

Soil Temperature and Rate of Colonization of *Ceratocystis wageneri* in Douglas-Fir

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## ABSTRACT

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The effect of soil temperature on infection success and rate of colonization of *Ceratocystis wageneri* in Douglas-fir (*Pseudotsuga menziesii*) seedling roots was studied in growth chambers, greenhouse, and field. In growth chambers at 10 and 17 C, 92 and 97% of the seedlings became infected; at 28 C, only 19% of the seedlings were infected. Vertical growth rate of *C. wageneri* in seedling xylem was two to three times faster at 17 than at 10 C; growth rate at 28 C was intermediate. Growth rates varied predictably with soil temperature fluctuations in the greenhouse. Increases in the proportion of days when soil temperatures were 15–18 C produced

increases in fungal growth rate in xylem; conversely, increases in the proportion of days when soil temperatures were > 18 C or < 15 C depressed growth rate. Soil temperatures > 15 C generally favored faster growth of *C. wageneri* in xylem. Growth rate of *C. wageneri* in roots of 20-yr-old trees averaged 2.2 m/yr, with a maximum of 3.6 m/yr; an average of eight successive annual rings were colonized within 3 mo. Results from these experiments indicate that fungal growth rate in roots is sufficient to explain observed radial spread of the disease in infection centers.

*Additional key words:* black-stain root disease, *Leptographium*, *Verticicladiella wageneri*.

*Ceratocystis wageneri* Kendrick (anamorph: *Verticicladiella wageneri* Goheen et Cobb), causal agent of black-stain root disease, is a vascular wilt pathogen of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), ponderosa pine (*Pinus ponderosa*), and several other species in the Pinaceae of western North America (2,4,8,16,17,19,20). In ponderosa pine, most new infections are initiated in small roots ( $\leq 5$  mm in diameter) growing within 15 cm of diseased roots (4). Root infections in Douglas-fir seedlings are initiated through wounds and natural openings to exposed xylem; living bark and cambial tissues are never directly penetrated by hyphae (14,15).

In each conifer host, mycelia of *C. wageneri* are confined to mature sapwood xylem tracheids (15–20), and radial colonization in ray tracheids is limited (15,17,18,20). Colonization is widespread in root systems of symptomatic trees; the root collar and many to most major roots are colonized to some degree before overall tree decline. Tree mortality results from extensive vascular tissue colonization of the lower stem and root system (13,14) that limits water uptake and impedes translocation of xylem sap to transpiring foliage (14).

Infected trees are attacked by various root- and stem-breeding bark beetles (Coleoptera: Scolytidae) and weevils (Coleoptera: Curculionidae) that contribute to rapid tree decline; root-feeding insects have been implicated as vectors of the disease (4–6,10,12,22–24).

Intertree spread of the pathogen can occur without insects, such as through root grafts and between small roots of healthy and diseased trees that are in intimate contact or proximity to each other (4,14,17). Healthy seedlings planted in the same pot with inoculated seedlings regularly become infected whether intertree root contact is allowed or is completely restricted (14).

Wagner and Mielke (20) reported that pinyon pine isolates of *C. wageneri* had an optimum temperature of 15 C for in vitro growth; others (4,18) have confirmed this observation, reporting an optimum range of 15–18 C for in vitro growth.

Several researchers have evaluated the relationship of edaphic factors to infection and colonization of Douglas-fir and ponderosa pine by *C. wageneri* (7,11,18,21). Goheen et al (7) examined the relationship of soil moisture to infection and colonization of ponderosa pine by *C. wageneri*. In greenhouse and lathhouse studies, mean values for vertical colonization of pine seedlings under different soil moisture treatments were 0.5–1.1 mm/day; high soil moisture (1/3 bar tension) favored seedling infection. Wilks et al (21) examined the relationship of redox potential and soil moisture to infection and colonization of ponderosa pine seedlings. Mean vertical colonization of pine seedlings placed in soils of varying redox potential was 1.2–2.7 mm/day. Intermediate redox and aeration conditions (300–700 mV potential) favored seedling infection; maximum vertical colonization occurred under slightly higher redox conditions (550–750 mV). Soil temperature was not a controlled variable in either study, and maximum vertical growth was measured in one direction from the point of inoculation.

Smith (18) was first to evaluate the role of temperature in infection of pine seedlings by *C. wageneri*. Ponderosa pine seedlings were inoculated with a hard pine isolate of *C. wageneri* and incubated for 2 mo at four constant temperatures. At 16 C, 90% of the seedlings were infected, and at 21 C, 30% were infected; no seedlings were infected at either 27 or 32 C. Harrington and Cobb (11) showed host preferences of three morphological variants of *C. wageneri* in inoculations of seedling and mature ponderosa pine and Douglas-fir. In the seedling inoculations, percent infection and extent of vertical colonization were evaluated at the same four soil temperatures studied by Smith (18). Neither Douglas-fir nor ponderosa pine seedlings were infected at 27 or 32 C by the hard pine, pinyon pine, or Douglas-fir variants. For all three variants, percent infection success and maximum vertical extension were greatest in Douglas-fir seedlings incubated at 21 C; infection and growth in pine seedlings was greatest at 16 C. Vertical colonization of pine seedlings by hard pine variants, measured in both directions from the point of inoculation, was 2.2–2.9 mm/day. Vertical colonization of Douglas-fir seedlings by Douglas-fir variants was 1.4–1.5 mm/day. In inoculations of mature trees of both species, vertical colonization of pine roots by hard pine variants was 2.3–6.4 mm/day; vertical colonization of Douglas-fir roots by Douglas-fir variants was 0.3–2.3 mm/day.

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Harrington and Cobb (11) confirmed Smith's results showing that infection success in pine seedlings was greatest at 16 C; however, they reported that percent infection and maximum vertical extension in Douglas-fir were greatest at 21 C. Our preliminary experiments indicated that soil temperatures  $\leq 18$  C favor infection of Douglas-fir and maximum vertical extension in xylem occurs when soil temperatures are within the optimum range for in vitro growth. Preliminary observations further indicated that previous estimates of maximum growth rate under optimum soil temperature conditions were conservative.

We studied the effects of three constant soil temperatures (10, 17, and 28 C) on infection and growth of *C. wagneri* in Douglas-fir; the lowest temperature was chosen to reflect soil temperature conditions in the Pacific Northwest, and the upper, to examine infection and establishment near the lethal limit. Infection success and maximum fungal growth in xylem were compared at each temperature. In vivo growth response to fluctuating soil temperature conditions was measured in greenhouse experiments. Fungal growth measurements from roots of field-inoculated trees gave estimates of in vivo growth rate under typical field conditions.

## MATERIALS AND METHODS

**Inoculation procedures.** Two-year-old seedlings used in greenhouse and growth chamber studies were wounded with a sterile scalpel at a right angle to the root axis, 3–10 cm below the root collar. A small wedge of bark and wood was removed from each seedling. Wound length was about equal to the diameter of the root, and wound width never exceeded 25% of the root circumference. A 1-cm square of *C. wagneri*-colonized potato-dextrose agar (Douglas-fir isolate VW-45) was placed on the wound and wrapped with a piece (3 × 10 cm) of sterile, moistened cheesecloth. The cheesecloth was covered with a piece (5 × 10 cm) of 0.5-mil polyethylene, and the ends were loosely wrapped with twist-ties. The seam and ends of the inoculum bandage were sealed with melted paraffin. Roots of large Douglas-fir trees in the field were inoculated similarly, but wound, inoculum, and bandage size were increased to accommodate the larger roots.

**Growth chamber studies.** Inoculated seedlings were placed in constant-temperature growth chambers set at 10, 17, or 28 C to maintain soil temperatures at their respective levels. Seedlings were individually transplanted with a cheesecloth wick (14) to plastic seedling transplant tubes (7 cm in diameter × 25 cm long) in a pasteurized potting medium (U.C. mix) composed of equal volumes of washed silica sand (E1-20) and peat (1). Potted seedlings were randomly sorted into three blocks of 36 seedlings each (108 inoculated seedlings); one block was placed in each of the three

growth chambers. A completely randomized block design was used in the study. Seedlings were wick-watered from a reservoir of 20% Hoagland's solution. Air temperature was continuously recorded, and soil temperatures were periodically monitored in each chamber. Lighting conditions were the same for the three growth chambers; light and dark periods alternated at 12-hr intervals.

At each sampling time, six randomly selected seedlings from each growth chamber, as well as other seedlings that were showing signs of wilting, were destructively sampled. Sampling was done 28, 43, 49, 57, 64, and 72 days after inoculation for the treatment at 10 C and 26, 41, 47, 55, and 62 days after inoculation for the treatments at 17 and 28 C. Sampling times were staggered between treatments to allow immediate dissection and examination after sampling. Six seedling samplings were done for the treatment at 10 C because the onset of tree mortality was slower; five samplings were done for the treatments at 17 and 28 C because the onset of tree mortality was more rapid. Seedling root systems were washed and decorticated, and maximum extension of the fungus was measured vertically, radially, and circumferentially. Maximum vertical extension was measured with a clear metric ruler; maximum radial and circumferential development were measured with prefabricated templates (14). Mean growth rates were computed for each sampling. Differences in percent infection and growth rates were compared using a *t* test for unpaired observations. Duncan's new multiple range test was used to compare treatment means.

**Greenhouse studies.** Growth rate of *C. wagneri* in seedling xylem was evaluated in the greenhouse, where soil temperature was allowed to fluctuate diurnally and seasonally. The greenhouse study, first conducted in 1981, was repeated in 1982. In 1981, 220 Douglas-fir seedlings were inoculated and transplanted to 2.5-L plastic planting containers in a pasteurized clay-loam forest soil. A randomized block design was used. Soil temperature in pots was measured continuously by soil thermograph. Seedlings were sampled at weekly intervals beginning 2 days and ending 151 days after inoculation. Ten seedlings were randomly selected at each sampling, as well as any other seedlings showing advanced stages of wilting. Preliminary evidence indicated that fungal growth rate declined rapidly with overall tree decline. Fungal growth was determined as for the growth chamber study. To test the hypothesis that soil temperatures higher and lower than the in vitro growth optimum range would depress the growth rate of *C. wagneri* in xylem, daily soil temperatures were partitioned as follows: proportions of the day when soil temperatures were < 15 C, 15–18 C, and > 18 C. Growth rates for each sample period were compared with the daily soil temperature profile for the same period.

In 1982, 100 seedlings were inoculated, transplanted, and

TABLE 1. Percent infection and growth rate of *Ceratocystis wagneri* in inoculated Douglas-fir seedlings at three soil temperatures

Sample (days after inoculation)	Temperature (C)	Seedlings sampled (no.)	Percent infected	Mean fungal growth rate (mm/day)		
				Vertical	Radial	Circumferential
28	10	6	83	0.9 a <sup>z</sup>	0.02 a	0.1 a
	17	6	83	2.1 b	0.02 a	0.1 a
	28	6	0	...	...	...
41–43	10	6	83	0.8 a	0.01 a	0.1 a
	17	9	100	3.1 b	0.03 a	0.3 b
	28	6	67	2.0 b	0.02 a	0.3 b
47–49	10	6	83	1.1 a	0.02 a	0.1 a
	17	7	100	3.5 b	0.04 b	0.3 b
	28	6	50	1.9 a	0.01 a	0.1 a
55–57	10	6	100	1.9 a	0.03 a	0.2 a
	17	9	100	4.6 b	0.04 b	0.3 b
	28	6	0	...	...	...
62–64	10	6	100	2.2 a	0.02 a	0.2 a
	17	5	100	4.3 b	0.03 a	0.3 b
	28	6	0	...	...	...
70–72	10	6	100	1.7	0.02	0.1
	17	...	...	...	...	...
	28	6	0	...	...	...

<sup>z</sup> Mean growth rates within sample followed by the same letter are not significantly different (*P* = 0.05). Means compared using the LSD multiple comparison procedure.

measured as in 1981. Soil temperature was measured continuously by soil thermograph, and seedlings were randomly sampled 24, 38, 48, 54, 65, 75, and 82 days after inoculation. Twelve seedlings were selected at each sampling, as well as other seedlings showing advanced wilting symptoms.

**Field study.** Roots of 20-yr-old Douglas-fir trees were wound-inoculated with the same isolate of *C. wagneri* used in the other studies. A total of 101 roots on 50 trees were inoculated at a soil depth of 20–40 cm and slope distance from the bole of 1–2 m. The test plantation was at Rock Creek in the Siuslaw National Forest near Corvallis, OR, at an elevation of 440 m. The stand was fully stocked on a southeastern exposure, and all inoculated roots were shaded by the fully closed canopy. Roots were inoculated in May 1982 and removed in August 1982 and February 1983. Soil temperature was measured continuously on the site by soil thermograph at a depth of 30 cm. Fungal growth was measured in infected roots at both sample times, and growth rates were computed. Differences in growth rates between sample times were compared using the *t* test for unpaired observations.

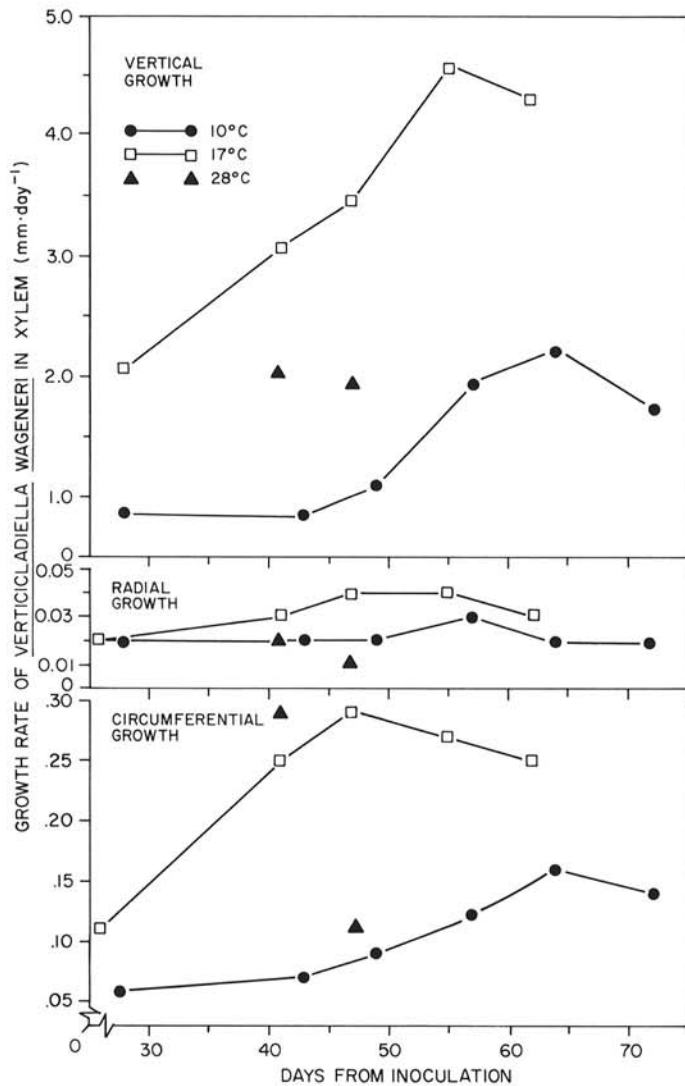
## RESULTS

**Growth chamber studies.** Cool soil temperatures favored seedling infection. Percent infection for all samples combined was similar at 10 and 17 C (92 and 97%, respectively) but was significantly ( $P = 0.05$ ) less at 28 C (19%) (analysis by *t* test). Time

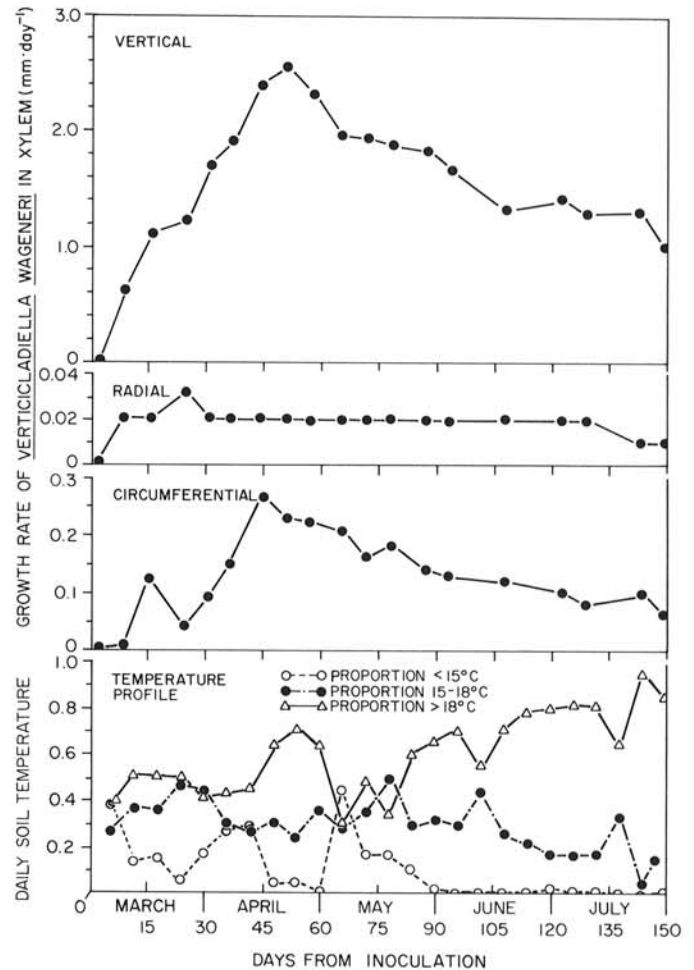
to establishment for all samples combined was similar at 10 and 17 C and greater at 28 C (Table 1). Vertical growth rate of *C. wagneri* in seedling xylem was two to three times more rapid at 17 than at 10 C; differences in growth rate were significant ( $P = 0.05$ ) for each sample period (Table 1). Circumferential growth rate was 1.5–3 times faster at 17 than at 10 C, and differences were significant for most sample periods. Radial growth rates at 17 C were greater than or equal to those observed at 10 C; in two instances, rate differences were significant. Growth rate at 28 C was intermediate to that observed at 10 and 17 C; lack of infection made it impossible to estimate growth rate in later samples. Peak growth rates were observed at 64 days at 10 C, 47–55 days at 17 C, and 41 days at 28 C (Fig. 1). All infected seedlings in the treatment at 28 C were sampled 47 days after inoculation.

**Greenhouse studies.** In 1981, soil temperatures in the greenhouse were frequently  $> 18$  C. The coolest soil temperatures occurred in the first month after inoculation (Fig. 2). In the second month, soil temperatures increased drastically. Vertical growth rate of *C. wagneri* peaked 51 days after inoculation at 2.5 mm/day and declined steadily with higher soil temperatures. Vertical and circumferential growth rates responded predictably to fluctuations in soil temperatures. Increases in the proportion of days when soil temperatures were 15–18 C produced increases in growth rate; increases in the proportion of days  $> 18$  C or  $< 15$  C depressed growth rate (Fig. 2). Radial growth was slow and fluctuations in growth rate were imperceptible with changes in soil temperature.

In 1982, greenhouse soil temperatures were generally  $< 18$  C. Percent infection of seedlings and time to establishment were similar to that observed in the greenhouse in 1981. Vertical growth rate of *C. wagneri* peaked 65 days after inoculation at 1.9 mm/day



**Fig. 1.** Vertical, radial, and circumferential growth of *Ceratocystis wagneri* in seedling Douglas-fir xylem at three constant soil temperatures. Data points are periodic sample means.



**Fig. 2.** Relationship of growth rate of *Ceratocystis wagneri* in seedling Douglas-fir xylem to soil temperature fluctuations in the greenhouse (1981). Soil temperature data points are 6-day periodic means.

(Fig. 3). The delay of 10–13 days in time to peak growth rate appeared to be related to the drastic cooling of soil temperatures 42–54 days after inoculation. Vertical growth rate responded to temperature fluctuations higher and lower than the growth

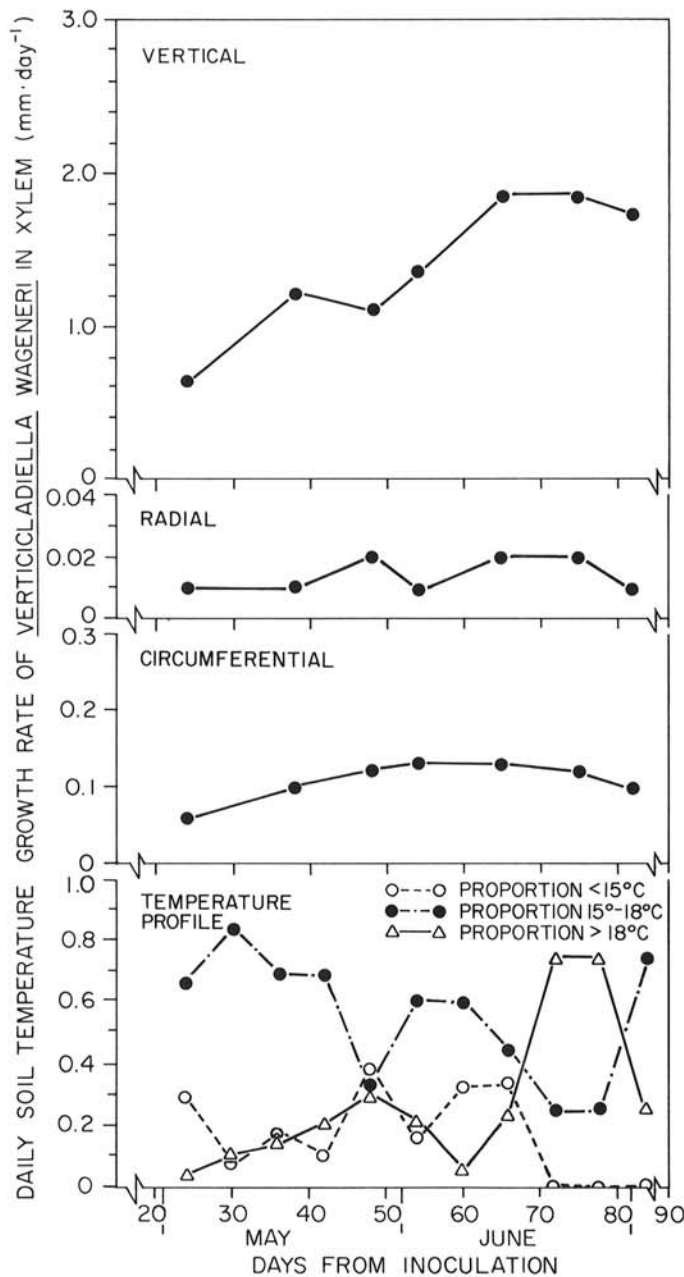


Fig. 3. Relationship of growth rate of *Ceratocystis wageneri* in seedling Douglas-fir xylem to soil temperature fluctuations in the greenhouse (1982). Soil temperature data points are 6-day periodic means.

optimum range; circumferential and radial growth rates were slow and unresponsive by comparison.

**Field study.** Soil temperatures were cool from May 1982 to May 1983 at the Rock Creek field plot (Fig. 4). Except for 20 days in late August and early September, soil temperatures were continuously  $< 15^{\circ}\text{C}$ ; during 7.5 mo, temperatures were  $< 10^{\circ}\text{C}$ . The highest soil temperature ( $16.5^{\circ}\text{C}$ ) was recorded in late August, and the lowest ( $2.5^{\circ}\text{C}$ ) was recorded in early December. Of the 101 roots inoculated, 25% (25 roots) were infected at the time of sampling. Resinosis and resin-soaked xylem were apparent around inoculation wounds of all 76 unsuccessfully inoculated roots; in one root, infection had occurred and 46 mm of vertical xylem was colonized after 270 days. Host resin response had apparently curtailed further spread of the fungus early in infection and it could not be reisolated; identity of the fungus was confirmed histologically. *C. wageneri* was reisolated and identified from each of the other 25 infected roots.

Low establishment success allowed only two sampling times: once in August during the period of highest soil temperatures and once in February during the lowest soil temperatures. Of the roots sampled 95 days after inoculation, 17% were infected and average vertical growth rate was nearly 6 mm/day (Table 2). In roots sampled 270 days after inoculation, 26% were infected and average vertical growth rate was 4.3 mm/day; the difference was not statistically significant. The threefold decrease in radial growth between the two samples was significant ( $P = 0.05$ , analysis by *t* test) (Table 2). Circumferential growth rate likewise decreased, but differences were not significant. An average of eight annual rings were colonized at either sample time (Table 2).

## DISCUSSION

In the greenhouse and growth chambers, soil temperatures  $\leq 18^{\circ}\text{C}$  favored infection by *C. wageneri*; temperatures  $> 18^{\circ}\text{C}$  decreased the likelihood of infection. Results from the growth chamber experiments indicated that the optimum temperature for in vivo growth was in the same range as that reported for in vitro growth.

Greenhouse soil temperatures in 1981 were most often higher than the growth optimum range. Soil temperatures in the 1982

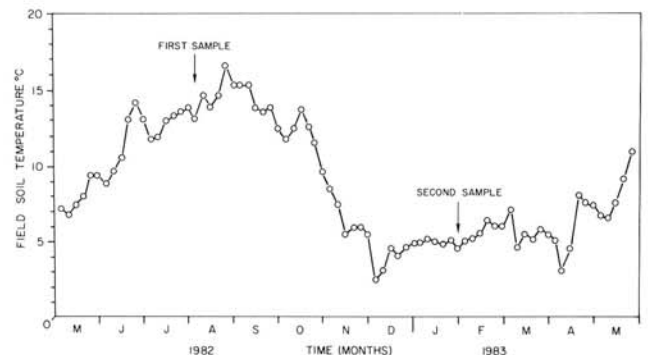


Fig. 4. Soil temperatures at the Rock Creek field plot from May 1982 to May 1983. Soil temperature data points are 5-day periodic means.

TABLE 2. Growth of *Ceratocystis wageneri* in roots of inoculated 20-yr-old Douglas-fir trees in the Rock Creek field plot

Root sample	Days after inoculation <sup>w</sup>	Number of roots sampled	Percent infected	Growth rate of <i>C. wageneri</i> in xylem <sup>x</sup>													
				Vertical		Radial		Circumferential		Annual rings colonized							
				mm/day	m/yr	mm/day	cm/yr	mm/day	cm/yr								
August 1982	95	23	17	5.9 a <sup>y</sup>	6.6	2.2	2.4	0.06 a	0.09	2.2	3.3	0.15 a	0.26	5.5	9.5	8.3 a	10
February 1983	270	78	26	4.3 a	10.0	1.6	3.7	0.02 b <sup>z</sup>	0.05	0.7	1.8	1.10 a	0.36	3.7	13.1	7.5 a	15

<sup>w</sup>Roots inoculated May 1982.

<sup>x</sup>Maximum extension in xylem measured in one direction from the point of inoculation.

<sup>y</sup>Means followed by the same letter are not significantly different ( $P = 0.05$ ), analysis by *t* test for unpaired observations.

<sup>z</sup>Difference in radial growth rate means between sampling is perhaps an artifact of a limit on the amount of possible radial penetration.

study were mostly within or lower than the optimum range. Vertical and circumferential growth rates in 1981 were greater than those observed in 1982. Temperature effects on radial growth rate were imperceptible in either study. Temperatures  $\leq 18$  C appear to favor infection, and temperatures  $\geq 15$  C appear to favor faster growth in xylem.

Peak vertical growth rate of *C. wagneri* in seedling xylem at a constant soil temperature of 17 C averaged 4.6 mm/day with a maximum of 7.2 mm/day. In the field, peak vertical growth rate in roots of 20-yr-old trees averaged 5.9 mm/day or 2.2 m/yr, with a maximum of 1 cm/day or 3.6 m/yr. Because soil temperatures in the field plot were lower than the growth optimum range for all but 20 days of the study year, growth rates in the field under optimum conditions might be greater than any that we observed.

Soil moisture also appears to be very important in the dynamics of infection and colonization (5,21); however, separating the effects of soil moisture and soil temperature is difficult, because the two are often inversely related in nature. Summer rains are infrequent during periods of near optimum soil temperature in the Pacific Northwest. In winter, soil moisture conditions may approach field capacity, yet soil temperatures are cool. Such conditions may be optimal for root infection by *C. wagneri* and suboptimal for growth. Concurrence of optimum conditions of both edaphic factors may be infrequent throughout the northern distribution of this disease.

In field-inoculated roots, an average of eight annual rings (beginning with the current year's xylem) were colonized after 95 days; after 270 days, there was no significant increase in the number of annual rings colonized. Although radial growth rate of *C. wagneri* in xylem is considerably slower than vertical growth rate, radial colonization of successive annual rings occurred early in root infections. Therefore, the number of years of infection cannot be estimated by the number of successive annual rings colonized in roots or stems of infected trees.

Hansen et al (9) and E. M. Hansen and D. J. Goheen (*unpublished*) monitored disease increase in natural infection centers of *C. wagneri* in 28 Douglas-fir plantations in Oregon and Washington over a 5-yr period and found that infection centers enlarged radially at an average rate of 1.5 m/yr. Cobb et al (3) determined the rate of spread of *C. wagneri* in ponderosa pine in the central Sierra Nevada from aerial photographs taken at 2-yr intervals during 11–15 yr. Data from 52 infection centers showed that the average rate of radial spread was 1 m/yr, but radial spread rates varied from 0 to 7 m/yr. Results of these two field studies are comparable with the results obtained in our field study in 20-yr-old Douglas-fir trees; growth rates were 1.6–2.2 m/yr. Although insects are undoubtedly involved in some intertree spread of the disease, the observed rates of infection center enlargement can be explained in terms of fungal growth rates in roots.

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