Effect of Flooding on Development of Phytophthora Root Rot in Fraser Fir Seedlings

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ABSTRACT

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The effect of flooding on root rot caused by *Phytophthora cinnamomi* in Fraser fir (*Abies fraseri*) seedlings was assessed. Soil infested with propagules of *P. cinnamomi* but without seedlings, and infested and uninfested soil into which 2-yr-old Fraser fir seedlings were transplanted, was continuously flooded for 0, 24, and 48 hr. Inoculum density of *P. cinnamomi* was quantified at 2, 9, and 23 days and seedling mortality was assessed 33 days after flooding. In treatments with infested soil and seedlings, inoculum density was greater ($P = 0.01$) with flooding than without flooding at days 9 and 23. Final seedling mortality and infection were also higher ($P = 0.01$) with the two flooding treatments (24 or 48 hr) compared to no flooding. Inoculum density was found to decline similarly among the treatments without the host regardless of the flooding period. A separate series of experiments demonstrated that significant increases in the production of secondary inoculum occurred within 3 days of flooding infested soil containing the host. The greatest rate of increase in secondary inoculum was recorded between days 5 and 7 following flooding. In experiments with zoospore suspensions used as inoculum, no difference in seedling infection was found between flooded or unflooded treatments at any inoculum level tested. Flooding (24 or 48 hr) appears to increase seedling infection and mortality by promoting production and dispersal of inoculum rather than predisposing the host.

Phytophthora cinnamomi Rands is the most important soilborne pathogen of Fraser fir (*Abies fraseri* (Pursh) Poir.) seedlings grown in North Carolina for the Christmas tree market. Phytophthora root rot of Fraser fir may occur either in nursery beds or in plantations; trees are susceptible to *P. cinnamomi* during the entire 12-yr production cycle (8).

Disease development in nursery beds or plantations generally begins in the spring with the death of a few scattered seedlings. As the season progresses, the number of seedlings displaying symptoms of Phytophthora root rot increases until a disease focus develops. The focus will generally expand slowly, encompassing more dead trees each year unless chemical control measures are initiated (3,11). In contrast to this typical pattern of disease development, there are occurrences in late spring of widespread and sudden mortality of seedlings or trees in nursery beds or plantations. This atypical pattern of disease development has been observed in nursery and plantation soils ranging from a loam to a loamy sand following a single flooding period. The results of numerous studies (20) have shown the role of excessive soil water in the development of diseases incited by *P. cinnamomi*; however, in these studies no naturally infested soils were used to examine the role of flooding in disease development and the production of secondary inoculum following flooding was not quantified. Also, the predispositional influence of flooding on disease development with *Phytophthora* spp. has been examined in only a few cases (2,12).

The objectives of this study were to determine the effect of flooding on Fraser fir seedling mortality in infested and uninfested soil and on the production of secondary inoculum of *P. cinnamomi*. Also, we wanted to ascertain whether a single flooding period would predispose the host to infection or primarily affect disease development by stimulating inoculum production and dispersal.

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MATERIALS AND METHODS

Seedling material and soil. All experiments were conducted in the greenhouse with 2-yr-old Fraser fir seedlings obtained from disease-free areas of nursery beds at the N. C. Forest Service, Linville River Nursery, Crossnore, NC. Dormant seedlings were collected in late October. To break bud dormancy, seedlings were heeled into a peat: vermiculite (3:1, v/v) growing medium and placed for 21 days in the greenhouse under a 9 ± 0.5 hr natural photoperiod plus a 3-hr night interruption with incandescent light (9). The ambient air temperatures were 24 ± 5 C during the day and 14 ± 4 C at night. After dormancy was broken, the seedlings were transplanted into plastic containers ($25 \times 15 \times 10$ cm) that had twelve 10-mm-diameter holes in the bottom to allow for drainage. Twenty-five seedlings were randomly selected from each of 500 seedlings transplanted and their entire root systems were disinfested (30 sec in 95% ethyl alcohol followed by a rinse in sterilized distilled water) and plated onto an agar medium selective for Phytophthora (PCH) (18) to assay for *P. cinnamomi*.

Flooding was accomplished by plugging the drainage holes in the containers with rubber stoppers and adding deionized water over a period of 1 hr until the water level was ~5 mm above the soil surface.

All soil (infested and uninfested) used in the various experiments was collected from nursery beds at the Linville River Nursery and was determined by physical analysis (1) to be a loamy sand (72.5% sand, 22.0% silt, and 5.5% clay). Organic matter content was determined to be 0.7%. A soil-drying curve was prepared for the soil (16). Infested soil was diluted with soil from the nursery beds in which no propagules of *P. cinnamomi* could be detected to establish desired inoculum densities prior to placing the soil in the containers.

Initial inoculum densities of *P. cinnamomi* in nursery bed and diluted soils were determined on three 10-g (fresh weight) samples per 2.0 kg of soil. Each sample was placed in a beaker containing 125 ml of deionized water with 5 μg of pimaricin (Delvocid Instant 50%, Enzyme Development Corp., NY 10001) and 10 μg penicillin per milliliter of water. The suspension was stirred for 45 sec and then dispensed as uniformly as possible onto a series of PCH plates (10 plates per 10-g sample). Plates were incubated in the dark at 20 ± 2 C for 48–56 hr, then gently rinsed under running tap water to remove the soil layer. Developing colonies of *P. cinnamomi* growing into the agar were counted microscopically and expressed as propagules per gram of oven-dried soil (inoculum density).

Seedling mortality and inoculum density treatments. Nine treatments were designed to determine the effect of flooding for 0, 24, or 48 hr on seedling mortality and inoculum density with the soil infested or uninfested, and host present or absent. The first set of three treatments consisted of transplanting Fraser fir seedlings into uninfested soil and varying the initial flooding period for 0, 24, or 48 hr. A second set of three treatments involved transplanting seedlings into infested soil and varying the length of the initial flooding period for 0, 24, or 48 hr. The third set of three treatments consisted of flooding infested soil without seedlings for 0, 24, or 48 hr.

Thirty seedlings that had broken bud dormancy were transplanted into each container for those treatments with seedlings. The nine treatments were arranged in a randomized complete block design with each block replicated five times. The entire experiment was repeated once. Seedling mortality was visually assessed daily for 33 days (7). Seedling infection was determined by plating entire disinfested root systems of each seedling in all treatments onto PCH medium 33 days after flooding. Inoculum density was quantified at 2, 9, and 23 days after flooding by plating three 10-g soil samples from each container on PCH medium as previously described.

The effect of flooding on seedling mortality and inoculum density (in infested and uninfested soil) was examined by partitioning the mean squares of each set of treatments into single degree-of-freedom linear contrasts (significance determined by F test). Analysis of variance was used to determine the effect of the presence or absence of the host on inoculum density and the presence or absence of the pathogen on seedling mortality (the mean of each set of three treatments was used).

Zoospore release and seedling infection. Two treatments were designed to examine the liberation of zoospores during the period of flooding. In the first treatment, 30 seedlings that had broken bud dormancy were transplanted into infested soil and then flooded for 24 hr. The second treatment consisted of flooding infested soil for 24 hr, allowing drainage to begin, and then transplanting 30 seedlings that had broken bud dormancy into this infested soil. A third treatment (seedlings transplanted into infested soil with no flooding period) was included to serve as a comparison for seedling infection.

To determine if zoospores were released during the 24-hr flooding period, rhododendron leaf disks (12-mm diameter) were used as baits (18). Fifty disks were floated on the water of each container during the 24-hr flooding period. Disks were surface disinfested with 95% ethyl alcohol for 30 sec and rinsed with deionized water prior to placement in the containers. After the 24-hr flooding period, the disks were rinsed in deionized water, blotted dry, and plated onto PCH medium to detect infection by *P. cinnamomi*.

Seedling infection was assessed by removing all seedlings from each container 9 days after flooding and placing their entire disinfested root systems onto PCH. Percent moisture of the soil in containers was determined at various time intervals following flooding and soil matric potentials calculated from the soil drying curve. The three treatments were replicated five times in randomized complete blocks and the experiment repeated once.

Secondary inoculum production. A time series experiment was designed to determine when the production of secondary inoculum (primarily chlamydospores, C. M. Kenerley, *unpublished*) occurred following infection. Seedlings were transplanted into infested soil and flooded for 24 hr. Following the flooded period, three 10-g soil samples were removed from each of three containers for each sampling day and assayed for *P. cinnamomi* on days 1, 2, 3, 5, 7, and 9 as previously described.

Predisposition. Predisposition of Fraser fir seedlings by flooding was examined by using zoospore suspensions as inoculum. Zoospore suspensions were prepared by placing mycelial disks into a 10% clarified lima bean broth for 3–4 days under continuous fluorescent light at 25 C. Sporangia formation was induced by rinsing the mycelial mats three times with deionized water and incubating them in 2% soil extract solution in the dark for 5–7 days at 25 C. Zoospore release was induced by rinsing sporangia three times with deionized water, incubating for 1 hr at 4 C, and returning to room temperature (~20 C). The experimental design consisted of a 2×4 factorial with two treatments (flooded for 24 hr and not flooded) and four concentrations of zoospores (2.1×10^6, 1.8×10^5, 1.2×10^5, and 1.2×10^4). In the treatment where seedlings had been flooded, the zoospore suspensions were added directly to the water following the 24-hr flooding period. Seedlings that were not flooded were inoculated by bringing the soil to saturation (requiring 1 hr) and then applying the zoospore suspensions. The inoculum was allowed to disperse for 20 min before draining all the containers. Roots were assayed for *P. cinnamomi* 7 days following the flooding period by plating entire disinfested roots on PCH.

TABLE 1. Infection and mortality of Fraser fir seedlings 33 days after flooding of soil either uninfested or infested with propagules of *Phytophthora cinnamomi*

Flooding period (hr)	Uninfested soil		Infested soil	
	Infected (%)	Mortality[a] (%)	Infected (%)	Mortality (%)
0	0	1.3	23	10.0
24	0	2.0	100	84.0
48	0	1.3	100	85.0

[a] *Phytophthora cinnamomi* was not isolated from any seedling that died when transplanted in uninfested soil.

RESULTS

Seedling mortality. Flooding in the absence of *P. cinnamomi* had no observable adverse effects on Fraser fir seedlings. Seedling mortality 33 days after flooding was 2% among treatments without the pathogen compared to 60% in treatments with the pathogen (Table 1). *P. cinnamomi* was not isolated from any of the seedlings that died in the uninfested soil. The root systems of flooded and unflooded seedlings were flexible and exhibited little or no root breakage. Seedlings from the unflooded and flooded treatments in uninfested soil had initiated new short roots and new root tips on the older roots. Flooding did, however, affect the color of the root system. Root systems of the seedlings that were flooded for 24 or 48 hr in uninfested soil were a dark brown whereas the roots of those seedlings in uninfested soil that was not flooded were a light brown. There was no difference in shoot growth among seedlings in uninfested soil from flooded or unflooded containers.

Within the set of flooded treatments there were differences in seedling infection and mortality. Final seedling mortality and infection (Table 1), and rate of seedling mortality were greater ($P =$

0.01) for transplanted seedlings in flooded (24 or 48 hr) infested soil compared to the unflooded treatment. However, there were no differences in final seedling infection, mortality or rate of mortality between the two flooding periods. Symptoms of Phytophthora root rot appeared within 9 days among plants in the two flooded treatments compared to 21 days in the unflooded treatment.

Inoculum density treatments. There were no differences in inoculum density among the two sets of treatments involving infested soil 2 days after flooding. However, by days 9 and 23 inoculum density was greater ($P = 0.01$) in the treatments with the host compared to the set of treatments without the host. Among the three treatments with flooded infested soil but without the host, there were no differences in inoculum density at any sampling date and inoculum density generally declined with time (Fig. 1). Among the three treatments with the host there was an increase ($P = 0.01$) in inoculum density between the flooded (24 or 48 hr) and unflooded treatment at 9 and 23 days (Fig. 2). However, no difference in inoculum density was found at any sampling period between soils flooded for 24 or 48 hr.

Zoospore release and seedling infection. Sixty-three and 62% of the leaf disks floated for 24 hr on the surface of the flooding water were infected with *P. cinnamomi* in treatments with and without the host, respectively. Twenty-two percent of the seedlings transplanted into the infested soil that was flooded for 24 hr prior to transplanting were infected with *P. cinnamomi*. Infection of seedlings transplanted into infested soil was 1.3% in the unflooded treatment and 97.3% in the treatment flooded for 24 hr after transplanting. The soil matric potential was greater than −25 mb for the first 5 hr after drainage began. An additional 21.5 hr of drainage were required before the matric potential was less than −50 mb. Forty-eight hours after drainage had begun, the matric potential was still greater than −100 mb.

Secondary inoculum production. The time sequence experiment demonstrated that a significant increase in inoculum density occurred during the 3 days after flooding (0.73 initially to 1.81 propagules per gram dry soil at 3 days). Inoculum density continued to increase (2.64 propagules per gram dry soil at 5 days) with the greatest increase detected between days 6 and 7 (inoculum density 22.37 at 7 days). In some containers, the greatest increase occurred between days 3 and 5. Seedlings were infected within 24 hr of flooding and infection was detected in 75–90% of the seedlings by the third day after flooding. Seven days after infested soil was flooded for 24 hr, 100% of the seedlings were infected with *P. cinnamomi*.

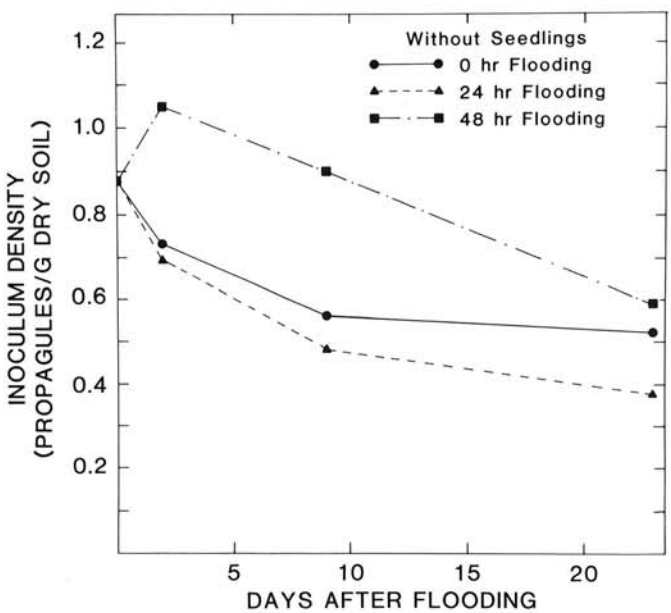

Fig. 1. Effect of flooding on inoculum density in soil infested with propagules of *Phytophthora cinnamomi* and not planted to Fraser fir seedlings.

An examination of the colonies of *P. cinnamomi* on PCH plates from soil samples removed on the seventh day showed that 80% of the colonies originated from chlamydospores. This was also the case for colony origin in the inoculum density experiment for soil samples taken at days 9 and 23 from containers with the host in infested soil that had been flooded for 24 or 48 hr.

Predisposition. Flooding seedlings for a 24-hr period prior to inoculating with zoospore suspensions did not increase the percent of seedlings infected (Table 2). Percent infection ranged 70–90% and no differences in percentage of seedlings infected were found between the flooded and unflooded treatments for any zoospore concentration (Table 2).

DISCUSSION

These results indicate that rapid and extensive mortality of Fraser fir seedlings in soils naturally infested with propagules of *P. cinnamomi* can occur following a single flooding period of 24 or 48 hr. Within 7 days of the flooding of infested soil (initial inoculum density of 0.73 propagules per gram of dry soil), 100% of the seedlings were found to be infected. Examination of root systems removed from containers with infested soil and plated onto PCH 24 or 48 hr after flooding showed multiple infection sites among the root systems. A significant increase in the production of secondary inoculum (primarily chlamydospores) was found as soon as 3 days

TABLE 2. The effect of flooding on infection of Fraser fir seedlings by zoospore suspensions of *Phytophthora cinnamomi*

	Infection (%) at inoculum density[a]:			
Treatment	2×10^6	1.8×10^5	1.2×10^5	1.2×10^4
No flooding	85[b]	85	90	80
Flooding (24 hr)	80	75	80	70

[a] Number of zoospores per container (25 × 15 × 10 cm). Inoculum was applied directly to the standing water in both treatments immediately after the no-flooding container was saturated (required 1 hr) and 20 min before all containers were drained.

[b] Percent seedlings infected determined 7 days after inoculation. There were no significant differences between treatments at any zoospore concentration (Student's *t*-test analysis).

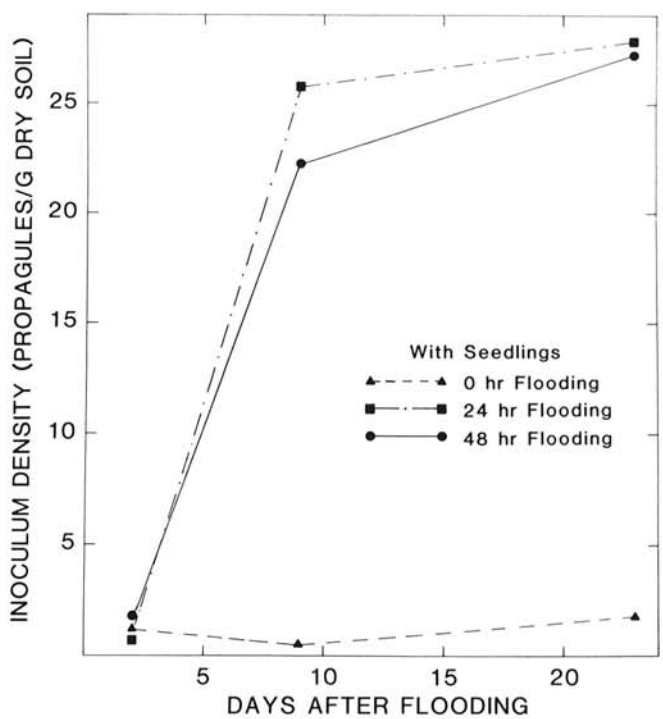

Fig. 2. Effect of flooding on inoculum density in soil infested with propagules of *Phytophthora cinnamomi* and planted to Fraser fir seedlings.

after flooding with the greatest increases between days 5 and 7. These findings are supported by the work of Weste and Cahill (19) in which they observed chlamydospore formation on root surfaces of several native Australian plants 48–72 hr following root-tip inoculations with zoospore suspensions. This rapid increase in the production of secondary inoculum reflects the ability of the pathogen to allocate energy reserves into survival units. Also, these large increases in secondary inoculum can further escalate spread within the nursery following subsequent flooding periods, increase the chances of spread of the pathogen to other nurseries and plantations, and serve as overwintering structures (11).

Further increases in secondary inoculum production did occur after day 7 as determined by repeated soil sampling, but at a much reduced rate. The reduction in rate may be attributed to inability of the fungus to colonize additional root tissue, a depleted food base, or an increase in propagule mortality. A more detailed examination of the extent and location of colonization within the root system than presented here may provide insight into the mechanism of secondary inoculum production by this pathogen. Also, the extent to which the mass and spatial distribution of root systems (as affected by seedling density) may affect secondary inoculum production needs to be examined to provide a better understanding of the population dynamics of this pathogen.

There was a general decline in inoculum density among the three treatments in which infested soil without the host was flooded for 0, 24, or 48 hr. More important, however, is that no significant difference in inoculum density was found among these three treatments at any sampling period following flooding. Baiting with leaf disks during the flooding period confirmed that the germination of some propagules resulted in zoospore liberation. These findings suggest that some zoospores colonized organic matter in the soil, allowing the fungus to survive as mycelium (21) or as newly-formed chlamydospores (C. M. Kenerley, *unpublished*). The report by Hwang and Ko (10) that zoospores of *P. cinnamomi* were unable to survive longer than 3 wk in a moist clay loam (60% water by weight) and our inability to detect any colonies on PCH plates from soil samples taken at 9 or 23 days arising from encysted zoospores supports this possibility. In the absence of a host, the ability of zoospores liberated following a flooding period to colonize organic matter with subsequent chlamydospore formation would enhance the survival capacity of this pathogen.

Flooding apparently increases the development of Phytophthora root rot of Fraser fir seedlings by a direct effect on the pathogen. Inoculation of flooded seedlings with zoospore suspensions at several different concentrations did not increase the infection compared to inoculation of unflooded seedlings. Blaker and MacDonald (2) also found that submerging the root systems of a susceptible rhododendron cultivar for 0, 24, or 48 hr prior to inoculation with zoospore suspension had no distinguishable effect on the subsequent rate of disease development. However, if plants of the resistant cultivar were flooded for 48 hr prior to inoculation with *P. cinnamomi*, they developed severe symptoms whereas the unflooded controls did not.

Shew (17) reported significant increases in infection by *Phytophthora parasitica* Dast. var. *nicotianae* (Breda de Haan) Tucker of a susceptible tobacco cultivar at varying inoculum densities when the infested soils were saturated. This led to the suggestion that the mechanism of enhancement of infection may be related to the stimulation of zoospore release and dispersal by flooding (17). These reports suggest that the increased disease development among susceptible hosts to Phytophthora root rots following flooding conditions is primarily due to the stimulation of zoospore production by the pathogen. Zoospore liberation was confirmed in this study by leaf baiting and in the tobacco study by direct plating of flooding water (17). It seems likely that sporangia formation occurred during the flooding period as the matric potential (less than −5 bars) of the soil prior to flooding was not favorable for sporangia formation (4,6,14,15). Also, no sporangia were found when colony origin was examined on PCH plates. Soil matric potentials required for the formation of sporangia from chlamydospores in naturally infected soils need to be determined.

Release of zoospores began during the flooding periods, and the soil matric potential would have been conducive to zoospore release (5,12,13) for at least an additional 5 hr during the drainage period. This single flooding event initiated environmental conditions that were conducive to the rapid buildup of zoospore inoculum in the presence of a very susceptible host. These results may explain the sudden and widespread mortality that often occurs in nurseries and plantations following flooding when only a few seedlings initially showed symptoms.

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