

Temporal and Spatial Epidemiology of Phytophthora Root Rot in Fraser Fir Plantations

D. M. Benson, L. F. Grand, and C. S. Vernia, Department of Plant Pathology, North Carolina State University, Raleigh 27695; and T. R. Gottwald, USDA ARS U.S. Horticultural Research Laboratory, Ft. Pierce, FL 34945

ABSTRACT

Benson, D. M., Grand, L. F., Vernia, C. S., and Gottwald, T. R. 2006. Temporal and spatial epidemiology of Phytophthora root rot in Fraser fir plantations. *Plant Dis.* 90:1171-1180.

In 1999, 19 plots of Fraser fir (*Abies fraseri*) with a disease focus were established in commercial plantings grown for Christmas tree production in the mountains of five western North Carolina counties. Progress of Phytophthora root rot caused by *Phytophthora cinnamomi* as estimated by mortality was followed in each plot over 3 to 4 years in an attempt to understand dispersal of inoculum. Slope, aspect, and field production age at the time plots were established were recorded. Rainfall estimated from National Weather Service stations each growing season also was recorded. The relationship of site parameters and rainfall to dispersal and disease was investigated. Disease incidence and mortality were assessed in June and September each year for 3 or 4 years depending on plot. Phytophthora root rot as estimated by mortality counts over time in a logistic regression model progressed in only five of 19 plots over 3 years. None of the site parameters correlated with mortality data, although slightly more disease was found in plots with a north aspect. Rainfall was below normal in the 3 years of the study and did not correlate with mortality in any year. Lack of disease progress in the majority of plots was attributed to drought conditions in the region. In the five plots where mortality increased over time, spatial analysis suggested an aggregated pattern of diseased plants. Aggregation was apparent but not very strong among nearest neighbors, but was considerably stronger among groups of trees within a local area. This aggregation within groups was stronger when larger group sizes were examined by beta-binomial analysis. A spatial analysis by distance indices method (SADIE) indicated the presence of secondary clusters occurring several meters away from the main focus. A stochastic model also was employed that indicated a combination of spatial processes were likely involved, specifically a tendency toward spread within a local area, but not necessarily to the nearest neighboring trees, combined with an influence of background inoculum that could not be accounted for within local areas and may have come from external sources. Thus, all sources of inoculum including infected planting stock, inoculum in soil, infected trees, and contaminated equipment were equally important in epidemics of Phytophthora root rot in Fraser fir and dispersal of *P. cinnamomi*.

Additional keywords: disease management, inoculum dispersal, soil moisture

Phytophthora root rot of Fraser fir (*Abies fraseri* (Pursh.) Poir.) caused principally by *Phytophthora cinnamomi* Rands is a serious disease in commercial Christmas tree production in western North Carolina where Fraser fir is produced outside its native habitat (3,17,22). Native stands of Fraser fir occur in organic soils above 1,525 m along the spine of the Appalachian Mountains in Virginia, Tennessee, and North Carolina without evidence of Phytophthora root rot. In contrast, Christmas tree production of the species typically occurs below 1,220 m on mineral soils that have poor internal drainage. A recent survey of Fraser fir in field plant-

ings in North Carolina found a 9% disease loss from Phytophthora root rot with more than 90% of the isolates recovered identified as *P. cinnamomi* (3). In nursery beds, 1.2% of Fraser fir seedlings were infected with *P. cinnamomi* but asymptomatic based on root isolations (3). Thus, *P. cinnamomi* may be introduced to field plantings of Fraser fir on infected but asymptomatic transplants. Other potential sources of inoculum include movement of infested soil on equipment between fields, movement of propagules in runoff water from adjacent fields or from infected nonfir hosts in adjacent forested areas, preexisting soilborne inoculum from earlier crops of Fraser fir, and tree-to-tree movement once an initial plant is infected.

Temporal and spatial analysis of Phytophthora root rot epidemics in Fraser fir has the potential to provide important information for management of the disease. Disease progress in a crop like Fraser fir with a production cycle of 8 to 10 years may be correlated with site parameters

such as slope, aspect, soil type, and rainfall. Since high soil moisture is correlated with increased incidence and severity of root rot caused by *P. cinnamomi* in many crops such as avocado (*Persea americana*) (36), jarrah (*Eucalyptus* spp.) (32), shortleaf (*Pinus echinata*) (35), and radiata (*P. radiata*) pine (24), as well as ornamental crops (1,5,25), site parameters associated with soil moisture conditions may be important in temporal progress of the disease.

Spatial analysis of Phytophthora epidemics can provide information on sources of inoculum. For instance, Benson and Campbell (2) found that occurrence of Phytophthora root rot of azaleas caused by *P. cinnamomi* in nursery blocks of containerized plants was random based on indices of dispersion and probability analysis, suggesting that inoculum was introduced on infected plants set out at random after transplanting or that sprinkler irrigation water contained inoculum. In pepper (*Capsicum* spp.), two-dimensional distance class analysis of plants infected with *P. capsici* detected a nonrandom pattern of diseased plants, suggesting the importance of nearest neighbor infections (31). For tobacco, ordinary runs analysis of black shank epidemics caused by *P. parasitica* (syn. *P. parasitica* var. *nicotianae*) detected either a prediction of random plant infections down the row (6) or a nonrandom pattern (11), possibly due to aggregated initial inoculum in the latter study.

The purpose of this study was to describe temporal and spatial epidemiology of Phytophthora root rot in field plantings of Fraser fir in relationship to dispersal of *P. cinnamomi* from disease foci and importance of inoculum sources. We correlated field parameters such as slope, aspect, and rainfall with Phytophthora root rot. Results of the analysis may provide insight into management considerations for growers. A preliminary report was published (4).

MATERIALS AND METHODS

Plots. In spring 1999, 19 plots of Fraser fir were established in Alleghany, Ashe, Avery, Jackson, and Watagua counties in western North Carolina. These five counties account for over 80% of the Fraser fir grown for Christmas tree production in North Carolina. Plots were selected in commercial fir plantings based on the presence of at least one disease focus in the field where trees had symptoms or had died from Phytophthora root rot. A focus

Corresponding author: D. M. Benson
E-mail: mike_benson@ncsu.edu

Accepted for publication 11 January 2006.

area in a plot with 3 to 11 diseased trees was marked by a 1.2-m-tall stake. A grid of trees centered on the focus was established and permanently marked based on field size, shape, and contour, thus resulting in a variable number of trees per plot which averaged 550 trees but ranged from 194 to 1,070 trees per plot. Although there were individual scattered trees with symptoms at the time of plot establishment, we did not observe other foci in the fields. Spatial scale was the same across plots as trees are typically planted on 1.5-m centers in the field. Maps were made of each plot based on tree and row number so that the same trees were assessed at each sampling date. Field production age, slope, and aspect at plot establishment were recorded for each plot (Table 1). Trees had been in field production 2 to 5 years when plots were established. Slope was determined with the aid of a Suunto clinometer (Forestry Suppliers, Inc., Jackson, MS).

Isolations. Samples of fine roots 1 to 2 mm in diameter were collected from trees in or near the disease focus in each plot at the time the plots were established. Samples were sealed in plastic bags and transported in an ice chest to the laboratory. Roots were washed under running tap water to remove adhering soil, and then blotted on paper towels to remove excess moisture. Roots from each plant were cut into about 1-cm-long segments, and five clumps of roots each containing several individual segments were placed on a semiselective pimaricin, penicillin, polymyxin (3-P) medium for recovery of *P. cinnamomi* (9). Within 48 h, the hyphae of developing colonies were observed under the microscope for the presence of typical hyphal swellings and branching pattern characteristic of *P. cinnamomi*. The pathogen was recovered from trees in 19 of the 21 plots. The two plots where *P. cinna-*

*mom*i was not recovered were dropped from the study.

Presence of other root pathogens. No evidence of basidiocarps of *Armillaria* spp. or *Heterobasidion annosum* (Fr.:Fr.) Bref. or other signs of these root pathogens were found in plots during initial site evaluation or subsequently over the 4 years of the study. *Armillaria* and *annosum* root rots have been seldom reported on Fraser fir in North Carolina plantations and are not considered major diseases (8,10,16). Records of the North Carolina State University Plant Disease and Insect Clinic contain 104 occurrences of *Phytophthora* root rot of Fraser fir in samples submitted between 1999 and 2002, but no occurrence of *Armillaria* or *annosum* root rot during this period.

Disease assessment. Disease incidence and mortality due to *Phytophthora* root rot were assessed twice each year after new growth had fully expanded in June and again in September from 1999 until fall 2001. Plots in which disease was observed to progress were assessed for a fourth growing season through fall 2002. Each tree was examined for symptoms of *Phytophthora* root rot. Although both disease incidence and mortality data were collected from each plot, only mortality data were analyzed since chlorosis and stunting symptoms were not consistent from one assessment date to the next. Trees killed by *Phytophthora* root rot turn a characteristic golden brown that progresses to brick red shortly thereafter. We observed trees that were green in June and brick red in September. When field trees are lost early in the production cycle, growers will often replant a Fraser fir or other species to replace the lost tree; whereas later in the cycle dead trees are removed and the spot is left vacant. Therefore, missing plants were counted as dead.

Data analysis. Mortality data from each sampling date were examined via linear regression with a logistic temporal model to describe disease progress over time. An increase in mortality was considered to have occurred if the significance value of the *F* test (*P*) was less than or equal to 0.05. In addition, residual plots from the regression model were examined to check the appropriateness of the model.

A correlation analysis was performed with Proc Corr (PC-SAS, SAS Institute, Cary, NC) to compare mortality and change in mortality over years with field production age in 1999 and 2002, aspect, and slope. A separate correlation analysis was done to compare mortality each year with annual rainfall each year. Total rainfall for each year was obtained from the National Weather Station closest to the plot. In most cases, this was a distance of 10 km or less.

Spatial analyses. Maps of *Phytophthora* mortality were examined at various spatial scales to assess their spatial structure. Ordinary runs analyses were performed on binary data from each row of each plot and assessment date to determine if aggregation existed between adjacent symptomatic trees within or across the rows. A Visual Basic Excel macro was used to conduct the analysis (T. R. Gottwald, unpublished software). Aggregation of dead trees was assumed for each row separately if the observed number of runs was less than the expected number of runs at *P* = 0.05 using a standard-normal test (7,23).

Beta-binomial distribution (BBD) analysis. For the second level of spatial hierarchy, the data were examined for the presence of aggregation at various quadrat sizes. The incidence data for each plot were partitioned into quadrats of 4 (2 by 2), and 16 (4 by 4) trees. When data are expressed as disease incidence, the beta-binomial distribution provides the best adjustment for random conditions (19). Thus, randomness within quadrats was assessed via beta-binomial analysis. The index of dispersion *D* was used to test for the presence of randomness of *Phytophthora* mortality in trees at each quadrat size. For the index of dispersion, a large *D* (>1) combined with a small *P* (<0.05) suggests aggregation of dead trees (19). Tests for aggregation were performed by comparison of $(N - 1) \times D$ with the chi-square distribution and with the *C*(α) test (*Z* statistic).

The binary power law was used as an assessment of overdispersion within sampling unit across all *Phytophthora* data sets. For disease incidence data, the modified power law (18) expresses the relationship between the observed sample variance of dead trees within sampling units (V_{obs}) and the theoretical variance of the random distribution (V_{bin}); that is, the binomial

Table 1. North Carolina county, aspect, sector, slope, and field production age of Fraser fir plots established to study disease progress of *Phytophthora* root rot epidemics

Plot	County	Aspect (degrees)	Sector ^a	Slope (%)	Plot (year)
77	Avery	170	S	7	4
78	Avery	258	W	13	5
79.1	Avery	78	E	27	5
79.2	Avery	180	S	11	5
82	Alleghany	70	E	7	5
83	Alleghany	350	N	12	5
85	Ashe	329	N	17	4
86	Ashe	52	E	4	4
87	Jackson	218	S	9	2
89	Jackson	242	W	7	4
90	Jackson	280	W	10	4
91	Watauga	356	N	3	3
92	Watauga	112	E	4	5
93	Watauga	256	W	16	4
94	Watauga	152	S	19	3
95	Watauga	240	W	15	4
96	Watauga	256	W	15	3
97	Watauga	150	S	14	4
98	Watauga	150	S	20	4

^a Sector assigned based on a 90 degree range with north set at 315 through 45 degrees, east 46 through 135 degrees, south 136 through 225 degrees, and west 226 through 314 degrees.

variance for binary data $[np(1-p)]$. It can be written as:

$$\ln(V_{\text{obs}}) = \ln(A_x) + b \ln(V_{\text{bin}})$$

in which $\ln(A_x)$ and b are the intercept and the slope of a straight line, respectively. When both A_x and b are equal to 1, $\ln(V_{\text{obs}}) = (V_{\text{bin}})$ and a random spatial pattern is suggested. When $b = 1$ and $A_x > 1$, there is overdispersion with no dependence to the mean incidence p . When both A_x and b are > 1 , the degree of aggregation varies with p . The parameters $\ln(A_x)$ and b were estimated by linear regression using the least squares method. The equality of $\ln(A_x)$ to 0 and b to 1 were tested by a t test using the parameter estimates and standard deviations.

SADIE. The spatial arrangement of quadrats with dead or missing trees was evaluated using the SADIE (Spatial Analysis by Distance IndicEs) method (26–28). The distance to regularity D_r is the minimum total distance that the occurrence of dead trees would need to move statistically to achieve the same number m in each quadrat. The degree of nonrandomness within a set of data is quantified by comparing the observed spatial pattern with rearrangements obtained after random permutations of the individuals among the quadrats. P_a , defined as the proportion of randomized samples with distance to regularity as large or larger than the observed value D_r , can be used for a one-sided test of spatial aggregation (at the significance level of 5%). An overall index of aggregation is given by

$$I_a = D_r/E_a$$

where D_r is the distance to regularity for the observed data and E_a the mean distance to regularity of the randomized samples.

An aggregated pattern is indicated by $I_a > 1$. The organization of clusters into patches (neighborhoods of units with counts larger than the average density m) or gaps (neighborhoods of units with counts $< m$) was analyzed by mapping clustering indices attributed to each quadrat (28). For each individual analysis, 2,028 randomizations were performed based on the 2×2 quadrat data for each plot. The number and the size of the patch clusters were computed using the clusterxyc.exe program (28) and visualized by mapping (bubble and contour plots) the clustering indices for each quadrat size. In addition, for each plot, the distance between the centroid of the main patch cluster to the centroid of the secondary main patch cluster was estimated in quadrat units. When several clusters of the same size were observed, the distance was calculated taking into consideration the more distal one.

Spatiotemporal analysis. Data for the Phytophthora epidemics were analyzed using the spatiotemporal stochastic model for disease spread, which was fitted using Markov Chain Monte Carlo (MCMC) stochastic integration methods. For a thorough description of the MCMC model, its application, and interpretation of results, refer to Gibson (12–14). The results of the spatiotemporal analysis can be viewed graphically in a two-dimensional parameter space representing a series of specific posterior density contours ranging from 0.1 to 0.9. The two parameters represent local (a_2) versus background (b) interactions. The parameter b represents the simple-interest or primary infection rate and quantifies the rate at which a susceptible individual acquires the disease due to primary infection from sources outside the host population or, in the case of soilborne

organisms, can also indicate an increase due to resident inoculum in the soil. Parameter b indicates the probabilistic rate at which the individual becomes infected independent of the infected trees in the plot or general area; whereas a_2 represents the secondary infection rate and quantifies the manner in which the infective challenge presented to a susceptible individual by a diseased individual in the population decreases with the distance between them. As a_2 increases, secondary transmissions occur over shorter ranges, and as long as b is not so large that primary infections dominate, disease maps generated by the model exhibit aggregation.

RESULTS

Temporal effects. When plots were established in the summer of 1999, mortality of Fraser fir from Phytophthora root rot ranged from 1 to 20% depending on plot with an average of 6.7% (Table 2). However, plots had at least one disease focus but a variable number of total trees, so comparisons of initial mortality can be misleading. Initially, mortality was less than 5% in eight plots, between 5 and 9% in seven plots, between 10 and 15% in three plots, and 20% in one plot.

Two patterns of disease progress based on mortality were observed. In 11 of the plots, mortality did not increase over time, with less than a 1% change in mortality over three growing seasons (Table 2). The static nature of Phytophthora root rot in these plots over time can be seen in a map of mortality for plot 91 over three growing seasons (Fig. 1). In eight plots, mortality increased 1% or more over time, with the most striking increase observed in plot 83, where mortality increased 7.2%. This increase in mortality was easy to see in the

Table 2. Trees, mortality, and change in mortality of Fraser fir caused by Phytophthora root rot over a 3-year or 4-year period (1999–2002) and corresponding with an apparent mortality rate ($\log y/(1-y)$), probability of greater t value and R^2

Plot	Trees	Mortality (%)				Mortality change (%)	Apparent mortality rate		
		1999	2000	2001	2002		$\log y/(1-y)$	$P > t ^a$	R^2
77	647	6.2	8.6	9.1	10.0	3.8	0.075	0.0008**	0.864
78	192	1.1	1.8	2.3	nt ^b	1.2	0.159	0.0365*	0.705
79.1	236	20.3	20.8	20.5	nt	0.2	0.014	0.5842	0.081
79.2	277	12.3	12.3	12.0	nt	-0.3	-0.007	0.1863	0.388
82	359	5.3	5.3	7.5	nt	2.2	0.098	0.1161	0.500
83	624	2.1	3.5	9.3	11.3	9.2	0.262	0.0007**	0.870
85	528	3.0	3.4	3.8	4.2	1.2	0.062	0.0014**	0.840
86	672	4.6	3.1	4.5	nt	-0.1	-0.091	0.4287	0.162
87	194	3.1	3.1	5.2	nt	2.1	0.107	0.4558	0.145
89	433	3.7	3.7	3.7	nt	0.0	0.002	0.9775	0.0002
90	360	2.5	2.5	2.8	nt	0.3	0.067	0.1626	0.422
91	912	7.2	7.5	7.5	8.4	1.2	0.006	0.8448	0.007
92	1,070	11.4	11.4	12.4	nt	1.0	0.040	0.2391	0.417
93	472	6.4	9.7	10.4	12.3	5.9	0.068	0.0096**	0.700
94	501	4.4	4.2	3.6	nt	-0.8	-0.037	0.3119	0.250
95	640	7.7	7.8	8.6	nt	0.9	0.003	0.8708	0.007
96	483	12.4	12.6	11.0	nt	-1.4	-0.026	0.2709	0.289
97	613	6.0	6.2	5.7	nt	4.1	0.025	0.6510	0.056
98	504	7.1	6.9	6.9	nt	-0.2	-0.140	0.1805	0.396

^a A single asterisk denotes a probability of a greater t value at $P = 0.05$ and a double asterisk denotes a probability of $P = 0.01$ for the given apparent mortality rate.

^b nt = not determined for year 2002.

map over time as the initial focus remained relatively unchanged in 2000, but symptomatic trees were found further downhill by the summer of 2001 and thereafter (Fig. 2). Across all plots, mortality averaged 7.7% by the end of the third growing season in 2001.

Disease progress in the 19 plots was estimated with the logistic model (34). In 14 plots, the slope parameter for the logistic model fitted to the mortality data over three growing seasons was not significantly different from 0 ($P > 0.05$). This supports the observation of a lack of disease progress as small changes in mortality occurred over this time in most plots (Table 2). Apparent mortality rates ranged from -0.007 to 0.262 per year across all plots. In plots 77, 78, 83, 85, and 93, however, mortality did increase over time and a significant fit was found (Table 2, Fig. 3). These five plots were scattered over four of the five counties where plots were established. In plots 78 and 85, the change in mortality over three or four growing seasons, respectively, was 1.2% compared with a 3.8, 9.2, and 5.9% increase over four growing seasons in plots 77, 83, and 93, respectively. Apparent mortality rates ranged from 0.062 to 0.262 per year across

the five plots (Table 2). Examination of graphs of standard residuals revealed no obvious patterns for the logistic model for the five field plots.

Correlation effects. Across all plots, mortality and change in mortality were not correlated with aspect, slope, or field production age in 1999 and 2001. When plots were grouped into sectors by aspect, those facing north had 10.9% mortality compared with 8.0, 8.3, and 6.5 for those facing east, south, and west, respectively. Slopes ranged from 3 to 27 degrees across all plots, with most plots having a slope of 7 degrees or steeper. When plot factors were compared for only the five plots where mortality increased over time, no relation between slope, aspect, or tree age and mortality was apparent.

Annual rainfall in the vicinity of most plots was below normal or extremely below normal each year. Annual rainfall deficits depending on year were 44 to 77 cm in Avery County, 12 to 38 cm in Alleghany and Ashe counties, 16 to 24 cm in Jackson County, and 5 to 19 cm in Watagua County. Across all plots, no correlation was found between annual rainfall in a given year and the mortality observed that year nor between annual rainfall in a pre-

vious year with mortality in the current year.

Spatial pattern effects. Spatial patterns were examined for the five plots in which mortality increased over time. The first level of spatial hierarchy examined was the association of disease status (mortality) between adjacent trees as tested by ordinary runs analysis. When individual within-row and across-row directions were examined, the majority of tests demonstrated some departure from randomness (Table 3). When plots were treated as a single long row or across row segment, a departure from randomness was indicated in 71 of 76 trials. There was also no indication of directionality of mortality down or across rows.

The next level of spatial hierarchy examined was the association of disease status within groups of trees in 2 by 2 and 4 by 4 quadrats. Interpretation of the index of dispersion values (D) suggested that nearly all plot/year combinations demonstrated aggregated spatial structure of *Phytophthora*-killed trees at both quadrat sizes tested (Table 4). When significant for both quadrat sizes tested, values for θ were higher for the smaller quadrat size, indicating aggregation within groups of four

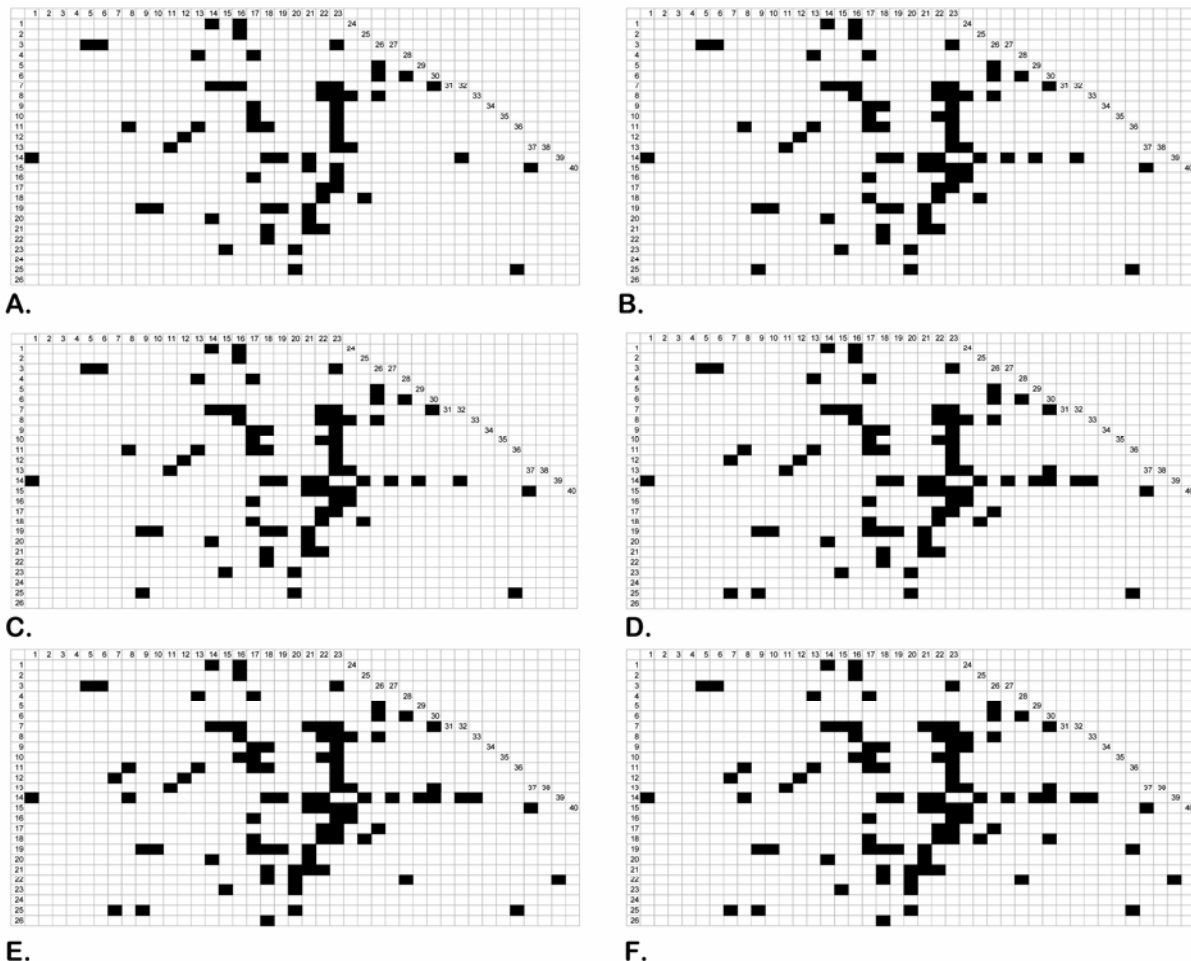


Fig. 1. Map of mortality of Fraser fir caused by *Phytophthora* root rot in plot 91. A, June 1999; B, September 1999; C, June 2000; D, September 2000; E, June 2001; F, September 2001. Initial focus was dead trees in columns 21 to 23 and rows 7 to 13. The plot had a 3% slope from top to bottom of the map.

trees, and were slightly stronger than for groups of 16 trees.

The relationship between $\log(V_{\text{obs}})$ and $\log(V_{\text{bin}})$, as demonstrated by a graph representing the binary power law, was highly significant ($P < 0.001$) for the two sizes of quadrat (Fig. 4A and B). The estimates of b and A were, respectively, 1.07 (SE = 0.04) and -0.09 (SE = 0.07) for the 2 by 2 quadrat ($R^2 = 0.94$) and 0.95 (SE = 0.05) and -0.47 (SE = 0.10) for the 4 by 4 quadrat ($R^2 = 0.91$). For the 2 by 2 quadrat size, the estimate for b was not significantly different from 1, whereas for the 4 by 4 quadrat size, estimates of b and A were

statistically different from 1 ($P < 0.05$), which indicated a general and significant pattern of aggregation of dead trees within all quadrat sizes tested. Values of b higher than 1 also indicated that the degree of aggregation was a function of mortality frequency in the quadrat. However, this was only true for the 2 by 2 quadrat size, which was not significant. Therefore, the results support those obtained with the beta-binomial index of dispersion, that plants killed by *Phytophthora* root rot showed aggregation across both quadrat sizes tested, and that aggregation tended to intensify with time

as a consequence of increase of *Phytophthora* mortality.

SADIE analysis provides a means to examine the entire plot for aggregation holistically, to estimate the number of individual clusters of dead trees, and to determine the distance between main and secondary clusters in quadrat units. Plot/year combinations were tested for those plots that demonstrated disease increase through time. The analyses performed indicated that in four of these five plot/year combinations tested, quadrats with dead trees had significant I_a values, indicating general heterogeneity and thus a nonrandom ar-

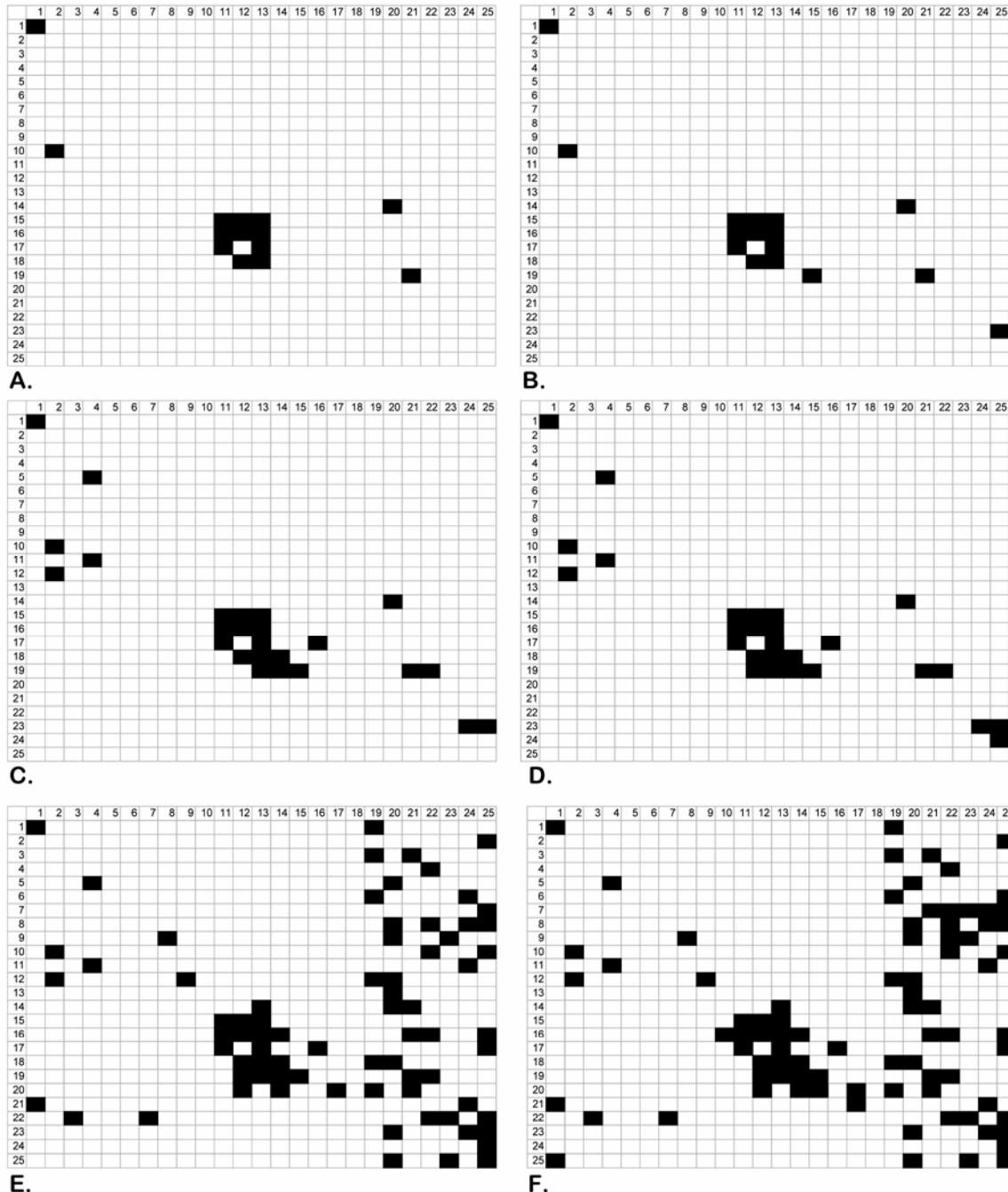


Fig. 2. Map of mortality of Fraser fir caused by *Phytophthora* root rot in plot 83. **A**, June 1999; **B**, September 1999; **C**, June 2000; **D**, September 2000; **E**, June 2001; **F**, September 2001. Initial focus was dead trees in columns 11 to 13 and rows 15 to 18. The plot had a 12% slope in a diagonal direction from the upper left-hand corner of the map to the lower right-hand corner of the map.

rangement of dead trees, supporting the ordinary runs and index of dispersion analyses. The pattern of dead trees in plot 85 was not distinguishable from a random arrangement, which is consistent with the

BBD analysis results. The number of distinct clusters of dead trees ranged from 4 to 10 among the plots. Where a nonrandom arrangement was indicated, the distance between the centroids of the main and

secondary clusters ranged from 3.3 to 3.9 in the X distance and 2.3 to 3.8 quadrat units in the Y distance. Because plants were uniformly planted on 1.5-m centers, this translates to 6.31 to 7.96 m between main and secondary clusters (Table 5).

Spatiotemporal stochastic model. In an attempt to investigate the influence of sources of inoculum and the spatial processes that gave rise to the spatial patterns of *Phytophthora* root rot observed in this study, we employed a stochastic modeling technique that has been useful in describing the spatiotemporal dynamics of various citrus tristeza pathosystems (15). For explanatory purposes, the resulting posterior density contour maps can be grouped in various ways indicating different combinations of spatial processes (Fig. 5).

For group one, in two of the nine contour maps, the background parameter, b , was clearly greater than 0, and a_2 ranged from about 1.0 toward the maximum value tested but had the highest posterior density near the middle of the a_2 range (Fig. 5A and C). A third contour map had commonality with this group in that b was most likely greater than 0 (all but the 15% or lower confidence regions), and a_2 was at or slightly lower than the middle of its parameter range (Fig. 5E).

A second grouping includes five of the nine contour maps, where the background parameter, b , was most probably greater than 0 (always falling in the 60% or greater confidence regions) (Fig. 5D, E, G, H, and I). In four of these cases (except for Fig. 5E), a_2 was more likely to occur at the upper half of its range (Fig. 5D, G, H, and I).

For a third grouping with two of the nine contour maps, the highest posterior density contours show that b was most likely (90 to 100% confidence region) at or near 0 but could be nonzero as well. The a_2 parameter ranged from about 0.7 toward the maximum value tested but had the highest posterior density near the middle of the a_2 range (Fig. 5B and F).

In all cases, the a_2 was always nonzero and >0.4 . Across all of the contour maps, the highest confidence region ($\geq 90\%$) always exceeded a value of $a_2 > 1.2$.

DISCUSSION

P. cinnamomi, as well as other root-infecting *Phytophthora* spp., are highly dependent on wet soils for production of sporangia and dispersal of zoospores. Over the course of three growing seasons, progress of disease mortality from foci in each plot was not observed in 74% of the plots. This result was unexpected since intuitively disease is expected to increase from year to year when susceptible hosts are present and the environment is favorable. Our results suggest that annual rainfall and subsequent soil moisture conditions were not favorable for disease development in most plots or *Phytophthora* infections

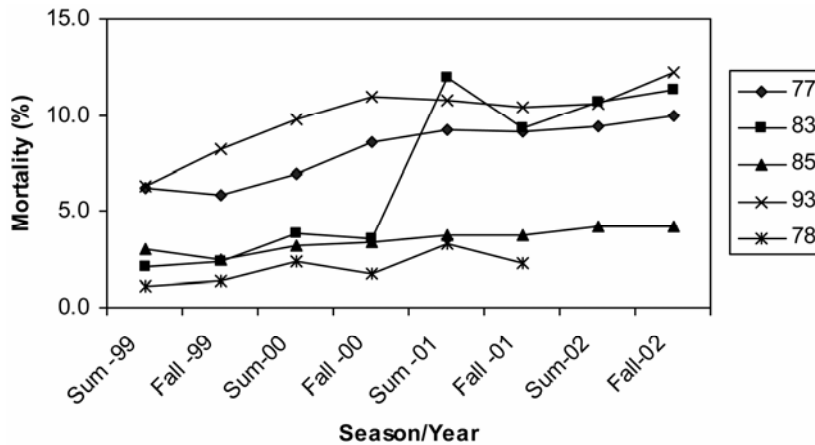


Fig. 3. Disease progress curves for mortality of Fraser fir caused by *Phytophthora* root rot between 1999 and 2002 for plots 77, 78, 83, 85, and 93.

Table 3. Ordinary runs analysis of mortality of Fraser fir caused by *Phytophthora* root rot

Plot	Disease incidence	Ordinary runs ^a			
		Row	Column	Row (all) ^b	Col (all)
77- 1999 summer	0.054	2/17	0/20	N	R
77- 1999 fall	0.062	1/17	0/22	N	R
77- 2000 summer	0.074	0/20	1/23	N	R
77- 2000 fall	0.097	3/21	2/26	N	R
77- 2001 summer	0.124	4/21	2/28	N	N
77-2001 fall	0.125	4/21	3/28	N	N
77-2002 summer	0.131	4/22	3/28	N	N
77-2002 fall	0.134	4/22	3/28	N	N
78-1999 summer	0.011	1/5	2/7	N	N
78-1999 fall	0.015	1/7	2/10	N	N
78-2000 summer	0.034	3/13	3/20	N	N
78-2000 fall	0.035	3/14	3/17	N	N
78-2001 summer	0.050	2/17	4/22	N	N
78- 2001 fall	0.052	2/18	4/22	N	N
83- 1999 summer	0.022	2/7	3/8	N	N
83-1999 fall	0.026	2/9	3/9	N	N
83-2000 summer	0.040	3/14	5/12	N	N
83-2000 fall	0.043	5/14	5/13	N	N
83-2001 summer	0.120	4/21	5/25	N	N
83- 2001 fall	0.134	6/22	6/25	N	N
83- 2002 summer	0.138	6/22	6/25	N	N
83-2002 fall	0.142	5/22	7/25	N	N
85-1999 summer	0.027	0/6	1/8	N	N
85-1999 fall	0.027	0/6	1/8	N	N
85- 2000 summer	0.032	0/7	1/9	N	N
85- 2000 fall	0.034	1/7	1/9	N	N
85- 2001 summer	0.038	3/7	1/9	N	N
85- 2001 fall	0.038	3/7	1/9	N	N
85- 2002 summer	0.042	3/9	0/9	N	N
85- 2002 fall	0.045	3/9	0/9	N	N
93- 1999 summer	0.065	2/11	0/18	N	R
93-1999 fall	0.092	4/11	1/19	N	N
93-2000 summer	0.117	5/14	2/21	N	N
93- 2000 fall	0.124	5/14	3/21	N	N
93- 2001 summer	0.135	5/15	3/21	N	N
93- 2001 fall	0.135	5/15	3/21	N	N
93-2002 summer	0.151	8/16	3/22	N	N
93- 2002 fall	0.160	8/16	3/23	N	N

^a Values shown for each plot in each assessment date are the proportion of the number of test rows with significant aggregation ($P = 0.05$) divided by the total number of rows tested (row with more than one diseased tree).

^b Row (all) and Col (all) tests consider the plot as one long row or column, respectively. R = random or nonaggregated situation indicated. N = nonrandom or aggregated situation indicated.

were subclinical and did not lead to tree mortality. Indeed, weather was dry or extremely dry for the 4 years of the study. This region of North Carolina experienced drought conditions that were not ameliorated until the 2003 growing season.

For the plots in which mortality increased over time, ordinary runs analysis does not support downhill movement of *Phytophthora* inoculum leading to mortality within the plots. In a native jarrah forest in Australia, Podger (29) observed that *P. cinnamomi* moved 40 m/year down drainages in poorly drained areas of the forest, but only 40 m per 5 years down drainages in well-drained areas based on symptom expression in susceptible hosts. Current management practices for Fraser fir, which include use of grass and legume groundcovers to minimize erosion, would tend to minimize long-distance dispersal of inoculum. In some plots where we observed expansion of the disease focus down the drainage feature, it is possible that inoculum already was distributed throughout the field on asymptomatic plants or as chlamydospores from previous crops of Fraser fir (3,33). In this situation, the role of water is not that of movement of inoculum but rather to extend periods of soil moisture favorable for sporulation and infection by existing inoculum.

Although there was some indication that single *Phytophthora*-infected trees did influence infection of nearby trees from the ordinary runs analysis, there was a much stronger and more general heterogeneity among trees within groups, especially 2 by 2 groups, and among nearby trees in the five plots. The BBD analysis clearly demonstrated that dead trees were aggregated in the plots where disease progressed over time. This was evident at quadrat sizes of both 2 by 2 and 4 by 4, but the smaller quadrat size had slightly stronger aggregation. This result could be interpreted to mean that conditions favorable for infection and disease development, such as high soil moisture, were present in fairly small sections of a field, since a 2 by 2 group of trees at 1.5-m spacing would occupy 2.25 m² and a 4 by 4 group only 20.25 m².

Mortality increased between 1 and 9% over the 4 years among the five plots. Aggregation of dead plants intensified in plots as mortality increased over time, as seen in the relationship between $\log(V_{\text{obs}})$ and $\log(V_{\text{bin}})$ when plotted as the binary power law. Increased aggregation over time suggests that plant-to-plant movement of inoculum continued, but the overall small change in mortality across the five plots implies that environmental conditions such as soil moisture were unfavorable during this period for *Phytophthora* sporulation and infection of Fraser fir. Plant-to-plant movement may have occurred both by root-to-root contact and by very limited dispersal of inoculum in water. Reynolds

et al. (30) demonstrated that mycelial growth through root-to-root contact contributed to dispersal for *P. cinnamomi* in the shallow rooted Fraser fir.

We chose plots based on a visual central disease focus from which additional trees were included with a grid size dependent on contiguous trees uninterrupted by

Table 4. Beta-binomial and index of dispersion analysis of mortality of Fraser fir caused by *Phytophthora* root rot

Plot	Disease incidence	Beta-binomial parameter (θ) ^a		Dispersion index (D) ^b	
		Quadrat 2x2	Quadrat 4x4	Quadrat 2x2	Quadrat 4x4
77- 1999 summer	0.054	0.142*	0.114*	1.30**	2.19***
77-1999 fall	0.062	0.094	0.088*	1.21*	2.04***
77-2000 summer	0.074	0.101	0.093*	1.22*	2.13***
77-2000 fall	0.097	0.088	0.092*	1.18*	2.03***
77-2001 summer	0.124	0.111*	0.067*	1.24**	1.86***
77-2001 fall	0.125	0.129*	0.073*	1.28**	1.93***
77-2002 summer	0.131	0.124*	0.079*	1.29**	1.94***
77-2002 fall	0.134	0.125*	0.077*	1.29**	1.89***
78- 1999 summer	0.011	0.401	0.156	1.85***	3.08***
78- 1999 fall	0.015	0.246	0.103	1.62***	2.45***
78- 2000 summer	0.034	0.231*	0.110	1.57***	2.52***
78-2000 fall	0.035	0.215*	0.104	1.54***	2.43***
78-2001 summer	0.050	0.188*	0.070*	1.46***	2.18***
78-2001 fall	0.052	0.177*	0.078*	1.44***	2.32***
83-1999 summer	0.022	0.728*	0.083	2.26***	2.00***
83-1999 fall	0.026	0.622*	0.102	2.17***	2.16***
83-2000 summer	0.040	0.492*	0.185*	2.01***	3.31***
83-2000 fall	0.043	0.450*	0.199*	1.96***	3.34***
83-2001 summer	0.120	0.237**	0.098*	1.61***	2.18***
83-2001 fall	0.134	0.308**	0.143**	1.71***	2.73***
83-2002 summer	0.138	0.295**	0.129**	1.69***	2.64***
83-2002 fall	0.142	0.259**	0.143**	1.64***	2.76***
85-1999 summer	0.027	0.182	0.115	1.51***	2.11***
85-1999 fall	0.027	0.182	0.115	1.51***	2.11***
85-2000 summer	0.032	0.285*	0.124	1.64***	2.13***
85-2000 fall	0.034	0.348*	0.148*	1.71***	2.49***
85-2001 summer	0.038	0.554*	0.196*	2.04***	3.09***
85-2001 fall	0.038	0.554*	0.196*	2.04***	3.09***
85-2002 summer	0.042	0.448*	0.140*	1.93***	2.79***
85-2002 fall	0.045	0.483*	0.181*	2.00***	3.43***
93-1999 summer	0.065	0.164*	0.068	1.40***	1.74**
93-1999 fall	0.092	0.204*	0.108*	1.46***	2.05***
93-2000 summer	0.117	0.329**	0.164*	1.63***	2.57***
93-2000 fall	0.124	0.435**	0.207*	1.81***	3.02***
93-2001 summer	0.135	0.324**	0.220	1.68***	2.98***
93-2001 fall	0.135	0.324**	0.220	1.68***	2.98***
93-2002 summer	0.151	0.377**	0.193*	1.76***	3.15***
93-2002 fall	0.160	0.376**	0.265	1.77***	3.40***

^a Maximum likelihood estimate of the beta-binomial aggregation parameter θ . Significant departures from zero were determined by a t test, $t = \theta/s.e.(\theta)$. Significance is indicated by *, **, *** at $P = 0.05$, $P = 0.01$, and $P = 0.001$, respectively.

^b Index of dispersion (D) values for the indicated quadrat size by plot and assessment date for *Phytophthora* root rot plots in Fraser fir. Values presented for each assessment date are D (=observed variance/binomial variance). Tests for aggregation were performed by comparison of $(N - 1) \times D$ with the chi-square distribution and with the $C(\alpha)$ test (Z statistic) as described in the text. Significance *, **, *** is indicated for the $C(\alpha)$ test. A large (>1) D and a small P (≤ 0.05) suggest rejection of H_0 (binomial distribution - random pattern of symptomatic trees) in favor of H_1 (overdispersion described by the beta-binomial).

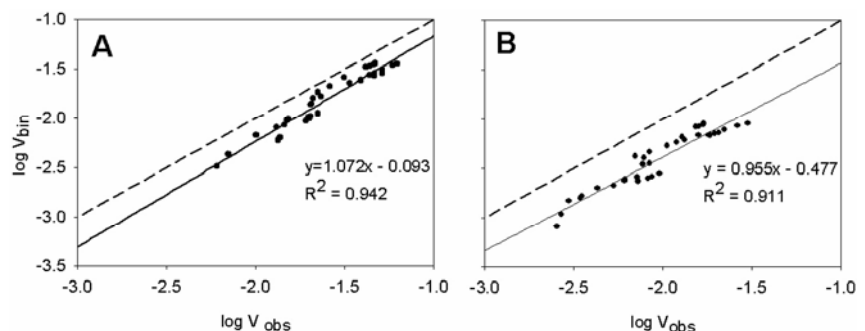


Fig. 4. Plot of the Binary Power Law of the log of observed versus log binary variance associated with *Phytophthora* root rot for five plots studied by assessment period combinations for **A**, 2 by 2 quadrat size, and **B**, 4 by 4 quadrat size.

equipment-access paths or fence lines in the field. However, SADIE analysis clearly demonstrated that four to 10 distinct clusters of mortality, depending on plot, developed

after 4 years. Again, this implies plant-to-plant dispersal of *Phytophthora* through either root-to-root contact or movement of inoculum from infected trees in water.

For Fraser fir, *Phytophthora* spread and subsequent mortality did not always appear to be to nearest neighboring trees but often did seem to occur within the same

Table 5. Spatial Analysis by Distance Indices (SADIE) analysis of mortality of Fraser fir caused by *Phytophthora* root rot

Plot	Year	Index of aggregation ^a		Patchiness ^b		Distance from main to secondary cluster(s) ^c	
		I_a	P_a	No. clusters	Size	X dist	Y dist
77	2002 fall	1.818*	0.0003	10	7 (1,2,3,5)	3.3429	2.9429
78	2001 fall	2.249*	0.0003	9	6 (1,2,4)	3.6667	3.8333
83	2002 fall	1.948*	0.0003	5	14 (1,11)	3.8702	2.8702
85	2002 fall	1.246	0.1094	4	2 (1)	1.5	4
93	2002 fall	1.440*	0.0116	4	6 (1,3)	3.5	2.3333

^a P_a is the proportion of 2,028 randomizations that are larger than D (moves to regularity of the observed data). Index of aggregation I_a is defined as $= D/E_a$ with E_a the mean distance to regularity of the randomized samples. Null hypothesis of spatial randomness is rejected if $P_a < 0.025$ (in favor of aggregation) or if $P_a > 0.975$ (for the alternative of regularity) at the usual 5% probability level.

^b Number and size of the patch clusters were computed using the clusterxyc.exe program and visualized by mapping (bubble and contour plots) the clustering indices for each quadrat.

^c Distance between centroid of the main patch cluster and centroid of the secondary main patch cluster in quadrat units. When several clusters of the same size were observed, distance was calculated taking into consideration the further one.

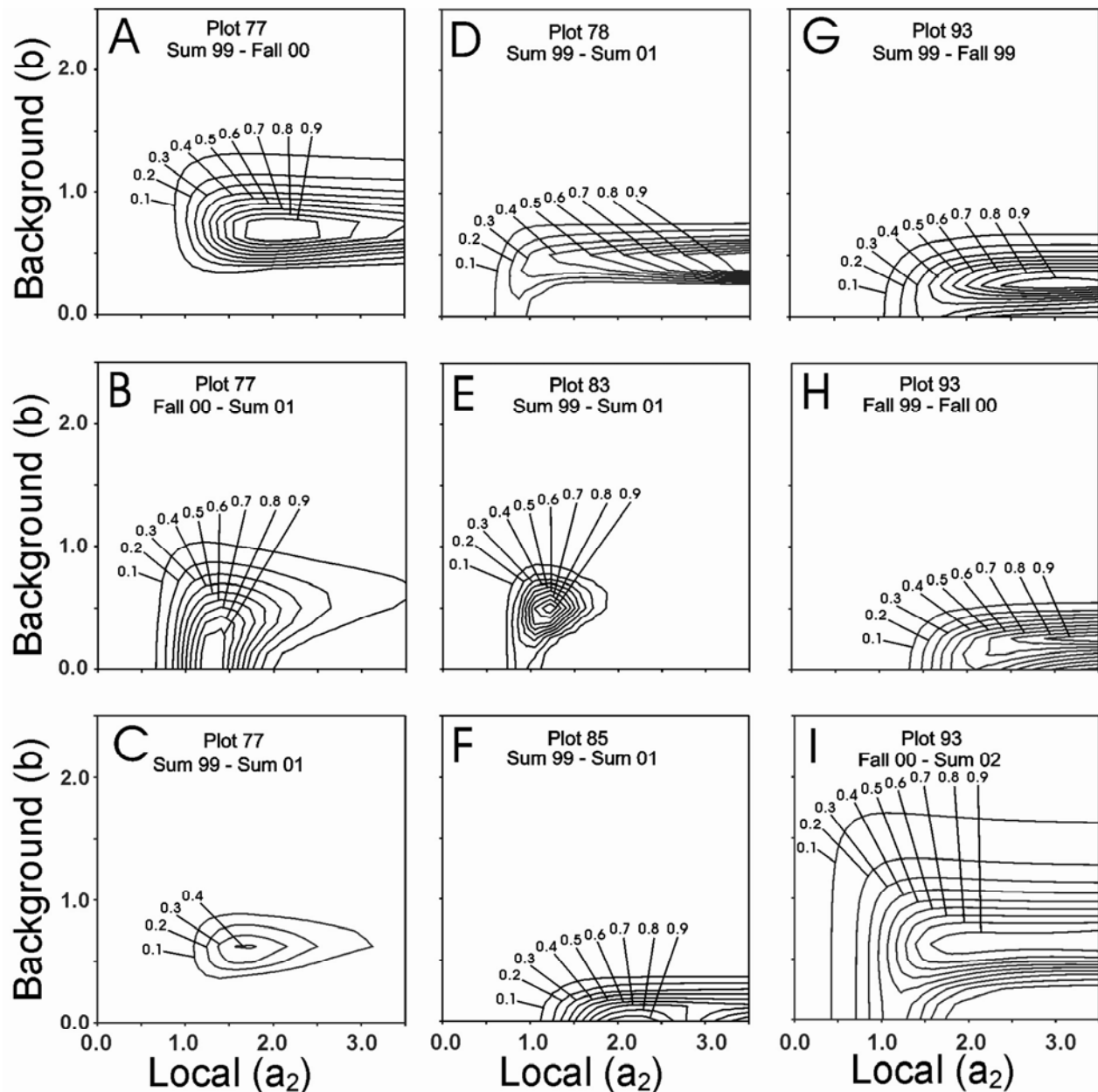


Fig. 5. Posterior density maps resulting from the Markov Chain Monte Carlo simulation spatiotemporal stochastic model of *Phytophthora* dynamics on Fraser fir.

vicinity of a few tree spaces away. Interpretation of the posterior density estimates of the contour maps generated in the spatiotemporal stochastic (MCMC) model highlights the tendency for the evidence to favor secondary spread via predominantly midrange local interactions for dispersal of inoculum through time with some indication of randomness (background transmissions of inoculum) as well. However, for a portion of the plots, the model provides some evidence that the background parameter b has a lower probability of being equal to 0 (Fig. 5D, G, H, and I), and in one plot the contour map suggests this probability is 90% or above (Fig. 5F). If we consider that for the majority of the plots we can make a case with at least some level of probability that the background parameter b could be 0, then for all but two of the plots (Fig. 5A and C), the model suggests that the spatial spread observed could be explained by midrange local interactions of secondary spread alone.

However, there is evidence from this analysis that random spread occurred, although the contribution of this random spread was not strong because in all cases the maximum confidence regions (90 to 100%) of the contour maps were always in the lower third of the range tested. For soil pathosystems, a random spatial increase in disease can mean either that inoculum originated outside the plot area or that it originated from resident inoculum in the plot in an apparently random pattern. To elucidate whether this random spread is a consequence of randomly distributed resident inoculum in the plot or from inoculum outside the plot, we must examine what we know of Fraser fir production practices and pathogen life cycle.

Potential sources of inoculum from outside the plot include the planting of infected asymptomatic trees (3), the replanting of asymptomatic trees as replacements for those lost, the introduction of inoculum carried into the plot in water (21), and/or by infested soil introduced from other fields on equipment or workers' shoes (21). Sources from inside the plot include colonized roots on infected trees (30), chlamyospores surviving in soil (30,33), and movement of infested soil or zoospores within the plot. In ohia forests, inoculum of *P. cinnamomi* was dispersed by soil on hikers' boots, wild animals, and moving water (21). Chlamyospores survived in ohia forest soils, but only zoospores were recovered in streams and puddles (21).

Correlations of mortality or change in mortality in plots with slope, aspect, tree production age, and annual rainfall were not significant over plots. In contrast, for tobacco black shank epidemics, multiple regression of disease incidence with environmental parameters found that temperature, annual rainfall, and days of drought

were consistently related (20). Cumulative daily soil water status expressed as matric potential was a better indicator of cycles of increase in mortality in black shank epidemics than chronological time where multiple cycles of increase in mortality were observed (11). For Fraser fir, years with higher annual rainfall conditions in general would be more favorable for disease development. Apparently, the general drought conditions of the area had an overriding effect, resulting in little disease progress and mortality of Fraser fir. It is possible that these parameters were not measured at a scale appropriate to the response of *P. cinnamomi* to subtle differences within a field. In any case, in the majority of plots during the study period, Phytophthora root rot had developed locally within a field prior to our assessments and then remained stagnant for the next 3 years. In the five plots where disease progressed, there was a clear indication of aggregation of infected trees with intensification of aggregation as disease increased over time. In this situation, both external and internal sources of inoculum may have been important in disease development, depending on plot. From a grower's perspective, all potential sources of inoculum were potentially indicated in these analyses, so strict sanitation measures would be important to minimize the threat of introduction of the pathogen and stimulation of development of Phytophthora root rot from resident sources in Fraser fir.

This study represents the dynamics of Phytophthora root rot on death of commercial Fraser fir during a multi-year period when lower than normal annual rainfall prevailed. Although impossible to anticipate at the beginning of a multi-year study, additional studies are desirable during multi-year periods when annual rainfall is at or above normal. Such additional studies should then be compared with the study presented in this paper to compare the spatial and temporal dynamics of the *Phytophthora*-Fraser fir pathosystem under differing long-term weather patterns. Reduced annual rainfall for multi-year periods may have affected inoculum production, inoculum movement via sheet flow and splash dispersal, infection, survival of new infections, and disease development leading to mortality. Multi-year precipitation levels at or above normal may increase mortality compared to that observed in the present study.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance provided by Jerry Moody (Avery County), David Tucker (Watauga County), Jerry Washington (Alleghany and Ashe counties), and Christy Bredencamp (Jackson County) in the field and by Barbara Shew, Kala Parker, and Jolene Taylor for help with the statistical analysis. This work was supported by the North Carolina Christmas Tree Association and the North Carolina Agricultural Research Service.

LITERATURE CITED

- Benson, D. M. 1986. Relationship of soil temperature and moisture to development of Phytophthora root rot of azalea. *J. Environ. Hort.* 4:112-115.
- Benson, D. M., and Campbell, C. L. 1985. Spatial pattern of Phytophthora root rot and dieback of azalea in container-grown nursery stock. *Plant Dis.* 69:1049-1054.
- Benson, D. M., and Grand, L. F. 2000. Incidence of Phytophthora root rot of Fraser fir in North Carolina and sensitivity of *Phytophthora cinnamomi* to metalaxyl. *Plant Dis.* 84:661-664.
- Benson, D. M., and Grand, L. F. 2003. Progress of Phytophthora root rot in plantations of *Abies fraseri*. (Abstr.) *Phytopathology* 93:S8.
- Benson, D. M., Shew, H. D., and Jones, R. K. 1982. Effects of raised and ground-level beds and pine bark on survival of azalea and population dynamics of *Phytophthora cinnamomi*. *Can. J. Microbiol.* 4:278-280.
- Campbell, C. L., Jacobi, W. R., Powell, N. T., and Main, C. E. 1984. Analysis of disease progression and the randomness of occurrence of infected plants during tobacco black shank epidemics. *Phytopathology* 74:230-235.
- Campbell, C. L., and Madden, L. V. 1990. *Introduction to Plant Disease Epidemiology*. John Wiley & Sons, New York.
- Cordell, C. E., and Astin, J. S., Jr. 1965. A new host for *Fomes annosus*, *Polyporus schweinitzii* and *Fomes pini*. *Plant Dis. Rep.* 49:360.
- Eckert, J. W., and Tsao, P. H. 1962. A selective antibiotic medium for isolation of *Phytophthora* and *Pythium* from plant roots. *Phytopathology* 52:771-777.
- Farr, D. F., Rossman, A. Y., Palm, M. E., and McCray, E. B., n. d. 2005. Fungal Databases, Systematic Botany and Mycology Laboratory, ARS, USDA. Online publication. Retrieved Oct. 25, 2005.
- Ferrin, D. M., and Mitchell, D. J. 1986. Influence of soil water status on the epidemiology of tobacco black shank. *Phytopathology* 76:1213-1217.
- Gibson, G. J. 1997. Investigating mechanisms of spatiotemporal epidemic spread using stochastic models. *Phytopathology* 87:139-146.
- Gibson, G. J. 1997. Markov chain Monte Carlo methods for fitting spatiotemporal epidemic stochastic models in plant pathology. *Appl. Stat.* 46:215-233.
- Gibson, G. J. 1997. Fitting and testing spatiotemporal stochastic models with applications in plant pathology. *Plant Pathol.* 45:172-184.
- Gottwald, T. R., Gibson, G. J., Garnsey, S. M., and Irely, M. 1999. Examination of the effect of aphid vector population composition on the spatial dynamics of citrus tristeza virus spread by stochastic modeling. *Phytopathology* 89:603-608.
- Grand, L. F., ed. 1985. *North Carolina Plant Disease Index*. 2nd ed. Tech Bull. 240. N.C. Agric. Res. Serv., North Carolina State University, Raleigh.
- Grand, L. F., and Lapp, N. A. 1974. *Phytophthora cinnamomi* root rot of Fraser fir in North Carolina. *Plant Dis. Rep.* 58:318-320.
- Hughes, G., and Madden, L. V. 1992. Aggregation and incidence of disease. *Plant Pathol.* 41:657-660.
- Hughes, G., and Madden, L. V. 1993. Using the beta-binomial distribution to describe aggregated patterns of disease incidence. *Phytopathology* 83:759-763.
- Jacobi, W. R., Main, C. E., and Powell, N. T. 1983. Influence of temperature and rainfall on the development of tobacco black shank. *Phytopathology* 73:169-143.
- Kliejunas, J. T., and Ko, W. H. 1976. Dispersal of *Phytophthora cinnamomi* on the island of Hawaii. *Phytopathology* 66:457-460.
- Kulman, E. G., and Hendrix, F. F., Jr. 1963.

- Phytophthora root rot of Fraser fir. Plant Dis. Rep. 47:552-553.
23. Madden, L. V., Louie, R., Abt, J. J., and Knoke, J. K. 1982. Evaluation of tests for randomness of infected plants. Phytopathology 72:195-198.
 24. Newhook, F. J. 1959. The association of *Phytophthora* spp. with mortality of *Pinus radiata* and other conifers I. Symptoms and epidemiology in shelter belts. N.Z. J. Agric. Res. 2:808-843.
 25. Ownley, B. H., and Benson, D. M. 1991. Relationship of matric water potential and air-filled porosity of container media to development of Phytophthora root rot of rhododendron. Phytopathology 81:936-941.
 26. Perry, J. N. 1995. Spatial analysis by distances indices. J. Anim. Ecol. 64:303-314.
 27. Perry, J. N. 1998. Measures of spatial pattern for counts. Ecology 79:1008-1017.
 28. Perry, J. N., Winder, L., Holland, J. M., and Alston, R. D. 1999. Red-blue plots for detecting clusters in count data. Ecol. Lett. 2:106-113.
 29. Podger, F. D. 1972. *Phytophthora cinnamomi*, A cause of lethal disease in indigenous plant communities in Western Australia. Phytopathology 62:972-981.
 30. 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.
 31. Ristaino, J. B., Larkin, R. P., and Campbell, C. L. 1994. Spatial dynamics of disease symptom expression during *Phytophthora* epidemics on bell pepper. Phytopathology 84:1015-1024.
 32. Shea, S. R. 1975. Environmental factors of the northern jarrah forest in relation to pathogenicity and survival of *Phytophthora cinnamomi*. For. Dep. Bull. 85. Perth W. Aust.:83 pp.
 33. Shew, H. D., and Benson, D. M. 1982. Qualitative and quantitative soil assays for *Phytophthora cinnamomi*. Phytopathology 72:1029-1032.
 34. Van der Plank, J. E. 1963. Plant Diseases: Epidemics and Control. Academic Press, New York.
 35. Zak, B. 1961. Aeration and other soil factors affecting southern pines as related to littleleaf disease. U.D. Dep. Agric. Tech. Bull. 1248.
 36. Zentmyer, G. A., and Richards, S. J. 1952. Pathogenicity of *Phytophthora cinnamomi* to avocado and the effect of irrigation on disease development. Phytopathology 42:35-37.