (12) reported that soil moisture and pathogen population density were correlated only when the soil matric potential was drier than -5 bars. Differences in soil texture (loam or loamy sand), seedling age (3- or 5-yr-old), or initial inoculum density (4.26 or 22.84 p/g) did not alter the pattern of decreasing inoculum density of *P. cinnamomi* with decreasing soil temperatures recorded in the field studies.

Further investigations into the role nonhosts may play in survival of this pathogen are warranted. Generally, inoculum densities were greater in the rhizosphere than in the nonrhizosphere soil from nonhost plots. Also, results from the study presented here suggest that nonhosts may differentially affect the survival of *P. cinnamomi*. An examination of root exudates and root morphology may explain these differences and provide a basis for selecting cover crops that will reduce the survival of *P. cinnamomi*.

LITERATURE CITED

1. Anonymous. 1972. Soil survey laboratory methods and procedures for collecting soil samples. Soil Survey Investigations Report No. 1. USDA, Soil Conservation Service. Stock No. 0107-0298, Superintendent of Documents, Washington, DC. 63 pp.
2. Benson, D. M. 1982. Cold inactivation of *Phytophthora cinnamomi*. Phytopathology 72:560-563.
3. Bevege, D. L. 1968. A rapid technique for clearing tannins and staining intact roots for the detection of mycorrhizas caused by *Endogone* spp., and some records of infection in Australasian plants. Trans. Br. Mycol. Soc. 51:808-810.
4. Hwang, S. C., and Ko, W. H. 1978. Biology of chlamydospores, sporangia, and zoospores of *Phytophthora cinnamomi* in soil. Phytopathology 68:726-731.
5. Marks, G. C., Kassaby, F. Y., and Fagg, P. C. 1975. Variation in population levels of *Phytophthora cinnamomi* in Eucalyptus forest soils of Eastern Victoria. Aust. J. Bot. 23:435-449.
6. Reeves, R. 1975. Behavior of *Phytophthora cinnamomi* Rands in different soils and water regimes. Soil. Biol. Biochem. 7:19-24.
7. Richards, L. A. 1947. A pressure membrane apparatus, construction and use. Agric. Engin. 28:451-454.
8. Shea, S. R., Gillen, K. J., and Leppard, W. I. 1980. Seasonal variation in population levels of *Phytophthora cinnamomi* Rands in soil in diseased freely-drained *Eucalyptus marginata* Sm sites in the northern jarrah forest of South-Western Australia. Protection Ecol. 2:135-156.
9. Shew, H. D., and Benson, D. M. 1983. Influence of soil temperatures and inoculum density on *Phytophthora cinnamomi* on root rot of Fraser fir. Plant Dis. 67:522-524.
10. Shew, H. D., and Benson, D. M. 1982. Qualitative and quanitative soil assays for *Phytophthora cinnamomi*. Phytopathology 72:1029-1032.
11. Steekelenburg, N. A. M., van. 1973. Influence of low temperatures on survival of *Phytophthora cinnamomi* Rands in soil. Meded. Ryksfac. Landbouwwet. Gent. 38:1399-1405.
12. Weste, G., and Ruppin, P. 1977. *Phytophthora cinnamomi*: Population densities in forest soils. Aust. J. Bot. 25:461-475.
13. Zentmyer, G. A. 1980. *Phytophthora cinnamomi* and the diseases it causes. Phytopathol. Monogr. 10, Am. Phytopathol. Soc., St. Paul, MN. 96 pp.
14. Zentmyer, G. A., and Mircetich, S. A. 1966. Saprophytism and persistence in soil by *Phytophthora cinnamomi*. Phytopathology 56:710-712.