Epidemiology of Phytophthora Root Rot of Fraser Fir: Root Colonization and Inoculum Production

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ABSTRACT

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Phytophthora cinnamomi grew equally well on attached primary roots of 3-yr-old Fraser fir seedlings and on excised roots. At optimal temperatures for fungal colonization (27 C), a Beta function fit to the data ($R^2 = 0.95$) predicted a linear colonization rate exceeding 18 mm/day. The effects of time and soil moisture content on chlamydospore production by *P. cinnamomi* on zoospore-inoculated 3-yr-old Fraser fir primary roots incubated in nonsterile sandy loam were examined. No chlamydospores formed in roots maintained at 5% soil moisture, and very few formed within roots maintained at 10 or 15%. Maximum numbers of spores were recovered from soil 17 days after burial of roots in the 10% soil moisture treatment. Production of chlamydospores occurred primarily at the root surface or in its immediate soil environment since large numbers of chlamydospores were obtained by soil sieving. A linear regression model for chlamydospore production as a function of incubation time in soil and soil moisture content was obtained ($R^2 = 0.91$): $C = 0.004(24.11 + 7.47D - 0.16D^2)M$ in which C = number of spores per millimeter of root, M = percent soil moisture content, and D = days following burial of roots in soil. Sporangium production on zoospore-inoculated 2-yr-old Fraser fir root tips was assessed on Büchner-funnel tensiometers. Sporulation was sparse and confined to the first 5-mm of root tip whether roots were placed directly on tensiometers following inoculation or preincubated in soil. Sporangia only occasionally formed on short roots and this appeared to be due to prior occupation of most short roots by other fungi. Sporangium production on root tips was extremely variable. A model for sporangium production as a function of soil matric potential, time, and preincubation treatment was developed by using stepwise regression ($R^2 = 0.66$): $S = B_\phi + B_1 T + B_2 T^2 + 0.000018 M^3$, in which S = number of sporangia produced, M = soil matric potential (mb), T = incubation time on tensiometer (hr), B_ϕ = a constant (−13.13 for preincubated roots, −14.13 for nonincubated roots and B_1 and B_2 are third-order functions of soil matric potential, the functional form depending on whether the roots had received a preincubation.

Since *Phytophthora cinnamomi* Rands was first reported as a pathogen of cinnamon (11), a voluminous literature has accumulated on virtually all aspects of pathogen physiology and ecology as well as on many host-pathogen interactions. No attempt has been made, however, to develop a detailed biological model that integrates the basic processes that drive an epidemic.

In general, the purpose of the research reported here was to examine certain aspects of host-pathogen interaction that are required for the development of a biologically-based epidemiological model. In particular, we based the model on environmental factor effects on rate of root colonization, chlamydospore production, and sporangia production in nursery beds of Fraser fir (*Abies fraseri* (Pursh.) Poir.) seedlings affected by *P. cinnamomi*.

MATERIALS AND METHODS

P. cinnamomi isolate PC1 (6), originally obtained from infested soil at the Linville River Nursery in Crossnore, NC, was utilized throughout the study. It was reisolated on a regular basis from infested soil (sandy loam) or root material. Agar cultures used for the production of inoculum were never more than 3 wk old.

Zoospore inoculum was prepared by transferring five cornmeal agar plugs from 7- to 14-day-old cultures into petri dishes containing 12 ml of 5% lima bean extract (LBE). After 3 days of incubation in darkness, mycelial mats were rinsed three times in deionized water. A soil extract solution prepared from nursery-bed soil (20 g of soil per liter) was added to the mats. Cultures were incubated for an additional 5 days in darkness and a large population of sporangia had formed. Soil extract was removed and mycelial mats were rinsed three times in deionized water, then incubated in deionized water. Cultures were chilled at 10 C for 45 min followed by 45 min at ambient laboratory temperatures (20–24 C) at which time maximum zoospore release was usually observed. Zoospores were collected by filtering the contents of the dishes through two layers of cheesecloth. Inoculum density was calibrated with a hemacytometer and inoculum was used immediately.

Root colonization. Rate of root colonization was assessed following root-tip inoculation. Roots of 3-yr-old Fraser fir seedlings were inoculated by immersing the first 10 mm of root tip in a zoospore suspension (66×10^3 zoospores per milliliter) for 10 min. Seedlings were wrapped in moist paper towel to prevent dessication of roots not actually immersed in the zoospore suspension. Roots were either left attached to seedlings or were excised from the tap root following inoculation. Single excised roots were placed in soil in individual 10-cm-diameter clay saucers while roots left attached to seedlings were planted in soil in 15-cm-diameter clay pots with the roots carefully positioned to prevent contact between them. Soil used in this experiment consisted of a 1:1 mixture (v/v) of a nonsterile, sandy loam nursery soil and washed sand. Potted seedlings were watered to soil saturation and soil was allowed to drain for 15 min to provide moisture conditions approximating field capacity. Soil in which excised roots were placed was saturated and allowed to drain before being placed in saucers. Pots and saucers were then enclosed in plastic bags to prevent further moisture loss. Pots and saucers were maintained at

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ambient laboratory temperatures (20–22 C) for 24 hr. Twenty attached and 20 excised roots were then removed, aseptically sectioned into 1-cm segments and placed on pimaricin-chloramphenicol-hymexazol agar medium (PCH) (15). Remaining pots and saucers were divided into three groups and placed in growth chambers maintained at either 20, 24, or 28 C. After 3 days of incubation in the growth chambers, all roots were removed, sectioned, and plated as above. Root segments from a given root were arranged on an agar plate according to distance from the root tip. Plates were examined after 2 days to determine the extent of colonization by *P. cinnamomi*. Extent of colonization was assessed by counting the number of consecutive segments from the tip from which the fungus could be isolated. Assessments made on roots plated 24 hr after inoculation were used to adjust the data from roots incubated in the growth chambers so that net colonization beyond the initial infection at time of inoculation could be determined. Mean net colonization by *P. cinnamomi* on 20 roots was used as a single replicate.

Radial colony growth of the fungus also was assessed on 5% lima bean agar (LBA) after 24 and 96 hr to provide a standard for comparison with results on in vitro fungal growth. Mean net radial colony extension for 20 plates incubated at either 16, 20, 24, 28, or 32 C was treated as a single replicate. The entire experiment was repeated three times.

Chlamydospore production on roots. Three-year-old Fraser fir seedlings were obtained from the Linville River Nursery at Crossnore, NC. Fifty seedlings were transplanted into a 2-L plastic tub which contained a soil mix consisting of one part natural nursery-bed soil (sandy loam, 67% sand) and one part washed sand. Zoospore inoculum was prepared as described above. Seedlings were inoculated by flooding the soil in the tub with a 500-ml suspension of zoospore inoculum (about 100 zoospores per milliliter). Additional deionized water was added to bring the water level 1 cm above the soil surface. The soil was drained 24 hr after infestation and the trees were incubated in the infested soil for an additional 4 days.

Seedlings were removed at the end of the incubation period and the roots were washed free of soil under running tap water. Roots were sectioned into five 5-cm segments. Five segments were placed in each of 18 petri dishes containing the 1:1 mix of nursery-bed soil and sand as used in the tub. Six plates each were adjusted to either 5, 10, or 15% soil moisture (corresponding soil matric potentials were −8.7, 0.25, and −0.10 bars, respectively). Petri plates containing soil (hereafter called soil plates) were enclosed in plastic bags to minimize water loss and stored under ambient laboratory temperatures. At 5 days, and at 7-day intervals thereafter, one plate was removed from each treatment. Soil and roots were gently washed for 5 min over a set of nested sieves (38 and 125 μm diameter opening) under running tap water. Material retained on the 38-μm sieve was backwashed into 0.5 L of deionized water containing 5 ppm of pimaricin and the entire suspension was plated onto PCH agar (40 plates per treatment). Colony counts were made after 24–36 hr of incubation. Counts were subsequently transformed into the expected number of chlamydospores based on a random sample of 500 colonies.

At each sampling date, all soil plates were weighed and deionized water was added to maintain the soil moisture content of the plates at their original levels. Average water loss per plate between sampling dates was approximately 1%. The study was repeated three times.

A histological study of root pieces used in the above study was conducted to ascertain the extent of chlamydospore production within infected root material. On two separate dates, subsamples of eight 5-mm root segments were taken from each soil plate of all replicates in the above study which gave a total of 960 mm of root. Root pieces within this sample had been infected from 10 to 31 days.

Infected root segments were fixed in formalin propionic-propanol (FPP) for 1 wk. Segments were dehydrated for an additional week in an isopropyl alcohol series and subsequently infiltrated and embedded in Paraplast (Sherwood Medical Industries, St. Louis, MO) (5). Sections 12 μm thick were cut with a rotary microtome, mounted on slides with Haupt's adhesive, and stained with Triarch's Quadruple Stain (Triarch, Inc., Ripon, WI).

Sporangium production on roots. Root tips from 2-yr-old seedlings, 10–20 mm in length, were excised from the seedlings. Zoospore inoculum used in this experiment was prepared as described above. Root tips became 100% infected after floating in the zoospore suspension for 1 hr. Infected roots were either incubated in nursery-bed soil at 10% soil moisture for 3 days prior to installation of the root pieces on Büchner funnel tensiometers (2) or placed directly onto the tensiometers with no incubation in soil.

Ten root pieces were installed on each 9.5-cm-diameter tensiometer. Prior to installation of the root pieces, a 1-cm layer of sandy loam soil was added to each tensiometer and the soil was lightly tamped. The soil in the tensiometers was saturated from below and 5 ml of soil extract was added to the tensiometers prior to root placement. Root pieces were placed on the saturated soil surface between two layers of 100-μm-mesh nylon screen. A second 1-cm layer of soil was added and lightly tamped and the water level was raised to the soil surface to drive out excess air. To further consolidate the soil mass, each tensiometer was adjusted to −100 mb for 4 hr then the tensiometers were watered to saturation from above with deionized water and adjusted to matric potentials of −10, −25, −50, and −100 mb. Sporangium production data for the 10 root pieces on an individual tensiometer were averaged and treated as a single replicate. Sporangium production by *P. cinnamomi* was assessed at 16, 28, 40, 52, and 64 hr following installation of root tips on the tensiometers. Each treatment combination was replicated two times.

RESULTS

Root colonization. The distribution of growth response was asymmetrical on either LBA or roots (Fig. 1). To develop a functional expression to characterize the temperature/growth-response data for use in disease simulation (13), a Beta function was chosen as the simplest model which would accurately reflect the asymmetric distribution of the data. A linearized form of the Beta function was fit by linear regression. This procedure required the insertion of estimates for T_{min} and T_{max} (minimum and maximum temperatures at which growth ceases, respectively) in equation 1:

$$y = \alpha(T - T_{min})^{\beta} (T_{max} - T)^{\gamma} \quad (1)$$

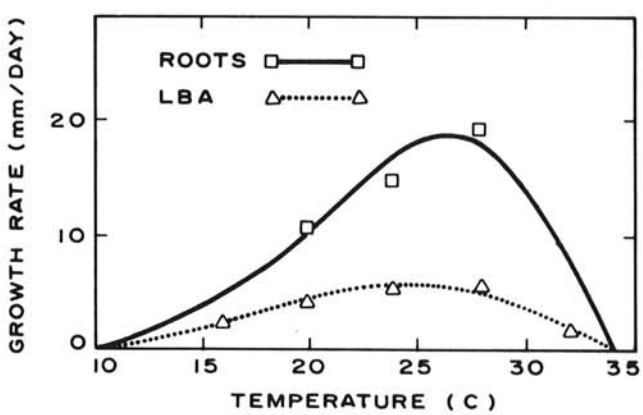

Fig. 1. Temperature effects on daily colonization rate (millimeters per day) of *Phytophthora cinnamomi* on roots of 3-yr-old Fraser fir seedlings and on 5% lima bean agar (LBA). A replicate consisted of the mean net linear growth of the fungus on 20 roots or mean net colony extension on 20 LBA plates. Growth rate of the fungus was measured on both attached and excised roots. The three treatments (attached roots, excised roots, and LBA) were each replicated three times. Results of regression analysis showed that temperature-growth response behavior was not significantly different for the two root types: therefore, root colonization data are pooled in the figure. Data points shown represent the mean of six replicates for fungal growth rate on roots and the mean of three replicates for colony extension on LBA.

in which: y = daily colonization rate (mm/day); T = temperature (C); and α, β, and γ are parameters to be estimated. Two critical assumptions were made in fitting equation 1 to the experimental data: a single, general model was equally appropriate for describing the temperature-growth response of *P. cinnamomi* on LBA and on Fraser fir roots; and the T_{min} and T_{max} at which fungal growth on roots could occur are the same as for fungal growth in vitro. The resulting general model accounted for 95% of the variation ($P = 0.0001$). Analysis of the model parameters demonstrated that the estimates a, b, and c of the parameters α, β, and γ did not differ significantly between attached and detached root types hence the root growth data for colonization of the two root types were combined (Fig. 1). However, both the a-parameter (which determines scale) and the b-parameter (which, together with the c-parameter determines the location of the mode) differed significantly for fungal radial growth on LBA and linear root colonization. The resulting models for growth on agar and on roots are given by equations 2 and 3, respectively:

$$y_{agar} = 0.0026(T - 10)^{1.85}\,(34 - T)^{1.21} \quad (2)$$

$$y_{root} = 0.0008(T - 10)^{2.70}\,(34 - T)^{1.21} \quad (3)$$

Chlamydospore production on roots. Careful examination of the longitudinal root sections prepared for histological study revealed that very few chlamydospores formed within infected Fraser fir root tissue within the first 4–5 wk following inoculation.

Colonization of root tissue was intensive 10 days after inoculation. The extent of colonization did not appear to be affected by soil moisture; however, soil moisture did affect root reaction to colonization. The phellem and endodermis of roots maintained at 5% soil moisture was necrotic and collapsed while the same kinds of tissues of roots maintained at 10 or 15% soil moisture were equally necrotic but had not collapsed (Fig. 2A). Hyphae were seldom observed in either the phellem or xylem. The absence of hyphae in the phellem may have been due to the advanced state of necrosis in this tissue. Numerous hyphae were observed in the more intact tissues of the phloem, endodermis, and cambial region of roots at all moisture levels (Fig. 2A and B). Tylose formation was commonly observed in tracheids adjacent to colonized cambium, and hyphae could occasionally be observed penetrating the tracheids, apparently through pits. Root sections from subsequent sampling dates (days 17, 24, and 31) showed little deviation from the pattern observed on day 10. No chlamydospores were observed in root sections at 5% soil moisture. Spores were observed most frequently on day 17 in roots maintained at 10% soil moisture (Fig. 2C). Necrosis and collapse of the phellem, endodermis, and phloem proceeded most rapidly in roots at 5% soil moisture (Fig. 2F). Severe disruption of these tissues was not seen in roots at 10 and 15% soil moisture until day 31 and even at this time the extent of disruption was still not as severe as at 5% moisture (Fig. 2E). Dense hyphal masses formed in the endodermis of roots at all moistures; however, the masses were most frequently observed in roots at 10 and 15% moisture (Fig. 2d).

In contrast to the absence of chlamydospore formation within root tissue, large numbers of chlamydospores were observed on the root surface or in the root-soil interface. On the basis of a random sample of 500 colonies obtained by soil sieving, 84% of the colonies on PCH agar originated from chlamydospores. The expected number of chlamydospores per colony was estimated to be 2.25. These data were used to transform the colony counts obtained by soil sieving into the expected number of chlamydospores per millimeter of root.

The production of chlamydospores on or near the root surface over time and under three soil moisture regimes was fit to a quadratic model by using linear regression (Fig. 3). The resulting model, which accounted for 91% of the variation ($P = 0.0001$), was:

$$C = 0.004(24.11 + 7.47D - 0.16D^2)M \quad (4)$$

in which: C = spores per millimeter of root, D = days of root incubation in soil, and M = percent soil moisture.

The form of equation 4 indicates that soil moisture affects the height, slope, and shape of the spore production curves (Fig. 3). According to this relatively simple model, the chlamydospore population is predicted to decline to zero by day 49. A more realistic representation of survival should probably allow for a much more gradual attrition in this population; however, for short-term simulations for which equation 4 is intended (13), the above model is a sufficiently accurate representation of chlamydospore survival.

Sporangium production on roots. Significant numbers of sporangia were produced only within the first 2-mm segment of inoculated root tips. As a general practice, however, sporangia were counted on the first 5-mm segment of root. The entire length of each root also was scanned to determine if short roots on the primary root also were suitable sites for sporangium production. Sporangium production was only observed on a few of the short roots. Hyphae of fungi other than *P. cinnamomi* were frequently observed growing from short roots. No attempt was made to identify these fungi, but it was noted that various hyphal types were commonly associated with the short roots and that, in the few cases in which sporangia of the pathogen were observed on short roots, no other mycelial forms were present.

Extreme variability in sporangium production was observed both for preincubated roots (Fig. 4a) and for roots placed directly on tensiometers following inoculation (Fig. 4b). A model for sporangium production as a function of time, soil matric potential, and preincubation treatment was obtained by using stepwise regression. The general form of the model, which accounted for 66% of the variation ($P = 0.0001$), was:

$$S = B_0 + B_1 T + B_2 T^2 + 0.000018M^3 \quad (5)$$

in which: S = number of sporangia formed on the first 5-mm of root, T = incubation time on the tensiometer (hr/100), and M = soil matric potential on the tensiometer (−mb).

For roots receiving a 3-day preincubation in soil at 10% soil moisture prior to installation on the funnels, the terms β_0, β_1, and β_2 in equation 5 are given by:

$$B_0 = -13.13,$$
$$B_1 = 12.81M - 0.32M^2 + 0.002M^3,$$
$$\text{and } B_2 = 21.30 - 18.22M + 0.48M^2 - 0.003M^3.$$

For roots that received no preincubation treatment the values are:

$$B_0 = -14.13,$$
$$B_1 = 7.54M - 0.18M^2 + 0.001M^3,$$
$$\text{and } B_2 = 21.30 - 9.79M + 0.23M^2 - 0.001M^3.$$

DISCUSSION

The minimum and maximum temperatures (T_{min} and T_{max}, respectively), employed in the Beta function used to describe fungal growth rate (Fig. 1), were obtained from the literature (17) and not experimentally. Nonetheless, examination of the growth response data and the fitted curve for growth on LBA (Fig. 1) provides convincing evidence of the accuracy of these estimates for growth on agar. Furthermore, the resulting set of cardinal temperatures for growth on LBA correspond extremely well with the midpoints of the cardinal ranges reported by Zentmyer (17). It would not generally be anticipated that the temperature limits for growth of a pathogen on its host would coincide with those for growth on an inert agar medium due to changes in host reaction with changes in temperature. Fraser fir roots, however, appear to be at least as favorable a substrate for growth as LBA (Fig. 2) and fungal growth on roots left attached to the seedling was as good or better than growth on detached roots. These results indicate a host-pathogen interaction in which host resistance mechanisms fail to influence pathogen-temperature interactions. Consequently, the assumption

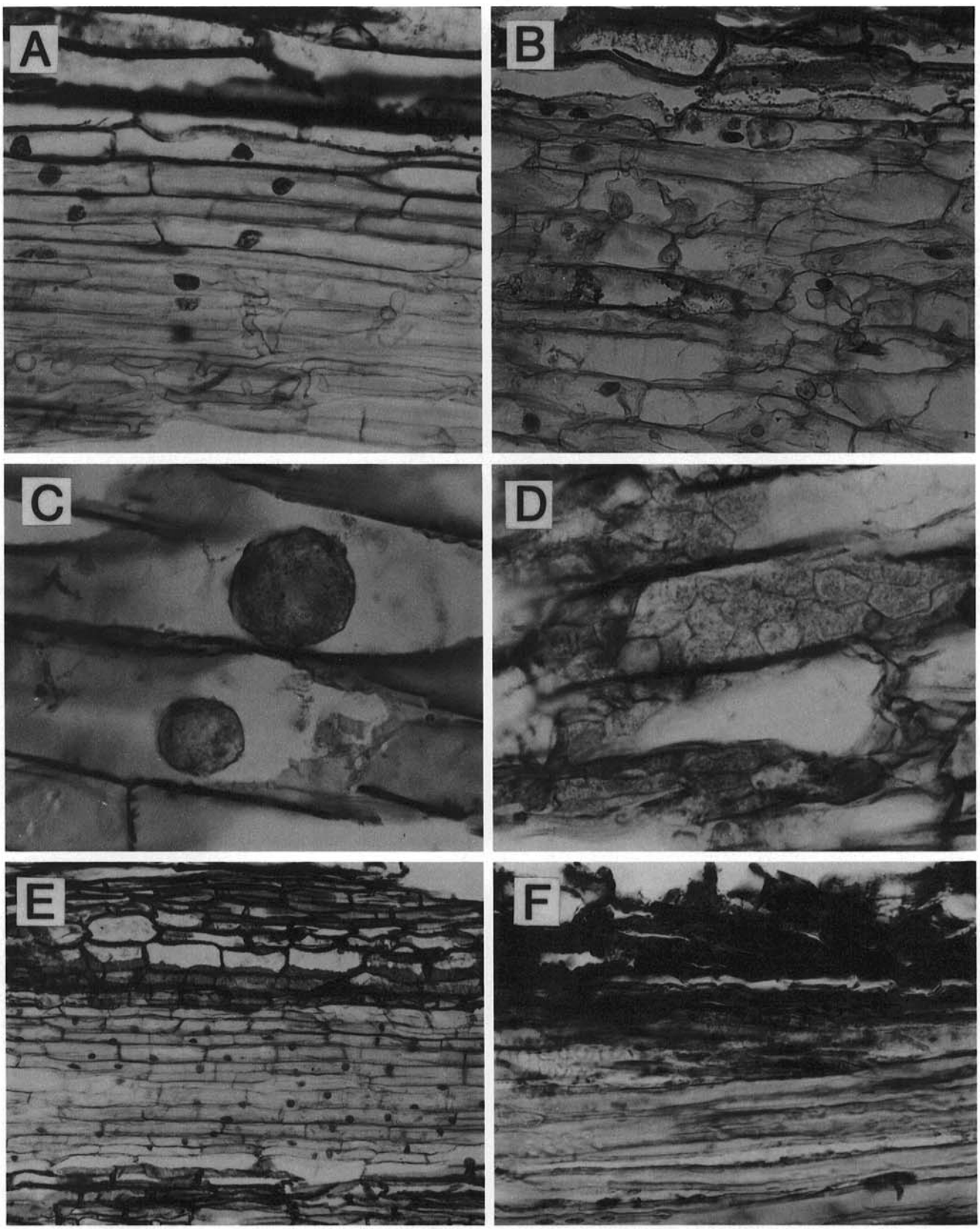

Fig. 2. Colonization of 2-yr-old Fraser fir roots by *Phytophthora cinnamomi*. **A,** Colonization of endodermis and phloem on day 10 in a root incubated in soil at 10% soil moisture (×450). **B,** Colonization of endodermis and phloem on day 17 in a root incubated in soil at 15% soil moisture (×450). **C,** Chlamydospores of *P. cinnamomi* in endodermis of root incubated for 17 days at 10% soil moisture (×720). **E,** Severe necrosis of phellem, endodermis, and phloem in root incubated for 31 days at 15% soil moisture (×180). **F,** Severe necrosis of phellem, endodermis, and phloem accompanied by disorganization and collapse of the tissue in a root incubated for 24 days at 5% soil moisture (×450).

that the limiting temperatures for colonization of Fraser fir roots by *P. cinnamomi* do not differ significantly from those obtained in vitro appears to be a reasonable one.

The most significant aspect of the rate of Fraser fir root colonization by the pathogen is the rapidity with which this process can proceed regardless of host root vigor. Linear rate of colonization can exceed 18 mm/day under optimal temperature conditions. This observation has two important consequences with respect to epidemic development in the nursery environment. Seedling densities in the nursery beds commonly exceed 100 seedlings per 0.1 m^2. The average distance between a seedling and its nearest neighborwill typically be less than 30 mm under these conditions. Simulations of root structural development indicate that physical root contacts among neighboring seedlings will be common in this situation and that focal points of infection may enlarge significantly due to strictly mycelial colonization among the seedling's root systems (13).

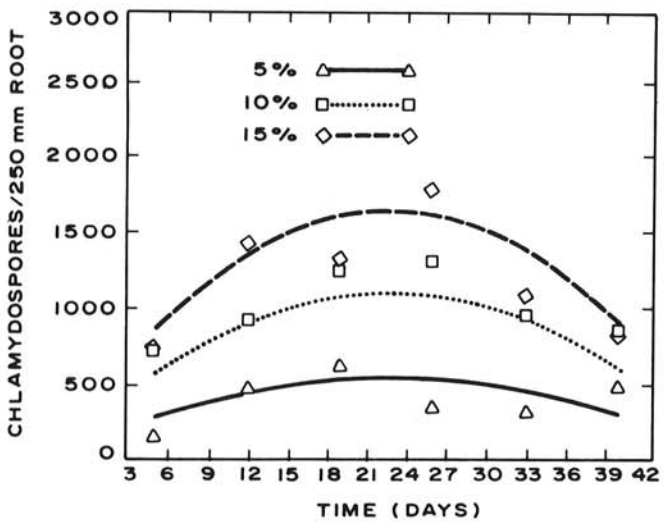

Fig 3. Chlamydospore production by *Phytophthora cinnamomi* on 3-yr-old Fraser fir roots over time at three soil moisture contents. Seedlings were inoculated by infesting soil with zoospore inoculum. Propagule counts were converted to numbers of chlamydospores per millimeter of root based on a random sample of 500 colonies from which it was determined that 84% of the colonies originated from chlamydospores. Each colony of this 84% had a mean of 2.25 spores per colony, and each data point is the mean of three replicates.

The second consequence of rapid colonization is a correspondingly rapid increase in chlamydospore inoculum. A second-year seedling may have as much as 600–700 mm of root by midsummer. Large numbers of chlamydospores of *P. cinnamomi* have been observed within infected root tissue of some hosts (7,12). Large numbers of chlamydospores also have been observed in the soil surrounding infected avocado roots (8). In all of the above cases, internally-formed spores were present within 4 wk of initial infection. In contrast, results of the present study indicate that, for at least the first 4 wk following inoculation of Fraser fir seedlings, very few chlamydospores form within infected root tissue. Within 4 wk following inoculation, however, chlamydospore counts exceeding seven spores per millimeter of root were obtained by sieving soil containing infected root material indicating that chlamydospores are formed by mycelium on the root surface. This conclusion is also supported by the observations on root colonization which demonstrated that very little phellem was sloughed off by the sieving process. Large increases in recovery of chlamydospores from soil containing infected Fraser fir seedlings were reported elsewhere (6). Similarly, Shew and Benson (15) have reported that chlamydospores of *P. cinnamomi* free in nursery soil constituted 87% of the recoverable propagules.

As many as 4.9×10^3 chlamydospores could be produced within a 4-wk period in the immediate soil environment of an infected

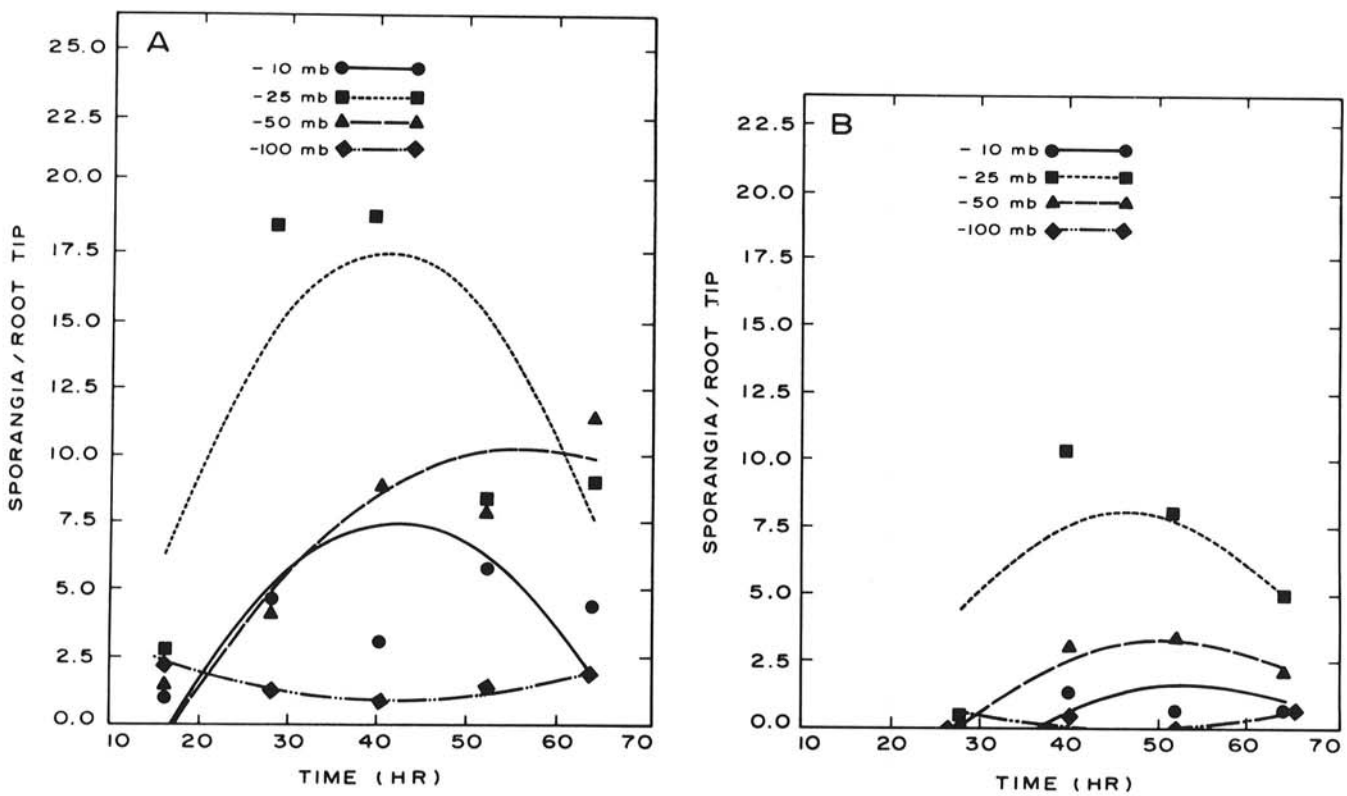

Fig. 4. Sporangium production by *Phytophthora cinnamomi* over time on root tips of 2-yr-old Fraser fir under different matric potential regimes. Root tips were inoculated by floating them in a zoospore suspension. Ten inoculated root tips were placed between two layers of 100-μm-mesh nylon screen and placed in nonsterile, sandy loam soil at a depth of 1 cm on Büchner funnel tensiometers. Mean sporangium production for 10 roots on a tensiometer was used as a single replicate. **A,** Inoculated roots placed on the tensiometer after 3 days of incubation in soil at 10% following inoculation.

Fraser fir seedling at midseason, based on an estimated seven chlamydospores per millimeter of root. If only 50% of this population germinated, produced sporangia, and liberated zoospores under suitable soil moisture conditions, 5×10^4 zoospores might be produced by a single infected seedling. A soil saturation event capable of producing surface runoff could have tremendous potential to initiate an explosive epidemic given a relatively small infection center involving as few as 50 seedlings.

Only the A^2 mating type of *P. cinnamomi* was used in this study. Homothallic oospore formation by *P. cinnamomi* both within avocado roots and in adjacent soil has been observed previously (8). This spore type, however, was never observed in the infected Fraser fir root material that had been prepared for histological examination, nor were oospores observed in the sample of 500 propagules which were recovered by sieving soil that had contained infected Fraser fir roots. It is possible that oospores are formed homothallically in Fraser fir roots but not in sufficient numbers to be detected in this study. It is at least as probable, however, that Fraser fir roots are not capable of stimulating homothallic oospore formation (17).

The production of sporangia on Fraser fir roots by the vegetative thallus of *P. cinnamomi* was sparse in comparison with the production of chlamydospores since the only significant production occurred within 5 mm of actively growing root tips. Only small clusters of sporangia were occasionally observed to arise from short roots. The sparse production of sporangia on short roots, which are histologically similar to the apical meristem of the primary root, may have been due to the occupation of the short roots by other fungi. As a result, production of sporangia appears to be limited to succulent, actively growing root tips which may be relatively free of prior colonization. Such an hypothesis would account for the observation that sporangium production is primarily confined to a primary root's apical meristem region.

The general model for sporangium production (equation 5) is complex, but still it accounts for only a modest amount of the variation seen in the data. It is possible that a larger number of replications would have improved the predictive ability of the model by reducing the somewhat erratic responses in sporangium production over time (Fig. 4a and 4b). However, the very low levels of sporangium production observed on roots suggest that this is a relatively unimportant source of zoospore inoculum in the pathosystem involving Fraser fir and *P. cinnamomi*.

The response of sporangium production to soil matric potential is in general agreement with preliminary studies conducted by Shafer (14). Optimal production in the present study occurred at −25 mb and production was severely restricted at −10 and −100 mb. These observations are similar to those reported by Benson (1) who used mycelial mats at constant matric potential in either a clay soil or pine bark substrate. Duniway (2) has suggested that the upper and lower matric potential limits for sporangial formation may be controlled by lack of oxygen and lack of free water, respectively. Maximum sporangium formation generally was observed to occur within 24–48 hr of installation of the roots on the tensiometers which is in general agreement with the results of numerous in vitro studies conducted with several *Phytophthora* spp. (4,10,16).

Although time trends in sporangium production were obscured by the extreme variability in the data, it nonetheless appears that maximum production at suboptimal potentials is delayed relative to the time at which maximum production is reached under optimal matric potential conditions, an observation also reported by Pfender et al (10) for *P. megasperma*. Pfender et al (10) found the optimum matric potential for this species to be 0 mb and the lower limiting value to be −250 mb. It is possible that the optimum potential of 0 mb observed for *P. megasperma* indicates a greater tolerance for reduced oxygen (9).

Other studies of relationships between sporangium production and matric potential in which mycelial mats of *Phytophthora* spp. were used rather than a natural host substrate have generally yielded significantly lower values for the optimum and lower limiting potentials (2,4,16). Gisi et al (4) suggested that reported differences in sporangium production in response to matric potential in *Phytophthora* spp. may be the result of variations in environment, substrate, experimental techniques, or some combination of these factors. Duniway (3) came to similar conclusions.

The study of root colonization and related histopathological events and inoculum production in the pathosystem involving Fraser fir and *Phytophthora cinnamomi* shows that it differs in significant ways from generally accepted views of pathosystem dynamics for *Phytophthora*. First, the rapid rate of root colonization together with frequent interplant root contact in the nursery bed (13) makes pathogen spread by mycelial growth appear to be a potentially significant component of the epidemiology of this pathosystem. Second, the production of large numbers of chlamydospores in the immediate soil environment of infected roots, along with with rapid colonization, provides a basis for explosive epidemics.

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