Epidemiology of Phytophthora Root Rot of Fraser fir: Estimates of Rhizosphere Width and Inoculum Efficiency

K. M. Reynolds, D. M. Benson, and R. I. Bruck

Graduate research assistant, professor, and assistant professor, respectively, Department of Plant Pathology, North Carolina State University, Raleigh 27695.

Journal Series Paper 9544 of the North Carolina Agricultural Research Service, Raleigh 27695.

Portion of a Ph.D thesis submitted by the first author to the North Carolina State University Graduate School.

Use of trade names implies neither endorsement of the products by the North Carolina Agricultural Research Service nor criticism of similar ones not mentioned.

This research was supported, in part, by a grant from the North Carolina Christmas Tree Growers Association.

Accepted for publication 16 April 1985 (submitted for electronic processing).

ABSTRACT

Reynolds, K. M., Benson, D. M., and Bruck, R. I. 1985. Epidemiology of Phytophthora root rot of Fraser fir: Rhizosphere width and inoculum efficiency. Phytopathology 75:1010-1014.

Fraser fir seeds were germinated for 12 days in a nonsterile, sandy-loam soil infested with different chlamydospore densities of *Phytophthora cinnamomi* in Büchner funnel tensiometers at −100 mb matric potential (ψ_m). After this time, tensiometers were either set to 0 mb for 16 hr and then reset to −100 mb; set directly to −10, −25, −50 mb; or left at −100 mb ψ_m for 5 days. The percentage of infected seedling radicles was determined by plating onto a selective agar medium. Seedling rhizosphere width, at the different inoculum densities and ψ_m, were calculated from infection levels. Rhizosphere width (W) did not depend on inoculum density. The model obtained for rhizosphere width as a function of ψ_m accounted for 82% of the variation ($P = 0.0001$) and is given by: $W = 4.01(1 - M)^{-0.72}$ in which W = rhizosphere width (mm) and $M = \psi_m$ (−mb). The same experimental system was used to study the effect of duration of soil saturation (2, 4, 8, and 16 hr) (P_s) on inoculum efficiency (E) by using Ferriss' model. The model developed, which accounted for 63% of the variation ($P = 0.0004$), was $E = 0.04\ P_s^{0.63}$.

Phytophthora cinnamomi Rands survives principally as chlamydospores in soil (10,14,24,26). Chlamydospores are important in the epidemiology of root rot caused in Fraser fir [(*Abies fraseri* (Pursh) Poir.)] by *P. cinnamomi* because root decay in 3-yr-old fir seedlings progresses rapidly, and survival time of the fungus as mycelium in roots or as sporangia on roots is quite limited (18). Hence, the role of chlamydospores may be more important in this pathosystem than has been previously recognized for other host-pathogen interactions involving *P. cinnamomi.*

Chlamydospores of the fungus are stimulated to germinate in the presence of amino acids as well as diffusates from avocado roots (17). Root exudates may be the primary factors that stimulate and regulate microbial activity on and near the surface of roots (12,20,21).

Baker (1) and Baker et al (2) developed the first models proposed to differentiate rhizoplane (root surface) effects from rhizosphere (volume of soil with altered microbial activity surrounding a root) effects. Gilligan (8) and Leonard (15) presented mathematical models for calculation of rhizosphere volume, and Gilligan (8) presented a method by which rhizosphere width could be estimated. Subsequently, Ferriss (7) showed that Gilligan's model (8) may yield negative values of rhizosphere width and proposed an alternative formula. Drury et al (5) described several criteria that an experimental design must include to ensure correct estimation of rhizosphere width with Ferriss' model.

The first objective of this study was to develop a functional relation between soil matric potential (ψ_m) and the rhizosphere width of Fraser fir seedling roots. Rhizosphere width is defined in this study as the maximum distance from the root that chlamydospores of *P. cinnamomi* can be stimulated to germinate and cause infection. The second objective was to develop a functional relation between the duration of soil saturation and chlamydospore inoculum efficiency. The term efficiency (that portion of inoculum in the rhizosphere that is capable of initiating successful infections on the root) is adapted from the study by Drury et al (5) to refer to the probability of a single propagule initiating a successful infection in this study. The results from this study are to be included in a simulation model for the spread of *P. cinnamomi* in Fraser fir nursery beds.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked *"advertisement"* in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

© 1985 The American Phytopathological Society

MATERIALS AND METHODS

Inoculum production and inoculation. Isolate PC1 (13) of *P. cinnamomi* was obtained from an infested nursery bed of Fraser fir at the Linville River Nursery in Crossnore, NC. Chlamydospore inoculum was prepared by infesting a 2-L plastic tub containing fifty 3-yr-old Fraser fir seedlings with 500 ml of a 6.6×10^4 suspension of zoospores per milliliter. Zoospore inoculum was prepared as described previously (19). The soil was a mixture of uninfested sandy loam from the Linville River Nursery and a washed, coarse sand (1:1, v/v). The soil was flooded for 24 hr (water level 1 cm above the soil surface) with deionized water and then drained. Seedlings were incubated in the infested soil for an additional 17 days at ambient laboratory temperatures (20–22 C). Chlamydospores were recovered on a semiautomatic elutriator described by Byrd et al (4) by using nested sieves with openings of 125- and 38-μm. Material retained on the 38-μm sieve was then mixed with uninfested, sandy loam soil which was adjusted to 10% moisture (w/w) (−0.25 bars) and incubated for 3 days at 15 C. At this time, five 10-g subsamples were assayed for inoculum densities as described previously (19). Inoculum densities were subsequently adjusted to desired levels by adding uninfested, sandy loam soil.

The inoculum densities used in the propagule efficiency experiment, were 2.7, 5.4, or 18.7 chlamydospores per cubic centimeter for soil saturation periods of 2, 4, or 8 hr (Table 1). In the 16-hr soil saturation experiment, 2.7 propagules per cubic centimeter were used. Inoculum was prepared freshly for each repetition of both experiments so that inoculum was of the same age.

Rhizosphere width experiment. Soil moisture was controlled in Büchner funnel tensiometers (6) which were maintained in a growth chamber at 24 ± 1 C. A 25-mm-deep layer of infested soil was added to each tensiometer (150 g dry weight). The infested soil was overlaid with a 5-mm layer of sand which was then smoothed and lightly tamped. Two-hundred Fraser fir seeds (percent

germination, 15%), which had been moist-stratified for 28 days at 5 C, were scattered on the surface of the sand and covered with an additional 5-mm layer of sand. Tensiometers were maintained at -100 mb ψ_m for 12 days to allow seeds to germinate and the radicles to grow into the infested soil. Soil was briefly saturated daily by addiing 25 ml of deionized water to the soil surface. To determine the proportion of propagules surviving at the end of the 12-day period, five 10-g subsamples of infested soil were removed from each of three tensiometers and assayed for *P. cinnamomi* on pimaricin-chloramphenicol-hymexazol agar medium (PCH) (23). Survival was estimated to be 80.7% (coefficient of variation in the 15 subsamples was $<1\%$). Initial propagule densities (Table 1) reflect values adjusted by the factor 0.807.

Tensiometers with germinated seedlings were adjusted after 12 days to 0, -10, -25, -50, or -100 mb ψ_m and maintained for 5 days. Additional tensiometers were adjusted to 0 mb ψ_m for 16 hr and then readjusted to -100 mb. Incidence of infection was determined after 5 days by gently removing seedlings, washing under running tap water, and plating onto PCH agar. Each tensionmeter contained 20 to 50 seedlings.

Propagule efficiency experiment. Flooding periods of 2, 4, 8, or 16 hr were applied to the seedlings in tensiometers on day 12. After the appropriate flooding period, tensiometers were returned to -100 mb ψ_m. After an additional 24 hr, seedlings were removed and plated on PCH agar to determine infection.

A single tensiometer in each experiment was considered a replicate. Treatments were replicated twice in each experiment and each experiment was repeated twice.

Calculation of rhizosphere width. Ferriss' (7) formula was modified as follows:

$$W = [(H/LMIE) - r]^{1/2} - r \quad (1)$$

in which: W = rhizosphere width (mm), H = total number of successful infections, L = mean length of susceptible root (mm), M = number of susceptible roots, I = inoculum density (propagules per mm), E = number of infections per propagule (efficiency), and r = root radius (mm).

The following data were obtained for each tensiometer: number of roots, mean length per root, and the number of infected roots. The latter value was converted to total number of infections by using Gregory's (9) multiple infection transformation (MIT). Measurements made on 100 Fraser fir radicles yielded a mean root radius (r) of 0.5 mm.

Reynolds (18) calculated an expected efficiency (E) of 0.32 for the propagule population of a Fraser fir root rhizosphere under saturated soil conditions (0 bars). A value of $E = 0.30$ was inserted in eq. 1 and equation 4 (below) for purposes of calculating rhizosphere width and maximum inoculum efficiency, respectively.

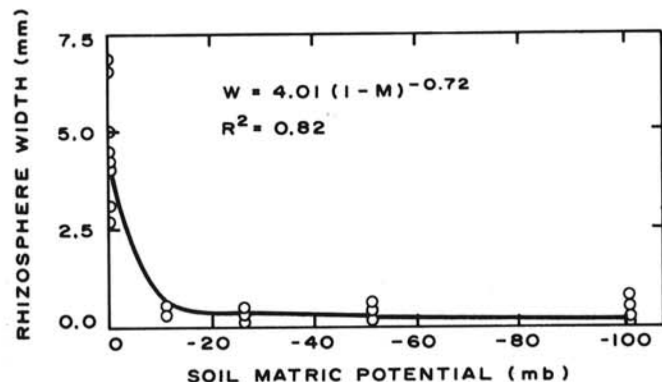

Fig. 1. Effect of soil matric potential on rhizosphere width of 2-wk-old Fraser fir seedling radicles. Data are based on infection of seedlings in soil infested with chlamydospores of *Phytophthora cinnamomi*, determined by plating onto a selective agar medium. Soil moisture was maintained at a constant level in Büchner funnel tensiometers. The data values indicated were calculated by using Ferriss' (7) model for rhizosphere width (equation 1 in the text). The regression line was generated by using equation 2 (in the text).

Ferriss' (7) model for calculating rhizosphere width (eq. 1) requires that only susceptible root length be included in the term L. To determine whether the mature region of 2-wk-old seedling radicles was susceptible to infection, seedlings at least 20 mm in length were inserted through 2-mm-opening wire-mesh screen with the radicle bent into a U-shape. Five seedlings were incubated in this manner for 1 hr in each of 10 petri dishes containing 15–20 ml of zoospore inoculum (100 zoospores per milliliter) so that only the mature region of the radicle was exposed to the inoculum. The seedlings were then transferred to petri dishes containing 15–20 ml of deionized water, incubated for an additional 24 hr, and the seedlings were plated onto PCH agar. Infection incidence was assessed after 48 hr by counting the number of radicles from which *P. cinnamomi* was recovered.

Statistical analyses. Parameter estimates, for the models that were developed, were obtained by least-squares linear regression by using the General Linear Model (GLM) program of the Statistical Analysis System (22).

RESULTS AND DISCUSSION

Use of Ferriss' model (equation 1) requires knowledge of the length, L, of root which is susceptible to infection. Our findings indicated that the entire length of radicle was susceptible; 96% of the radicles became infected when only the mature region was exposed to zoospore inoculum. The mean root length exposed to infested soil on Büchner funnel tensiometers was, therefore, used in equation 1.

A marked reduction in predicted rhizosphere width occurred with small reductions in ψ_m below 0 mb (Fig. 1 and Table 2). Rhizosphere width was reduced from a predicted value of 4.0 mm at 0 mb to approximately 0.75 mm at -10 mb ψ_m (Fig. 1 and Table 2). However, little change in rhizosphere width occurred over the interval from -10 to -100 mb ψ_m. The following model was obtained by regression analysis:

$$W = 4.01(1 - M)^{-0.72} \quad (2)$$

in which W = rhizosphere width (mm) and $M = \psi_m$ ($-$mb). The regression model (eq. 2) accounted for 82% of the variation ($P = 0.0001$). The analysis included tests for additive and interaction effects of inoculum density (I), which were found to be insignificant. The independence of W and I suggests that the use of Gregory's MIT may be appropriate. If the transformation had failed to properly estimate the number of successful infections per root at either very low or very high disease incidence, this would have been reflected in a dependence of W on I.

The predicted rhizosphere widths (Table 2) and the resulting model (Fig. 1) are strongly conditioned by the assumption that inoculum efficiency, E, is constant over 0 to -100 mb ψ_m (18). It is therefore, useful to consider alternative assumptions about the relationship between E and ψ_m. The most probable alternative is that E varies as some negative power of ψ_m:

$$E = 0.32\,(1 - M)^{-\alpha} \quad (3)$$

TABLE 1. Propagules densities of *Phytophthora cinnamomi* used in study of rhizosphere width of 2-wk-old Fraser fir seedlings[a]

Matric potential (mb)	Propagules densities (propagules per cm^3)
0	2.7, 5.4
−10	6.7
−25	6.7, 26.8, 53.5
−50	9.4, 18.7, 26.8, 53.5
−100	9.4, 18.7, 26.8, 53.5

[a] Previous work (18) indicated that 84% of propagules originated from clusters of one or more chlamydospores. For colonies originating from chlamydospores, the number of spores per colony ranged from 1 to 9 with a mean of 2.25 spores per colony.

in which: α a parameter that describes a family of curves and E and M are as previously defined. Corresponding to the family of curves represented by equation 3, is a family of curves for the relation between W and M of which equation 2 can be regarded as a specific case ($\alpha = 0$). Note that, for any relation with the general form of equation 3, predicted rhizosphere widths will be larger (equation 1). Consequently, equation 2 can be viewed as defining a lower bound on the set of possible relations between W and M.

Calculated values for inoculum efficiency (E) (Fig. 2) were obtained by rearranging equation 1 to express E as a function of the other variables:

$$E = H / \; MLI[(W + r)^2 - r^2] \tag{4}$$

All the variables are defined as in equation 1, except that W is now defined to be a constant equal to 4.01 mm at $\psi_m = 0$. It should be noted that, just as in the case of equation 2, predicted values of E (equation 4) will depend upon the estimate, $E = 0.30$, for the case when saturation period, or time in general, is not limiting. This must be true, since the predicted value, $W = 4.01$ mm, when $\psi_m = 0$ mb, also depends on our initial assumption that $E = 0.30$ when time is not a limiting factor. The fitted regression line (Fig. 2) which is based on the calculated values for E (equation 4 and Table 3), is represented by the following model:

$$E = 0.04 \; P_s^{0.63} \tag{5a}$$

in which: E = infections per propagule (inoculum efficiency) and P_s = length of saturation period (hr). The regression model for equation 5a accounted for 63% of the variation ($P = 0.0004$).

The fitted regression line (Fig. 2) suggests that efficiency increases rapidly during the first 4 hr of saturation and subsequently increases in a nearly linear fashion up to 16 hr. Since data were not obtained for flooding periods of less than 2 hr, the predicted behavior in the interval of 0 to 2 hr (Fig. 2) is strictly a consequence of the assumed model form (equation 5a). The distribution of data points, within the range of saturation periods studied, clearly suggests that a linear model would be equally appropriate to describe the relation between E and P_s. Under the latter assumption the model becomes:

$$E = 0.043 + 0.015 \; P_s \tag{5b}$$

This model accounted for 77% of the variation. Comparison of the two models (equations 5a and 5b) shows that differences occur only with respect to how the curve is represented in the interval from 0 to 2 hr (Fig. 2). Equations 5a and 5b can be interpreted, approximately, as lower and upper bounds, respectively, on the set of possible relations between E and P_s (Fig. 2), since both models belong to the family of relations given by:

$$E = \alpha + \beta \; P_s^{\gamma} \tag{5c}$$

in which α, β, and γ are parameters of the general model. No attempt was made to determine E for periods less than 2 hr, because calculated values of E (Table 3) were obtained on the assumption that rhizosphere width is 4.01 mm under saturated soil conditions (eq. 4) and there is an increasing likelihood that such an assumption would not be valid with shorter saturation periods.

The validity of the regression models developed in this study for calculating rhizosphere width of Fraser fir roots and chlamydospore inoculum efficiency of *P. cinnamomi* depends upon the degree to which the experimental conditions satisfy five criteria outlined by Drury et al (5). The following conditions need to be satisfied if the models are to be good predictors: a cylinder should be a good geometric model for a root; only the susceptible root length should be included in equation 1; the number of infections per root should be accurately estimated by Gregory's (9) MIT; the estimate of I should be accurate; and the single value for inoculum efficiency required in equation 1 must, in some sense, be representative of the entire propagule population in the rhizosphere.

The preceding criteria appear to be fulfilled in our study. First, examination of the shape of Fraser fir radicles indicated that a cylindrical model for representation of root shape was adequate. In relation to the second criterion, previous studies on zoospore attraction to roots have shown that zoospores are primarily

TABLE 2. Calculated values of Fraser fir rhizosphere width[a] at selected matric potentials (ψ_m) and inoculum densities (I) of chlamydospores of *Phytophthora cinnamomi*

ψ_m (mb)	I (P/cm^3)	Observations (no.)	Rhizosphere width (mean mm)
0	2.7	4	5.18
0	5.4	3	4.43
0	6.7	4	4.35
−10	6.7	3	0.46
−25	6.7	3	0.35
−25	26.8	2	0.25
−25	53.5	2	0.23
−50	9.4	1	0.42
−50	18.7	1	0.30
−50	26.8	2	0.27
−50	53.5	2	0.38
−100	9.4	2	0.26
−100	18.7	2	0.30
−100	26.8	2	0.06
−100	53.5	2	0.39

[a] Calculated values for rhizosphere width were obtained from the model of Ferriss (7).

TABLE 3. Calculated values for inoculum efficiency[a] of chlamydospores of *Phytophthora cinnamomi* under different soil saturation periods

Saturation period (hr)	Observations (no.)	Mean efficiency
2	4	0.10
4	3	0.12
8	3	0.12
16	4	0.29

[a] Inoculum efficiency of chlamydospores was calculated from the model of Ferris (7) after rearrangement of terms (equation 4 in the text). A rhizosphere width of 4.01 mm was substituted in equation 4; this width is the predicted value for equation 7 when the soil is saturated.

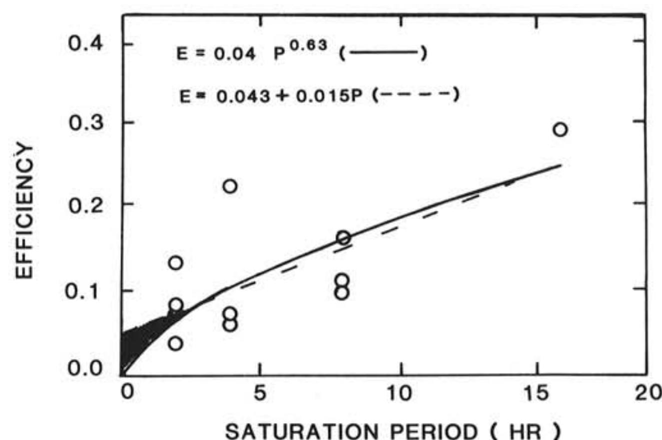

Fig. 2. Linear and nonlinear representations of the functional relation between chlamydospore inoculum efficiency of *Phytophthora cinnamomi* and soil saturation period. These two functions provide approximate bounds on the set of all possible relations which should be considered due to uncertainty of model behavior in the interval from 0 to 2 hr (indicated by the shaded area). The model of Ferriss (7) for calculation of rhizosphere width was rearranged to express inoculum efficiency as a function of the other variables (equation 3 in the text). The value 4.01 (equation 2 in the text) was substituted for rhizosphere width. Data points were calculated by using equation 3. The regression line was generated by using equation 4 (in the text).

attracted to either tissues immediately behind the root tip or the zone of elongation and in some instances to the root hair zone (11,16,25). However, no studies have reported on the attractiveness of relatively mature tissues independently of attraction to the root tip region. We found that when only the mature region of Fraser fir root radicles was exposed to zoospore inoculum, a sufficient number of zoospores were attracted to this region to initiate infection. The entire length of radicle at this stage of growth (2 wk), however, is comprised of very succulent tissue, so these observations should not imply that mature regions of roots are generally attractive to zoospores. Results on radicle susceptibility indicate, however, that we can consider the entire length of radicle to be susceptible to infection.

In the third criterion of Drury et al (5), W would be underestimated if the number of infections received per root is also underestimated. Underestimation of the number of infections per root, in turn, occurs when the assumptions of Gregory's MIT are not fulfilled. All the requirements needed to fulfill these assumptions (3) appear to have been met in the present experimental system: assays of the infested soil used in the experiments demonstrated that inoculum was approximately Poisson-distributed; inoculum was even-aged and obtained from a single isolate of *P. cinnamomi* so that uniform virulence is probably a reasonable assumption; Fraser fir seedlings do not exhibit differential susceptibility to the pathogen; occurrence of seedling infection on the tensiometers appeared to be Poisson-distributed; and infection was assessed by plating experimental root material so that lack of symptom expression at the time of infection assessment was not a factor. It has already been pointed out that W (equation 2) was independent of I which provides further evidence that the assumptions of the MIT are basically fulfilled.

Accurate assessment of I is problematic as Drury et al (5) have pointed out. They also point out that assessment problems may be increased by using a selective medium, such as PCH agar. The value of W will be overestimated if I is underestimated. However, a consistent error in estimation of I does not result in a consistent error in estimation of W. Therefore, it is useful to examine the error pattern that may result. In the predicted curve for rhizosphere width (Fig. 1), W is relatively large at or near 0 mb ψ_m, decreases rapidly between 0 and -10 mb, but then changes little over the interval -10 to -100 mb. Equation 1 can be approximated by the following formula when soil moisture conditions approach saturation (0 ψ_m):

$$W = (a/1)^{1/2} - b \qquad (6)$$

in which: W = rhizosphere width; $a = H/(\pi LE)$; $b = r$ (mm); I = inoculum density (propagules/mm^3); and H, L, and E are as defined in equation 1. Similarly, for values between -10 and -100 mb, equation 1 can be approximated by:

$$W = a/I \qquad (7)$$

If it is now assumed that the actual I in the soil is $2I$, the actual rhizosphere width is only one-half the predicted value, based on a sampling estimate of I for I (eq. 7). In contrast, similar calculations for equation 6 show that the actual value of W will be two-thirds that of the predicted one. This should not be construed as a problem inherent in Ferriss' model. Rather, the problem is inherent in soil assay techniques that do not accurately measure the actual I in soil. We do not know whether estimates of chlamydospore numbers obtained by soil sieving and plating on PCH agar provide accurate estimates of the true I of *P. cinnamomi* in soil. This is perhaps the greatest source of uncertainty in the validation of the rhizosphere width model (equation 2). Additionally, propagules smaller than 38 μm (such as zoospores) were not collected; the role that these propagules may play as a source of inoculum for infection is not known.

The final point raised by Drury et al (5) concerns the problem of specifying a single value for propagule efficiency in the rhizosphere when, in fact, a distribution of efficiency most likely exists, with the efficiency of a propagule being a function of its distance from a root as well as existing soil moisture and temperature conditions. In principle, this is not a problem; an expected efficiency ($E = 0.32$) for the population of propagules in the rhizosphere was previously reported (18). This estimate of E was obtained from our experiments on zoospore movement and from data available in the literature on the probability of various events occurring during the infection process under saturated soil conditions (18). Various types of compensation phenomena may act to maintain efficiency at a fairly constant level over a range of varied environmental conditions (18). Since complete data on infection processes are lacking, the assumption of a constant efficiency is simply an hypothesis.

Thus, it is clear that equations 2 and 5 must be regarded only as tentative models since their predictive accuracy is uncertain. Most important, however, is that the models draw attention to the extreme sensitivity of changes in rhizosphere width and inoculum efficiency in response to changes in ψ_m and soil saturation period, respectively, since the relative changes can be considered to be reasonably accurate. Further, this analysis of the models has served to emphasize areas of research that must be addressed if continued progress is to be made toward understanding the dynamics of rhizosphere host-pathogen interactions.

LITERATURE CITED

1. Baker, R. 1971. Analyses involving inoculum density of soilborne plant pathogens in epidemiology. Phytopathology 61:1280-1292.
2. Baker, R., Maurer, C. L., and Maurer, R. A. 1967. Ecology of plant pathogens in soil. VIII. Mathematical models and inoculum density. Phytopathology 57:662-666.
3. Bald, J. G. 1970. Measurements of host reaction to soilborne inoculum. Pages 37-41 in: Root Disease and Soil-Borne Pathogens. T. A. Toussoun, R. V. Bega, and P. E. Nelson, eds. University of California Press, Berkeley. 252 pp.
4. Byrd, D. W. Jr., Barker, K. R., Ferris, H., Nusbaum, C. J. Griffin, W. E., Small, R. H., and Stone, C. A. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. J. Nematol. 8:206-212.
5. Drury, R. E., Baker, R., and Griffin, G. J. 1983. Calculating the dimensions of the rhizosphere. Phytopathology 73:1351-1354.
6. Duniway, J. M. 1975. Limiting influence of low water potential on the formation of sporangia by *Phytophthora drechsleri* in soil. Phytopathology 65:1089-1093.
7. Ferriss, R. S. 1981. Calculating rhizosphere size. Phytopathology 71:1229-1231.
8. Gilligan, C. A. 1979. Modeling rhizosphere infection. Phytopathology 69:782-784.
9. Gregory, P. H. 1948. The multiple-infection transformation. Ann. Appl. Biol. 35:412-417.
10. Hendrix, F. F., Jr., and Kuhlman, E. G. 1965. Factors affecting direct recovery of *Phytophthora cinnamomi* from soil. Phytopathology 55:1183-1187.
11. Hinch, J., and Weste, G. 1979. Behaviour of *Phytophthora cinnamomi* zoospores on roots of Australian forest species. Aust. J. Bot. 27:679-691.
12. Katznelson, H. 1963. Nature and importance of the rhizosphere. Pages 187-207 in: Ecology of Soil-Borne Plant Pathogens: Prelude to Biological Control. K. F. Baker and W. C. Snyder, eds. University of California Press, Berkeley. 571 pp.
13. Kenerley, C. M. 1983. Epidemiology and control of *Phytopthora cinnamomi* root rot of *Abies fraseri*. PhD. dissertation. North Carolina State University, Raleigh. 100 pp.
14. Kenerley, C. M., and Bruck, R. I. 1983. Overwintering and survival of *Phytophthora cinnamomi* in Fraser fir and cover-cropped nursery beds in North Carolina. Phytopathology 73:1643-1647.
15. Leonard, K. J. 1980. A reinterpretation of the mathematical analysis of rhizoplane and rhizosphere effects. Phytopathology 70:695-696.
16. Milholland, R. D. 1975. Pathogenicity and histopathology of *Phytophthora cinnamomi* on highbush and rabbiteye blueberry. Phytopathology 65:789-793.
17. Mircetich, S. M., Zentmyer, G. A., and Kendrick, J. B. Jr. 1968. Physiology of germination of chlamydospores of *Phytophthora cinnamomi*. Phytopathology 58:666-671.
18. Reynolds, K. M. 1984. Epidemiology of Phytophthora root rot of Fraser fir. PhD. dissertation, North Carolina State University, Raleigh. 215 pp.

19. Reynolds, K. M., Benson, D. M., and Bruck, R. I. 1985. Epidemiology of Phytophthora root rot of Fraser fir: Root colonization and inoculum production. Phytopathology 75:1004-1009.
20. Rovira, A. D. 1963. Plant root exudates and their influence upon soil micro-organisms. Pages 170-186 in: The Ecology of Soil-Borne Plant Pathogens. K. F. Baker and W. C. Snyder, eds. University of California Press, Berkeley. 571 pp.
21. Rovira, A. D. 1969. Plant root exudates. Bot. Rev. 35:35-57.
22. SAS Institute Inc. 1982. SAS User's Guide: Basics. 1982 Edition. Statistical Analysis Systems Institute Inc., Cary, NC. 923 pp.
23. Shew, H. D., and Benson, D. M. 1982. Qualitative and quantitative soil assays for *Phytophthora cinnamomi*. Phytopathology 72:1029-1032.
24. Shew, H. D., and Benson, D. M. 1983. Influence of soil temperature and inoculum density of *Phytophthora cinnamomi* on root rot of Fraser fir. Plant Dis. 67:522-524.
25. Zentmyer, G. A. 1961. Chemotaxis of zoospores for root exudates. Science 133:1595-1596.
26. Zentmyer, G. A., and Mircetich, S. M. 1966. Saprophytism and persistence in soil by *Phytophthora cinnamomi*. Phytopathology 56:710-712.