Relation of Soil Redox Potential to Infection of Ponderosa Pine by *Ceratocystis wageneri*

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ABSTRACT

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Influences of soil aeration and associated properties, as reflected by soil redox potential, on infection and colonization of inoculated ponderosa pine seedlings by *Ceratocystis wageneri* were studied under controlled conditions in the greenhouse. Two field soils with markedly different structures yielded differing infection percentages under established moisture regimes. When the two soils were mixed in varying proportions, a range of soil aeration and redox potential conditions was produced under a single moisture regime. Disease severity was greatest under conditions of restricted (intermediate redox potentials) but not completely impeded aeration (very low redox potentials). In the more strongly reduced soils, colonization by the fungus down the taproots of infected seedlings generally extended to the depth at which calculated gas-phase oxygen levels were continuously at or near zero. Implications for spread of field disease centers are discussed.

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

Ceratocystis wageneri Goheen et Cobb (*Verticicladiella wageneri* Kendrick), the causal agent of black-stain root disease of ponderosa pine (*Pinus ponderosa* Laws.) and other conifers (4,21,24), usually occurs in disease foci or centers originating from single, infected trees. In adjacent, healthy trees of ponderosa pine, the fungus often infects small (5 mm in diameter) rootlets within 15 cm of infected roots and 20–50 cm from the soil surface (8). Although transmission sometimes occurs via direct root contact, fungus hyphae can grow through soil for distances of 15 cm or more from infected roots (12,13); this could be the major means of dispersal from diseased to healthy roots. Therefore, edaphic factors may have a direct effect upon the fungus as well as upon host susceptibility.

Severe tree mortality due to black-stain root disease in southern California occurs in years with heavy, well-distributed summer rains (24). In Colorado, the disease occurred most frequently on trees in cooler, wetter sites (15). Similarly, in the central Sierra Nevada, most of the largest infection centers occur in cool, moist, low-lying areas, and the spread of the pathogen appears to be more rapid in gullies and small creek drainages. We have also noted that high levels of buried organic matter and soluble and exchangeable soil manganese are associated with disease centers in the central Sierra Nevada (*unpublished*). High levels of soil moisture and oxidizable organic matter lower soil redox potential by impeding aeration and by donating electrons, respectively. One general

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consequence of this is an increase in the more soluble, reduced forms of transition metal elements such as iron and manganese (3).

Results of our greenhouse and laboratory studies tend to corroborate these field observations. Relatively high levels of soil moisture greatly enhance infection of inoculated pine seedlings (9), and infection decreased at temperatures greater than 16 C (21). Optimum temperature for growth of *C. wageneri* cultures was reported to be 15–16 C (21,24), although researchers in this laboratory have found that maximum growth of most isolates occurs around 18 C. Increases of 50 and 20% in linear growth rates are obtained in culture media containing 100 ppm and 10^3 ppm Mn^{++}, respectively (12).

Thus, it was hypothesized that soil redox potential should be a useful integrative indicator of soil conditions conducive to growth, spread, and infection by the pathogen. Accordingly, the present study was undertaken to determine the effect of soil moisture and aeration levels, as reflected by soil redox potential, on infection and colonization of inoculated ponderosa pine seedlings.

MATERIALS AND METHODS

The A12 horizons of two soils (Musick and Horseshoe) common to the mixed conifer forests of westside, central Sierra Nevada were collected for the experiments. The Musick series soil was collected from a large, active *C. wageneri* infection center at an altitude of 1,320 m. The A12 horizon occurred at a depth interval of 10–40 cm. The Horseshoe series soil was collected from a disease-free area several kilometers away from the Musick site at an elevation of 1,250 m. The A12 horizon at the Horseshoe site occurred at 5–15 cm. In the central Sierra Nevada, the disease has not been observed in ponderosa pines growing either in Horseshoe soil or below 1,300 m elevation.

Each soil material was passed through a 6-mm sieve to remove large gravel and root fragments, and then thoroughly mixed. After this treatment, the Musick soil was comparatively well-aggregated and contained an appreciable volume (~25%) of pores having an effective radius larger than 0.015 mm ("noncapillary pores"). In contrast, the Horseshoe soil was poorly aggregated and exhibited few (~5%) pores in this size class (Fig. 1). The texture of both soils was classed as a loam, and the pH (saturation paste) of each was 5.9.

Experiment I. Forty-two 11.3-L (3-gal) plastic pots were filled with 8.0 kg (oven-dry basis) of each soil. Five 1-yr-old ponderosa pine seedlings were planted in each of the 84 pots, and seven moisture regimes (six pots per regime) were imposed on each of the two soils. Seedlings in four pots of each treatment were inoculated, and those in the remaining two served as uninoculated controls. Individual pots were assigned to greenhouse bench positions according to a table of random numbers.

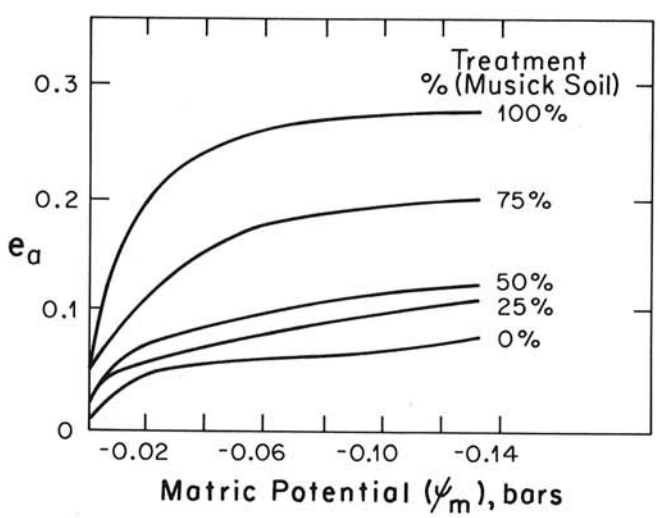

Fig. 1. Moisture characteristic (desorption) curves for two experimental soils (Horseshoe and Musick) and known mixtures of them in terms of proportional volumetric air-filled porosity (e_a). Unmixed Horseshoe soil (with no Musick soil) is represented by curve "0" and unmixed Musick soil by curve "100."

Seven moisture regimes, ranging from very dry to fully saturated, were imposed as follows:

A. Pots were watered thoroughly (ie, saturated) with tapwater at the time of transplanting and allowed to drain and dry until the soil reached a matric potential of −15 bars, as determined gravimetrically. Water contents at −15 bars were determined with a ceramic plate pressure extractor (2) to be 13.3 and 21.0% for the Horseshoe and Musick soils, respectively. The soil was then allowed to dry for an additional 10 days before the seedlings were inoculated. After drying for an additional 15 days (ie, 25 days after reaching −15 bars), the soil was thoroughly watered. During the remainder of the experiment, the soil was thoroughly watered whenever it reached a matric potential of −15 bars.

B. Pots were treated as in regime A except that the soils were thoroughly watered immediately following inoculation. Subsequently, the soil was thoroughly watered each time moisture reached −15 bars for the remainder of the experiment. The initial drying cycle of these first two treatments governed the timing of inoculation for the entire experiment.

C. Pots were watered daily until inoculation. After inoculation, they were watered whenever the soil dried to −15 bars, as with regimes A and B.

D. Pots were watered daily throughout the experiment.

E. Prior to inoculation, the soil was kept saturated by blocking drainage holes and applying sufficient water to maintain a free-water surface at or near the soil surface. Following inoculation, the drainage holes were opened, and the soil was subsequently watered daily.

F. Pots were watered daily until inoculation. After inoculation, the soil was kept saturated, as described for regime E.

G. Soil was kept saturated from the time of transplanting until the end of the experiment.

Soil redox potential was monitored in preselected pots by means of bright, 0.56-mm-diameter (24-gauge) platinum wire electrodes (25) permanently implanted at a depth of 5 cm, a saturated calomel reference electrode and a millivoltmeter (model 126A; Photovolt Corp., Indianapolis, IN 46268). One electrode was implanted in each of two pots treated under regimes A, B, and C, and in each of three pots treated under regimes D, E, F, and G. When readings were made, the reference electrode was carefully inserted into the top of the soil in an area away from the immediate vicinity of the seedlings to a depth sufficient to support it and complete the circuit. Measurements were made at 3- to 7-day intervals at the beginning of the experiment, and at 14-day intervals after the readings had stabilized.

Concentrations of soluble and exchangeable manganese at the depth of inoculation were determined by analyzing triplicate samples taken at the time of inoculation. Ammonium-acetate extraction (1.0 N, pH 7.0) was performed several days after sampling was done according to standard methods (2), and determinations were made by atomic absorption spectrophotometry.

Soil temperatures were measured in each of 13 randomized pots at the time of redox measurements with permanently implanted (5 cm depth) thermistor probes and a calibrated ohmmeter (model YSI 42 SC; Yellow Springs Instrument Co., Yellow Springs, OH 45387). Ambient air temperature was recorded continuously with a hygrothermograph (Henry Green model 621; Weather West, Oakland, CA 94610).

Inoculum blocks were prepared from freshly cut segments (3 cm long × 0.5–1.0 cm in diameter) of ponderosa pine branches according to the procedure of Goheen et al (9). Sterile blocks to be used for uninoculated controls, as well as those inoculated with *C. wageneri*, were incubated for 10 wk at 18 C. The fungus isolate had been obtained 3 mo earlier from a dying ponderosa pine in the central Sierra Nevada.

Inoculations were performed approximately 45 days after transplanting the seedlings by placing half of a longitudinally split inoculum block vertically against the taproot of each seedling at a

depth interval of 2–5 cm. Care was taken to avoid wounding the roots. Seedlings were harvested 87–92 days after inoculation. The bark of each was removed to observe presence of infection and measure extent of colonization in the xylem. Presence of the fungus was confirmed by isolation from stained tissue.

Independence of count data for numbers of infected seedlings was measured by chi-square analysis with continuity correction (16). Treatment means for redox, manganese, and soil temperature data were tested using analysis of variance and Duncan's multiple range test. Least significant differences for redox data were calculated from the equation, $LSD = t(s_1^2/n_1 + s_2^2/n_2)^{0.5}$ because of unequal sample sizes (16). Manganese data showed significant heterogeneity and were first transformed logarithmically as required by Bartlett's test for homogeneity of variances (22).

Experiment II. The same soil materials used in experiment I were mixed dry in volumetric ratios of 4:0, 3:1, 2:2, 1:3, and 0:4, in a large V-blender. These mixtures were identified according to the percentage of Musick (more strongly aggregated) soil incorporated (ie, 100, 75, 50, 25, and 0%, respectively). This was done to obtain a wide range of pore-size distributions among treatments that, when subjected to the same moisture regime, would yield a correspondingly wide range of gas-phase oxygen concentrations (14). A correspondingly wide range of redox potentials was expected to follow since oxygen is the ultimate electron acceptor in soil systems (3).

Moisture characteristic (desorption) curves for the five soil mixtures (within the range of matric potentials encountered in the experiment) are shown plotted against proportional volumetric air-filled porosity (e_a) in Fig. 1. These were measured on a tension table by equilibration with a hanging water column on samples with bulk densities representative of the soil mixtures. Non-zero values of e_a at zero water potential represent volumetric proportions of entrapped air at water "saturation." Apparent asymptotic values of e_a at potentials more negative than approximately −0.10 bars represent volumes comprised of pores having effective radii larger than 0.015 mm.

Each treatment consisted of eight 11.3-L plastic pots containing 10.0 kg (oven-dry basis) of the prepared soil mixtures. Five 2-yr-old ponderosa pine seedlings were planted in each pot. Seedlings in six of the eight pots of each treatment were inoculated, with those in the other two pots serving as uninoculated controls. After the seedlings were transplanted, pots in all treatments were watered daily with 0.5 L of tapwater for the first 14 days and thereafter with the same volume every other day. Inoculum blocks were prepared as described above using the isolate obtained from a seedling infected in experiment I. Blocks were incubated for 11 wk, and inoculations were performed 46–48 days following transplanting.

Soil redox potentials and temperatures at 2–5 cm depths were measured daily, and ambient air temperature was recorded continuously, as described above. A platinum electrode was implanted in each of three pots containing inoculated seedlings of each of the five treatments. Two electrodes were implanted in each of the remaining three pots of treatments 25, 50, and 75. Electrodes were concentrated in these latter three treatments since it was anticipated that redox potentials of these would fluctuate more widely. Thermistor probes were implanted in 13 randomized pots for the soil temperature measurements.

Estimated depths to zero gas-phase oxygen concentrations were derived from calculated oxygen depth functions (18). This method has been shown to yield data comparable to directly measured values (10). Representative oxygen diffusion coefficients were calculated from average air-filled porosities in the upper 5 cm of soils in the pots using the diffusion-porosity equation of Bakker and Hidding (1) for puddled topsoils, since soils in this experiment were observed to have been puddled by watering. Soil oxygen consumption was estimated from manometric measurements to be 0.015 μmoles/g/hr, which agrees with published values for the average temperature of the soils during the experiment (10). Calculation of the oxygen depth functions were made for "equilibrium" cases immediately following watering and drainage (Fig. 2), as well as after 200 and 400 ml of water loss by evapotranspiration from each pot. These quantities of water were

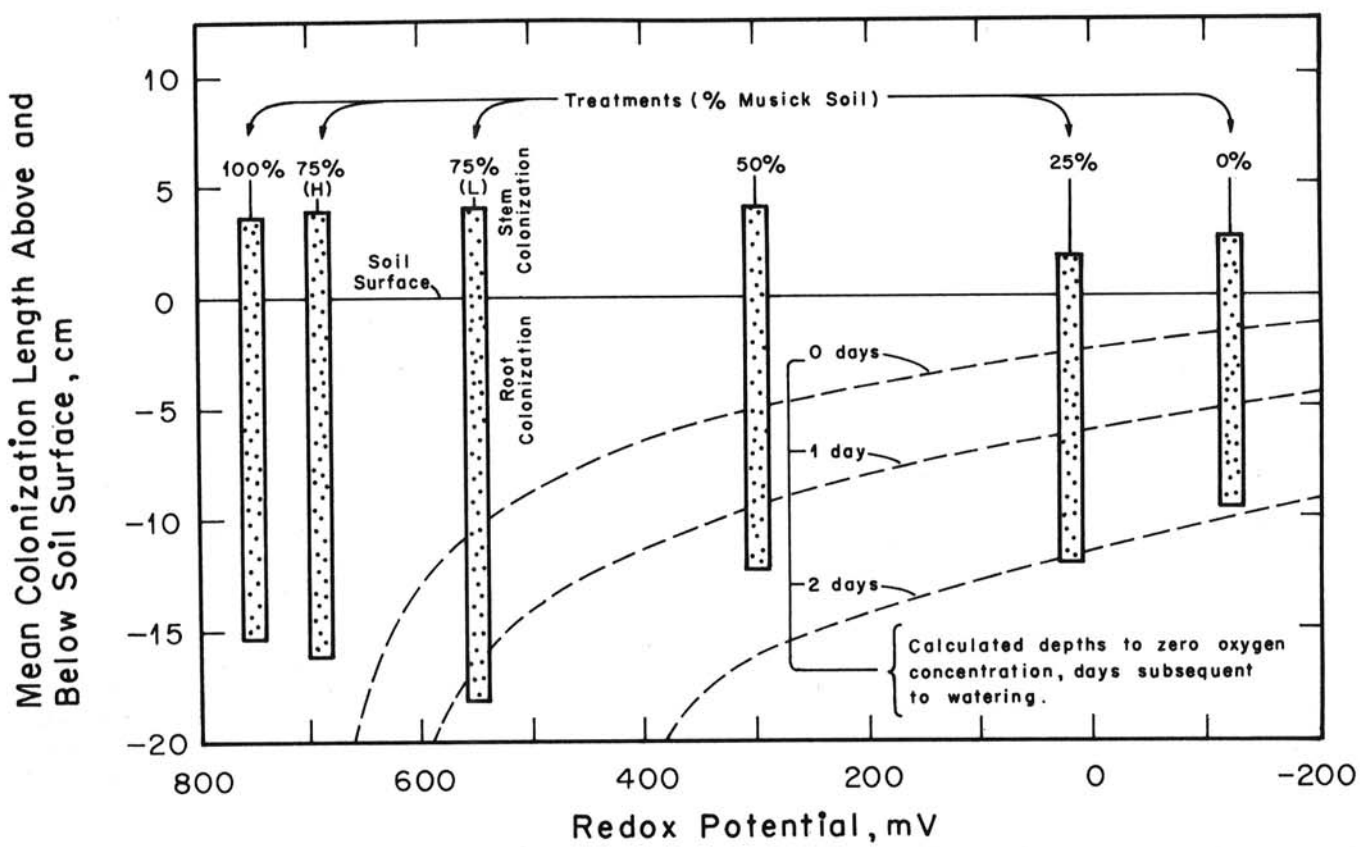

Fig. 2. Mean colonization of *Pinus ponderosa* seedlings by *Ceratocystis wageneri* above and below the soil surface with bars placed according to mean redox potentials subsequent to infection, and corresponding depths of zero oxygen concentration for 0, 1, and 2 days subsequent to watering.

assumed to have been removed in equal proportion from all soil volume elements in each pot, and represents estimated typical rates of evapotranspiration during periods of 1 and 2 days, respectively. Concomitant water potentials were never more negative than −0.10 bars except day 2 values for treatments 25 and 0, which were −0.12 and −0.18 bars, respectively.

The experiment was terminated 52–56 days after inoculation. Presence and extent of seedling colonization above and below inoculum blocks were measured. Independence of count data for numbers of infected seedlings, and mean separations for redox, soil temperature, and colonization data were tested statistically as described above. Logarithmic transformation of data for distance of downward colonization of taproots and square-root transformation of data for total colonization length were found to be necessary according to Bartlett's test for homogeneity of variances (22).

TABLE 1. Relation between soil moisture regime in two soils (of the Horseshoe and Musick series), soil redox potential, and ponderosa pine seedling infection by *Ceratocystis wageneri*

Moisture regime		Seedling infection (%)[a]		Mean soil redox potential (mV)[b]	
Treatment	Before/after inoculation	Horseshoe	Musick	Horseshoe	Musick
A	Dry/very dry	40 b	15 cd	+763 a	+733 a
B	Dry/dry	85 a	45 bc	+741 a	+671 a
C	Moist/dry	60 ab	60 ab	+731 a	+750 a
D	Moist/moist	20 cd	65 ab	−168 b	+727 a
E	Flooded/moist	35 bc	60 ab	−193 b	+553 a
F	Moist/flooded	20 cd	15 cd	−146 b	−156 b
G	Flooded/flooded	5 d	5 d	−183 b	−174 b

[a] Figures are percentages of seedlings infected of a total of 20. Percentages followed by the same letter are not significantly different, $P = 0.05$.
[b] Figures followed by the same letter are not significantly different, $P = 0.001$.

TABLE 2. Mean ammonium acetate-extractable manganese (ppm, oven-dry basis) and corresponding mean redox potentials (mV), in two soils prior to ponderosa pine seedling inoculation with *Ceratocystis wageneri*

	Mn^{++} (ppm)[a]		Redox potential[b]	
Treatment	Horseshoe	Musick	Horseshoe	Musick
A	3.2 ab	2.4 ab	+719 a	+717 a
B	4.4 ab	2.5 ab	+720 a	+663 a
C	23 bc	1.7 a	+508 ab	+723 a
D	75 cd	2.5 ab	−106 c	+692 a
E	205 d	···	− 59 c	− 82 c
F	16 abc	2.3 ab	+346 b	+716 a
G	203 d	315 d	− 89 c	−118 c

[a] Figures followed by the same letter are not significantly different, $P = 0.05$.
[b] Figures followed by the same letter are not significantly different, $P = 0.01$.

TABLE 3. Mean soil redox, average ponderosa pine seedling infection, and mean length of colonization by *Ceratocystis wageneri* as affected by mixtures containing different proportions of Musick and Horseshoe soils

	Treatment (% Musick soil)[z]					
Parameter	100	75H	75L	50	25	0
Soil redox potential (mV)	+737 a	+695 b	+552 c	+306 d	+15 e	−120 f
Seedlings infected (%)	33 ab	60 a	60 a	63 a	23 b	43 ab
Colonization						
Stem (cm)	3.6 a	3.8 a	4.1 a	3.8 a	1.6 a	2.5 a
Root (cm)	15.3 b	16.2 ab	18.1 a	12.3 bc	12.2 bc	9.8 c
Total (cm)	18.9 ab	20.0 ab	22.2 a	16.1 b	13.8 b	12.3 b

[z] Means not designated by the same letter were significantly different, $P = 0.05$ (redox potential means, except 100 versus 75H, were significantly different, $P = 0.01$).

RESULTS

In the first experiment, 106 seedlings were infected by *C. wageneri*, and the fungus was easily reisolated from infected plant parts. No control seedlings became infected. Most of the infected seedlings became thoroughly colonized by the fungus before the experiment was terminated, and no comparison of colonization rates could be made.

Percentage of seedlings that became infected correlated well ($r = 0.74^{**}$) with mean redox potentials after inoculation (Table 1), but not those ($r = 0.42$) prior to inoculation (Table 2). Except for seedling infection in the very dry Musick soil (regime A), infection was high in well-aerated treatments characterized by high redox potentials, and it was generally low in treatments where very low redox potentials indicated strongly reducing conditions. In the intermediate moisture regimes (D and E), the well-aggregated Musick soil exhibited much higher redox potentials than the poorly aggregated Horseshoe soil, and percent infection was correspondingly higher in the Musick soil. Soluble and exchangeable manganese levels were inversely correlated ($r = -0.86^{**}$) with mean redox potentials prior to inoculation (Table 2).

Soil temperatures ranged from 12.4 to 15.4 C among pots. Drier soils tended to be slightly warmer, but mean differences between pots or treatments were not statistically significant. Maximum diurnal variation in ambient temperature ranged from 4 to 21 C.

In experiment II (Table 3, Fig. 2), mixtures of the two soils in 25% increments from 100% Musick to 100% Horseshoe resulted in a range of mean redox potentials from +737 mV (treatment 100) to −120 mV (treatment 0). Treatment 75 was divided into two subtreatments (75H and 75L) with three pots in each because the three nearest the greenhouse fan (75H) consistently exhibited higher redox potentials, probably caused by a higher evapotranspiration rate. Differences in redox potential means for all treatments were highly significant ($P = 0.001$), except for one pair, 100 versus 75H, for which the difference was significant ($P = 0.05$).

Percentage infection was highest at the intermediate redox and aeration conditions (treatments 50, 75H, and 75L), although most differences were not significant (Table 3). Total colonization (stain length) was greatest in treatments 75L, 75H, and 100. There were no significant differences among treatments in amount of stem colonization above the soil surface. However, root colonization below soil exhibited several significant differences among treatments, with greatest colonization in treatment 75L and least in treatment 0.

Calculated depths to zero gaseous oxygen concentration immediately after watering and for 1 and 2 days later progressively decreased in treatments 75L through 0 (Fig. 2). For treatments 100 and 75H, the calculated depths exceeded depth of soil in the pots, indicating a likely continuous presence of gaseous oxygen throughout these soils over the duration of the experiment. At the conclusion of the experiment, soil below the calculated depth of zero oxygen 1 day after watering in treatments 50, 25, and 0 was mottled in color, generally had low-chroma color, and had a "marshy" smell, compared to soil above this depth in these treatments and to the entire soil mass of other treatments. These conditions are indicative of strong reducing conditions.

The fungus was reisolated easily from infected seedlings, and no control seedlings became infected. Mean soil temperatures were 16.3 to 16.6 C, and there were no significant differences among treatments. Maximum soil temperatures ranged from 23.5 to 26.3 C. Usual diurnal variation in ambient temperature was 10–25 C.

DISCUSSION

Results of experiment I clearly show a highly significant positive correlation between soil redox potential and pine seedling infection by *C. wageneri*. Percentage infection was consistently low whenever the redox potential after inoculation was negative, indicating very poor soil aeration. Infection was also reduced somewhat under the driest regime (A), which was characterized by high redox potentials. Apparently this treatment adversely affected

the inoculum.

The same number of seedlings, 53, became infected in each of the two soils. However, distribution of infected seedlings among treatments differed between soils as a result of their differences in structure. Maximum infection in the Horseshoe series occurred in relatively dry soil; in the Musick series, it occurred in the intermediate moisture treatments. This difference was also reflected in the redox potential values; the Musick soil required more water than the Horseshoe soil before the redox potential indicated poor aeration and a strongly reduced condition. Thus, under the daily watering regime, the strong structure of the Musick soil resulted in aeration, which was comparable to that in drier treatments of both soils, whereas the same regime in the Horseshoe soil resulted in the poor aeration and low redox potentials associated with low infection rates.

The moisture regime before inoculation and the corresponding redox potential appeared to have no influence on subsequent infection, nor did the the amount of extractable manganese at time of inoculation. While these findings do not eliminate host predisposition as a potential factor, they do indicate that predisposition had little influence in this experiment.

In experiment II, differences in soil structure that resulted from mixing the two soils acted together with the water potential regime to produce a range of redox potentials and soil aeration conditions. Hence, differences in both percentage infection and extent of colonization of *C. wageneri* were directly related to soil structure and aeration as reflected by redox potential, and not to the water regime-soil interaction of experiment I. The results support those of experiment I, particularly those indicating below-ground colonization to be higher under the moist, well-aerated conditions characterized by moderate redox potentials than under near-anaerobic conditions characterized by very low redox potentials or under dry conditions characterized by high redox potential. The relatively high percentage of infected seedlings in treatment 0, compared to the results of experiment I, may be due to the combination of less-frequent watering and higher experimental temperatures, which resulted in sufficient temporary aeration at inoculum block depth to allow infection. Redox potentials in treatment 0 pots occasionally jumped to +125 or +200 mV during warm weather.

Results of experiment II also appear to be consistent with those of Goheen et al (9). Results of a separate experiment with the same soil material as that used by those investigators indicated that the highest moisture treatments in which they obtained maximum disease severity was likely to have occurred at redox potentials ranging from +550 to +650 mV (25).

Colonization increased significantly from a minimum in treatment 0 to a maximum in treatment 75L. For these treatments, depth of colonization corresponded well with calculated depths of zero oxygen concentrations. Oxygen concentrations within roots are expected to decrease to zero under these conditions as well (11). This may indicate diminished host resistance under these conditions, with growth of the fungus limited only by oxygen availability, and could result from toxicity due to high concentrations of soluble manganese or other transition metal species. Similarly, higher disease severity in treatments 75L and 50 may indicate that the fungus becomes more virulent as soluble manganese concentrations increase. Growth of *C. wageneri* in vitro is stimulated by elevated soluble manganese concentrations (12). Significant manganese reduction and solubilization at the pH of our soils begins to occur between +600 mV and +300 mV (5,6,17,20,23), as was observed in experiment I. Maximum disease severity thus occurred as soluble manganese levels were elevated, but before growth could be limited by absence of oxygen.

Results of this study indicate that the observed increase in disease severity in ponderosa pine associated with moist field conditions may be due at least in part to concomitant effects on soil aeration. In the central Sierra Nevada, most infection by *C. wageneri* apparently occurs during winter and spring when soil moisture levels are highest, and the most rapid rates of spread are exhibited on trees growing in moist sites (8,9). Since most growth of the fungus in expanding disease centers appears to occur within host roots (8), vigorous root colonization associated with decreased oxygen or increased soluble Mn levels should have a significant effect on disease center enlargement.

Presence of Mn concretions in some soils in affected areas indicates the probability of seasonal aeration impairment sufficient for significant Mn reduction and solubilization and may be an indicator of sites particularly vulnerable to rapid disease spread.

When the fungus must grow through the soil from root to root, stimulation by redox-active products such as Mn^{++} might be a competitive advantage. Reports (7,19) indicate Mn^{++} optima for other fungi to range upward to a few parts per million. Growth through soil may be an important mode of spread for *C. wageneri* (12,13), and field excavations showing few root contacts at apparent infection courts (8) indicate that the fungus may grow perhaps 15 cm through soil before infecting a root. Microhabitat distributions and properties related to redox potential might be particularly important to this mode of growth and spread.

Whether the observed differences in structure and aeration between the two soils can help to explain the large differences in incidence of disease in ponderosa pine with locality will require further study. However, if root infection occurs in the Horseshoe soil principally when soil moisture is relatively low, this could be a limiting factor. By the time soil moisture content is reduced sufficiently in the spring, soil temperatures at the lower elevations where the Horseshoe soil occurs could be above the 15–18 C optimum for growth of *C. wageneri* and infection. With the more highly aggregated Musick soil, the redox potential increases more rapidly in the spring and probably becomes favorable for infection while temperatures are still optimal for the pathogen and disease development.

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