Influence of Soil Temperature and Inoculum Density of *Phytophthora cinnamomi* on Root Rot of Fraser Fir

H. D. SHEW, Assistant Professor, and D. M. BENSON, Associate Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27650

ABSTRACT

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 Disease 67:522-524.

Known numbers of *Phytophthora cinnamomi* chlamydospores were added to a sandy loam soil maintained in 10-cm-diameter pots in constant temperature water baths in the greenhouse. Soil temperatures of 14, 16, 19, 22, and 25 C were studied. Inoculum densities of 10, 50, 100, 500, 1,000, and 5,000 chlamydospores per kilogram of soil resulted in 3, 10, 22, 60, 75, and 100% infection of Fraser fir seedlings, respectively, at optimum temperatures for disease development. Optimum temperatures for infection were between 16 and 25 C. An ID_{50} value for infection (inoculum density required for infection of 50% of the total number of plants) of 322 chlamydospores per kilogram of soil was obtained when inoculum density effects were averaged over temperatures of 16–25 C. Optimum temperatures for mortality of Fraser fir seedlings were between 19 and 25 C. Soil temperatures below 19–25 C delayed the onset of foliar symptoms from an average of 16 days at 25 C to 34 days at 16 C. Although significant root infection took place at 12 and 14 C, foliar symptoms were not expressed by infected seedlings when incubated below 16 C. The minimum level of *P. cinnamomi* inoculum in soil required for infection and mortality of Fraser fir seedlings was determined to be fewer than 10 chlamydospores per kilogram of soil.

Fraser fir (*Abies fraseri* (Pursh) Poir.) is the most important species in the Christmas tree industry in North Carolina, and production of the species in nurseries (3–5 yr) and plantations (6–9 yr) is limited in some locations by root rot caused by *Phytophthora cinnamomi* Rands (2). Fraser fir is highly susceptible to Phytophthora root rot from seeding until the tree is cut for the Christmas tree market. Knowledge of the effect of initial inoculum density of *P. cinnamomi* in soil on infection and mortality of Fraser fir is required to understand the epidemiology of Phytophthora root rot under nursery conditions and to identify low-hazard planting sites for Fraser fir plantations.

Chlamydospores of *P. cinnamomi* serve both as a primary survival structure and as a primary form of inoculum in soil (6,12,15,17). Eighty-seven percent of the *P. cinnamomi* propagules recovered in assays of soil obtained from a Fraser fir nursery and an azalea bed were chlamydospores free of organic matter (12). Similarly, Hwang and Ko reported that an average of 71 and 84% of the *P. cinnamomi* propagules from two ohia and two avocado soils, respectively, were chlamydospores (6). Hence, the use of chlamydospores in inoculum density-infection tests reflects the most common propagule of *P. cinnamomi* in soil.

The influence of inoculum density of *P. cinnamomi* on disease incidence and severity has been investigated for several hosts, but quantitative information on inoculum density effects is generally lacking for this important pathogen. Hendrix and Kuhlman (3) reported inoculum densities of 1–30 propagules per gram of soil from a Fraser fir nursery where 50% of the trees were killed by *P. cinnamomi*, but the relationship of initial inoculum density to infection and death of Fraser fir was not studied. Sterne et al (13,14) reported that an inoculum density of 10 chlamydospores per gram of soil resulted in little infection of avocado (*Persea indica* L.) seedlings but at 15 chlamydospores per gram of soil, up to 100% infection of the root system occurred. An inoculum density of 250 chlamydospores per gram of soil was required for 11% mortality of ohia seedlings (*Metrosideros collina* subsp. *polymorpha*) (6). In contrast with the relatively high initial inoculum densities used in tests, inoculum densities of less than five propagules per gram of soil are commonly encountered for *P. cinnamomi* in natural soil (8,15).

The influence of soil temperature on development of *P. cinnamomi* root rot of various hosts has also been investigated (9,10,15,16). There has been considerable interest in the minimum temperature at which infection occurs, primarily relative to predicting the occurrence and severity of root rot. Results from previous studies indicate that significant root infection occurs at 15 C (15,16) but not below 15 C (9,10). Additional information on the influence of soil temperatures on *P. cinnamomi* root rot of other host plants is needed, especially for host plants grown in cool soils and in relation to possible interactions with inoculum density. The purpose of this study was to quantitatively describe the relationship between inoculum density of *P. cinnamomi* chlamydospores in soil and the infection and mortality of Fraser fir seedlings at controlled soil temperatures.

MATERIALS AND METHODS

An isolate of *P. cinnamomi* (CN-1) obtained from Fraser fir roots was used in all experiments. Cultures were maintained on 5% clarified V-8 juice agar (5) at 25 C. Chlamydospores for infesting soil were produced by growing *P. cinnamomi* cultures in 9-cm-diameter petri plates containing 16–20 ml of 10% clarified lima bean broth (LBB) for 2–4 wk at 25 C in continuous light. LBB was prepared by autoclaving 100 g of frozen baby lima

Journal Series Paper 8201 of the North Carolina Agricultural Research Service, Raleigh 27650.

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

Accepted for publication 30 September 1982.

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.

beans for 15 min in 500 ml of deionized water, clarified by filtering through filter paper and a 3-cm layer of Celite 545 (Fisher Scientific), brought to the volume of 1 L by adding deionized water, and reautoclaved for 25 min. To collect chlamydospores, the cultures were blended at high speed for 1 min in sterile deionized water in a Waring Blendor and the resulting suspension poured through nested sieves to remove mycelial fragments and collect chlamydospores between 38 and 75 μm in diameter. Differential settling of the mycelial fragments and chlamydospores (4) followed by resieving was used to remove mycelial fragments, and the concentration of chlamydospores in the suspension was determined by making spore counts in at least 10 fields of a hemacytometer. The chlamydospore suspension was stored at 6 C until used but never longer than 4 hr after collection.

A sandy loam greenhouse soil (steamed 30 min at 80 C) amended with a coarse builders' sand and peat moss (3:1:1, v/v) was infested with a known number of chlamydospores to give inoculum densities of 10, 50, 100, 500, 1,000, and 5,000 chlamydospores per kilogram of soil. The inoculum was thoroughly mixed into the soil with a twin-shell blender before transplanting Fraser fir seedlings (produced in the greenhouse or obtained from the Linville River Nursery, Crossnore, NC [11]) into the infested soil contained in 10-cm-diameter plastic pots. Pots were maintained at 14, 16, 19, 22, and 25 C in constant-temperature water baths. The pots had a sealed drainage system that allowed any excess water from the daily irrigations of 50–100 ml of water to drain through the soil and out of tanks. Tests were conducted for 60–80 days in a greenhouse with a maximum ambient temperature range of 22–35 C (night-day) temperatures. There were seven seedlings per treatment and the experiment was repeated at least once at every temperature except 14 C. The percentage of the total number of plants infected was determined by carefully removing the seedlings from the soil, thoroughly washing the roots in running tap water, and dipping the roots in 70% alcohol before drying and plating the entire root system of each seedling onto a pimaricin-chloramphenicol-hymexazol (PCH)-selective medium (12). The plates were observed for growth of the pathogen from any part of the root system into the medium. A similar test was conducted at 12 C, except seedlings were transplanted into a naturally infested soil at 15,000 propagules per kilogram of soil. Percent infection was determined as described previously.

The length of time required for infection of seedlings after transplanting into infested soil was determined by incubating seedlings in infested soil at controlled temperatures for a 3-wk period. Seedlings were removed every 2 or 3 days and assayed for infection as described previously.

To further define the effect of soil temperature on development of Fraser fir root rot, 1-yr-old Fraser fir seedlings were transplanted into soil infested at 10,000 chlamydospores per kilogram of soil. The seedlings were incubated in the soil at 25 C for 1 wk, then 10 pots were selected at random and placed at 16, 19, 22, and 25 C (total 40 pots), observed over a 45-day period for symptoms, and assayed for infection as described previously.

Final inoculum density of *P. cinnamomi* in soil from pots where seedlings were killed was determined at the end of each experiment. Soil assays were completed by a wet-sieving-selective medium technique (12).

RESULTS

The effect of soil temperature on Fraser fir root rot was determined by averaging percent infection or mortality over all inoculum densities tested at each temperature (Fig. 1). Optimum temperatures for infection were between 16 and 25 C (no significant difference in infection at 16, 19, 22, and 25 C) ($P = 0.05$) and for mortality, between 19 and 25 C ($P = 0.05$). At 25 C, all seedlings that became infected died within 41 days. At 14 C, a minimum of 500 chlamydospores per kilogram of soil was required to initiate detectable infection. No mortality occurred at 14 C. At 12 C, 33% (four of 12) of the Fraser fir seedlings incubated in the naturally infested soil at 15,000 chlamydospores per kilogram of soil became infected but none expressed symptoms. Lower inoculum densities were not tested at 12 C.

A highly significant effect ($P = 0.01$) of inoculum density of *P. cinnamomi* on infection of Fraser fir was observed as inoculum density increased from 10 to 5,000 chlamydospores per kilogram of soil. At optimum temperatures, infection incidence increased from 3 to 100% and mortality from 0 to 100% with increasing inoculum density (Fig. 2). The ID_{50} values (inoculum density required for 50% infection) were obtained from log-log transformations (1) of inoculum density vs. infection. An ID_{50} value for infection of 322 chlamydospores per kilogram of soil was obtained for soil temperatures between 16 and 25 C in these tests. Seedlings became infected as soon as 2 days after transplanting into infested soil in some tests, but generally at least 10 days were required for 100% of the plants to become infected. There was no difference observed in the number of days required for infection to occur at soil temperatures between 16 and 25 C.

Lowering soil temperature delayed the onset of symptoms. The average time required for mortality of Fraser fir seedlings after placing at soil temperatures of 16, 19, 22, and 25 C was 34, 22, 16, and 16 days, respectively. Only 60% of the seedlings incubated at 16 C were killed after 45 days, whereas 100% of the seedlings at the higher temperatures were killed. All seedlings were infected.

Final inoculum density of *P. cinnamomi* was not correlated with either soil temperature or initial inoculum density. Inoculum densities ranged from 1,400 to 9,200 propagules per kilogram of soil from pots where seedlings were killed.

DISCUSSION

Root rot of Fraser fir developed with an initial inoculum density as low as 10 chlamydospores per kilogram of soil (Fig. 2). At optimum temperatures for disease development, 1,000 chlamydospores per kilogram of soil resulted in 76% infection and 49% mortality. The ID_{50} value obtained for infection of Fraser fir by *P. cinnamomi* chlamydospores was similar to those obtained for chlamydospore inoculum of other *Phytophthora* spp. on other hosts (4,7). The ID_{50} values for other host-pathogen systems ranged from 132 chlamydospores per kilogram of soil for *P. parasitica* var. *nicotianae* on tobacco (4) to 900

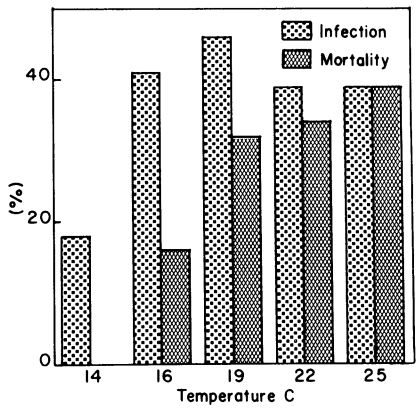

Fig. 1. Influence of soil temperature on infection and mortality of Fraser fir caused by *Phytophthora cinnamomi* when averaged over inoculum densities of 10 to 5,000 chlamydospores per kilogram of soil at each temperature.

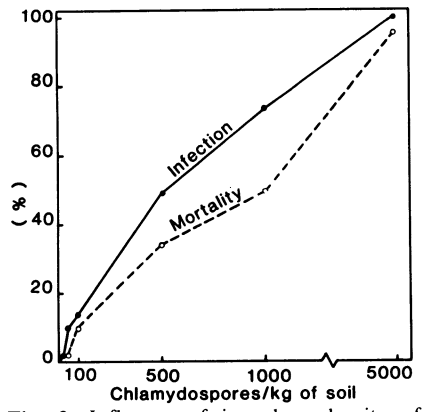

Fig. 2. Influence of inoculum density of *Phytophthora cinnamomi* on infection and mortality of Fraser fir at optimum temperatures of 16–25 C for infection and 19–25 C for mortality.

chlamydospores per kilogram of soil for *P. palmivora* on papaya (7), compared with 322 chlamydospores per kilogram of soil for *P. cinnamomi* on Fraser fir.

Inoculum density of *P. cinnamomi* in naturally infested soil in a Fraser fir nursery ranged from 1,000 to 30,000 propagules per kilogram of soil in an earlier study (3). We have observed similar as well as higher inoculum densities in soil from a Fraser fir nursery where root rot was present, but inoculum densities recovered from soil in Fraser fir plantations were always <5,000 propagules per kilogram of soil and generally <1,000 propagules per kilogram of soil (*unpublished*). Initial inoculum densities of *P. cinnamomi* in a Fraser fir nursery bed were recently reported to be 20 propagules per kilogram of soil (C. M. Kenerley, *personal communication*). Similar inoculum densities, <3,000 propagules per kilogram of soil, were found in soils from avocado groves (15), and in soils from stands of pine, an inoculum density of <1,000 propagules per kilogram of soil was detected (8). The inoculum densities used in our experiments thus appear to be in line with those of *P. cinnamomi* found in naturally infested soils on various hosts and illustrates that even at low initial inoculum densities (eg, 500 chlamydospores per kilogram of soil), a high percentage of Fraser fir seedlings can become infected and die if soil temperatures are favorable (Fig. 2). The minimum inoculum level of *P. cinnamomi* required for infection of Fraser fir lies below 10 propagules per kilogram of soil. Based on our results, recovery of even very low densities of *P. cinnamomi* in soil from a potential plantation site would indicate a disease hazard.

Optimum temperatures for development of *P. cinnamomi* root rot on various hosts are between 19 and 27 C (15). Optimum temperatures of 19–25 C for mortality and 16–25 C for infection were found in our studies of the *P. cinnamomi* root rot of Fraser fir. Temperatures above 25 C were not studied because average soil temperatures seldom exceed 25 C in the mountainous region where Fraser fir is grown. Although infection by *P. cinnamomi* does not occur below 15 C on several hosts (9,10), our results agree with those of Zentmyer (15,16) for avocado. He found substantial infection at 15 C (the lowest temperature tested). In our tests, we detected infection at both 12 and 14 C. Because isolates of *P. cinnamomi* show a wide variation in minimum temperatures for growth and sporulation (15), isolate differences may explain some of the differences reported by researchers in the ability of *P. cinnamomi* to infect at low soil temperatures. Foliar symptoms were not observed on fir seedlings incubated below 16 C in this study even when infected seedlings were incubated for 130 days. This may be due to the good growth of Fraser fir at and below 16 C compared with the relatively slow growth of the pathogen at those temperatures.

As discussed by Kannwischer and Mitchell (4), it is desirable to use raw soil in studies dealing with the epidemiology of root diseases. They found, however, that by allowing recolonization of autoclaved soil by airborne microorganisms for 30 days before infesting the soil, similar inoculum densities were required for infection of tobacco in both autoclaved and raw soil. In this study, soil was heated with aerated steam to 80 C for 30 min, which does not completely sterilize soil. In addition, soil was stored for at least 1 mo in open soil bins in the headhouse before use in these tests. We therefore believe our results are very similar to results that would be observed in Fraser fir nursery soil, which is treated with methyl bromide before seeding or transplanting Fraser fir.

Soil temperature is a limiting factor in the development of *P. cinnamomi* root rot on some hosts (8). In locations where soil moisture is not a limiting factor for infection, such as in a Fraser fir nursery bed, soil temperature may be the critical environmental factor that determines when infections take place and thus may aid in the timing of control measures.

LITERATURE CITED

1. Baker, R. 1971. Analyses involving inoculum density of soilborne plant pathogens in epidemiology. Phytopathology 61:1280-1292.
2. Grand, L. F., and Lapp, N. A. 1974. *Phytophthora cinnamomi* root rot of Fraser fir in North Carolina. Plant Dis. Rep. 58:318-320.
3. Hendrix, F. F., Jr., and Kuhlman, E. G. 1965. Factors affecting direct recovery of *Phytophthora cinnamomi* from soil. Phytopathology 55:1183-1187.
4. Kannwischer, M. E., and Mitchell, D. J. 1981. Relationships of numbers of spores of *Phytophthora parasitica* var. *nicotianae* to infection and mortality of tobacco. Phytopathology 71:69-73.
5. Hoitink, H. A. J., and Schmitthenner, A. F. 1969. Rhododendron wilt caused by *Phytophthora cinnamomi*. Phytopathology 59:708-709.
6. Hwang, S. C., and Ko, W. H. 1978. Biology of chlamydospores, sporangia, and zoospores of *Phytophthora cinnamomi* in soil. Phytopathology 68:726-731.
7. Mitchell, D. J. 1978. Relationships of inoculum levels of several soilborne species of *Phytophthora* and *Pythium* to infection of several hosts. Phytopathology 68:1754-1759.
8. Otrosina, W. J., and Marx, D. H. 1975. Populations of *Phytophthora cinnamomi* and *Pythium* spp. under shortleaf and loblolly pines in little leaf disease sites. Phytopathology 65:1224-1229.
9. Roth, L. F., and Kuhlman, E. G. 1966. *Phytophthora cinnamomi*, an unlikely threat to Douglas-fir forestry. For. Sci. 12:147-159.
10. 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, West Australia. 83 pp.
11. Shew, H. D., and Benson, D. M. 1981. Fraser fir root rot induced by *Phytophthora citricola*. Plant Dis. 65:688-689.
12. Shew, H. D., and Benson, D. M. 1982. Qualitative and quantitative soil assays for *Phytophthora cinnamomi*. Phytopathology 72:1029-1032.
13. Sterne, R. E., Zentmyer, G. A., and Kaufmann, M. R. 1977. The effect of matric and osmotic potential of soil on *Phytophthora* root disease of *Persea indica*. Phytopathology 67:1491-1494.
14. Sterne, R. E., Zentmyer, G. A., and Kaufmann, M. R. 1977. The influence of matric potential, soil texture, and soil amendment on root disease caused by *Phytophthora cinnamomi*. Phytopathology 67:1495-1500.
15. Zentmyer, G. A. 1980. *Phytophthora cinnamomi* and the Diseases it Causes. Monogr. 10. American Phytopathological Society, St. Paul, MN. 96 pp.
16. Zentmyer, G. A. 1981. The effect of temperature on growth and pathogenesis of *Phytophthora cinnamomi* and growth of its avocado host. Phytopathology 71:925-928.
17. Zentymyer, G. A., and Mircetich, S. M. 1966. Saprophytism and persistence in soil by *Phytophthora cinnamomi*. Phytopathology 56:710-712.