Influence of Soil Moisture on Infection of Ponderosa Pine by Verticicladiella wagenerii

Donald J. Goheen, Fields W. Cobb, Jr., and George N. McKibbin

Plant Pathologist, U.S. Department of Agriculture, Forest Service, Forest Insect and Disease Management, Portland, OR 97208; Associate Professor and former Research Associate, respectively, Department of Plant Pathology, University of California, Berkeley 94720.

Accepted for publication 8 December 1977.

ABSTRACT

GOHEEN, D. J., F. W. COBB, JR., and G. N. MC KIBBIN. 1978. Influence of soil moisture on infection of ponderosa pine by Verticicladiella wagenerii. Phytopathology 68:913-916.

To test the hypothesis that high soil moisture favors infection of ponderosa pine by *Verticicladiella wagenerii*, 160 1-yr-old ponderosa pine seedlings were inoculated with *V. wagenerii* and subjected to two relatively dry and three relatively wet soil moisture treatments in the greenhouse. When examined after 12 wk, the largest numbers of infected seedlings were observed in the wettest soil treatments with numbers decreasing significantly ($P=0.05$) as soil treatments became drier (12 of 15 to 0 of 20 seedlings infected).

Verticicladiella wagenerii Kendrick causes a wilt-type root disease of conifers in the western United States. Principal hosts include Douglas - fir [*Pseudotsuga menziesii* (Mirb.) Franco], ponderosa pine (*Pinus ponderosa* Laws.), Jeffrey pine (*P. jeffreyi* Grev. and Balf.), and the piñon pines. Other conifers, for example sugar pine (*P. lambertiana* Dougl.) and white fir (*Abies concolor* Lindl. and Gord.), have been infected on rare occasions.

Foliar symptoms caused by *V. wagenerii* include reduction of terminal growth, crown thinning, and needle chlorosis. These are similar to symptoms associated with other root pathogens of conifers. Diagnostic evidence of infection by *V. wagenerii* is a dark purple-brown to black stain in the sapwood of host roots and lower bole.

The fungus invades host root systems and moves rapidly up to the root crown. The darkly pigmented hyphae grow through the tracheids and interfere with host water uptake (18). Infected trees usually die within 2-3 yr. Bark beetles are often involved in the actual killing.

Cool temperatures (18 C or lower) favor infection by some strains of *V. wagenerii* (18), but little is known about other environmental conditions that influence infection. In the central Sierra Nevada where ponderosa pine is the main host affected, many of the largest and most active infection centers have been found in low-lying sites. In such locations, soil moisture content generally is high, especially in winter and spring when observations indicate that the fungus is most actively infecting and colonizing roots. The studies reported here and in an earlier abstract (9) were initiated to test the hypothesis that high soil moisture may favor infection and disease development.

00032-949X/78/000157$03.00/0

MATERIALS AND METHODS

The study involved two similar experiments conducted in the spring of 1972 and the spring of 1973. A Holland sandy loam, A_{12} horizon, from the Blodgett Research Forest, El Dorado County, California, was used in the investigation. A soil moisture release curve was prepared (16); the soil contained 31.2% water (w/w) at 1/3 bar and 17.6% at 15 bars in a pressure membrane apparatus.

One-yr-old ponderosa pine seedlings were planted one seedling each in equal amounts of soil in containers 18 cm tall and 11 cm in diameter. Seedlings were inoculated by taping a 3-cm-long inoculum block to the roots 3 cm below the root crown. In 1972, seedlings were planted and inoculated simultaneously. In 1973, seedlings were established in pots for 1 mo prior to inoculation. To prepare inoculum blocks, pine dowels were boiled in a 10% solution of malt extract for 2 hr, placed in glass jars and sterilized for 30 min at 121 C. A piece of agar colonized by *V. wagenerii* was introduced into each jar, and jars were stored at a constant temperature of 18 C for 8 wk. At the end of that time, the dowels were covered with *V. wagenerii* hyphae and appeared to be thoroughly colonized. The isolate used to prepare the inoculum was obtained from a dying ponderosa pine east of the Blodgett Research Forest.

A variation of the frequency of irrigation technique was used to generate the soil moisture treatments employed (8, 11, 13). Soil moisture in all treatments commenced at 1/3 bar tension and plants were allowed to extract water from the soil until preselected stresses below 1/3 bar were reached. When these points were attained, the entire system was watered back to 1/3 bar.

The treatments used were as follows:

1972 Experiments.—Seedlings were inoculated at the beginning of moisture treatments. Each treatment consisted of 15 inoculated seedlings and three controls (seedlings with noncolonized dowels taped to the roots).

Treatment 1A.—Soil moisture was maintained near saturation.

Treatment 1B.—Soil moisture was maintained near 1/3 bar tension (fluctuating between 1/3 and 1.0 bar).

Treatment 1C.—Soil moisture was allowed to decrease from 1/3 bar to 15 bars tension and then was immediately watered back to 1/3 bar.

Treatment 1D.—Soil moisture was allowed to decrease from 1/3 bar tension to 15 bars and was kept at or below this tension for 10 days before watering.

1973 Experiments.—Unless otherwise noted, seedlings were inoculated at the beginning of moisture treatments. Each treatment included 20 inoculated seedlings and three controls.

Treatment 2A.—Soil moisture was maintained near 1/3 bar.

Treatment 2B.—Soil moisture fluctuated between 1/3 bar and 5 bars.

Treatment 2C.—Soil moisture was allowed to decrease from 1/3 bar to 15 bars and then was immediately watered back to 1/3 bar.

Treatment 2D.—Soil moisture was allowed to decrease from 1/3 bar to 15 bars tension and seedlings then were inoculated. The pots were watered back to 1/3 bar immediately after inoculation, and moisture treatment was continued as in 2C.

Treatment 2E.—Soil moisture was allowed to decrease from 1/3 bar to below 15 bars tension for 10 days; pots were rewatered to 1/3 bar, and the moisture treatment was continued as in 2C.

Treatment 1A was watered twice daily. Other treatments were weighed every other day to determine whether or not additional watering was required. In 1972, potted seedlings were kept in a lathhouse where the average ambient temperature was 18.3 C and the highest temperature recorded was 22.2 C. In 1973, potted seedlings were kept in a greenhouse where the average ambient temperature was 21.1 C and the highest temperature recorded was 26.7 C. Soil temperatures in different treatments were never found to differ by more than 1 C.

Eighty days after inoculation, all seedlings were removed from the soil and examined. Foliar symptoms, presence or absence of stained sapwood, percentage of stem circumference stained, and extent of stained tissue above and below the inoculum block were recorded for each seedling. Isolations were made from seedlings with stained tissue to confirm the presence of the fungus.

Fisher-Yates tests of significance in 2 × 2 contingency tables (1, 14) were used to compare numbers of seedlings infected in different treatments. One-factor analyses of variance were used to compare lengths of stained tissue above and below the inoculum block, total stain lengths, stain midpoint locations, and arc-sine transformation of the percentage of circumference stained of infected trees in the various treatments. For purposes of pooled comparisons, treatments 1A, 1B, 2A, and 2B were considered to be wet (nonstressed) treatments, while 1C, 1D, 2C, 2D, and 2E were considered to be dry (stressed) treatments.

RESULTS

A total of 31 of 60 ponderosa pine seedlings inoculated in 1972 and 17 of 100 inoculated in 1973 exhibited stained sapwood. *Verticicladiella wagenerii* was isolated easily from stained seedlings. The largest numbers of infected seedlings were in the wettest soil treatments with numbers decreasing rapidly with increased dryness of the soil treatments (Fig. 1). The smallest numbers of infected seedlings occurred in treatments subjected to the greatest moisture stresses; treatments 2D and 2E resulted in no infected seedlings. Differences were significant ($P = 0.05$) between numbers of trees infected in treatments 1A and 1D, and 1B and 1D in the 1972 experiment, between 2A and 2C, 2A and 2D, 2A and 2E, 2B and 2D, and 2B and 2E in the 1973 experiment, and between pooled wet treatments and pooled dry treatments for 1972 and again for 1973.

Comparison of mean values for colonization patterns (Fig. 2, Table 1) showed that with successively drier soil treatments: (i) colonized circumference decreased, (ii) total vertical colonization decreased, (iii) colonization above the inoculum block decreased, (iv) colonization below the inoculum block increased, and (v) the stained tissue midpoint shifted downward. However, all colonization parameters were extremely variable within treatments, and the analyses of variance showed that only the F-value for stained tissue midpoint comparisons was high enough to reject the null hypothesis of no significant differences. The only significant differences were between stained tissue midpoints of treatments 1A and 1D and treatments 1B and 1D, which suggested that stained tissue midpoints of wet treatments were higher than those of

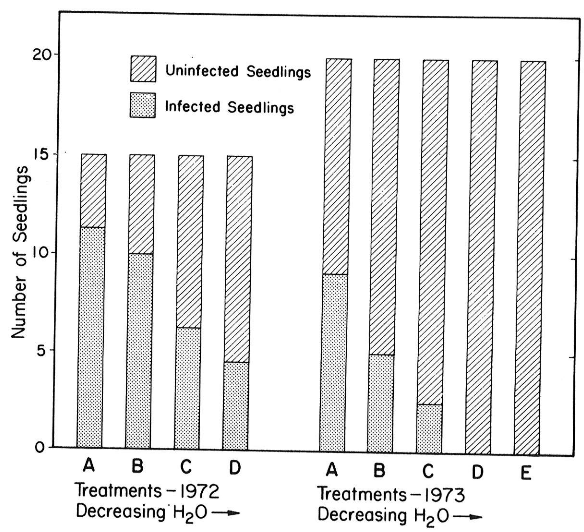

Fig. 1. Numbers of inoculated ponderosa pine seedlings infected by *Verticicladiella wagenerii* under different soil moisture treatments. Treatments are: 1972 Experiments: A above 1/3 bar tension, B near 1/3 bar tension, C 1/3 bar to 15 bars tension, D 1/3 bar to 15 bars tension plus 10 days; 1973 A near 1/3 bar tension, B 1/3 bar to 5 bars tension, C 1/3 bar to 15 bars tension, D 1/3 bar to 15 bars tension, inoculation at 15 bars, E 1/3 bar to 15 bars tension plus 10 days, inoculated at 15 bars.

treatments that involved moisture stress below 15 bars soil water tension.

There were no apparent differences in foliar symptoms between infected seedlings in different soil moisture treatments. The most common symptom observed was a failure of new needles to attain normal length accompanied by a slight yellowing of older needles. Scattered dead needles appeared on some infected seedlings, but only one exhibited total needle necrosis. A few infected seedlings showed no external symptoms before the experiment was terminated.

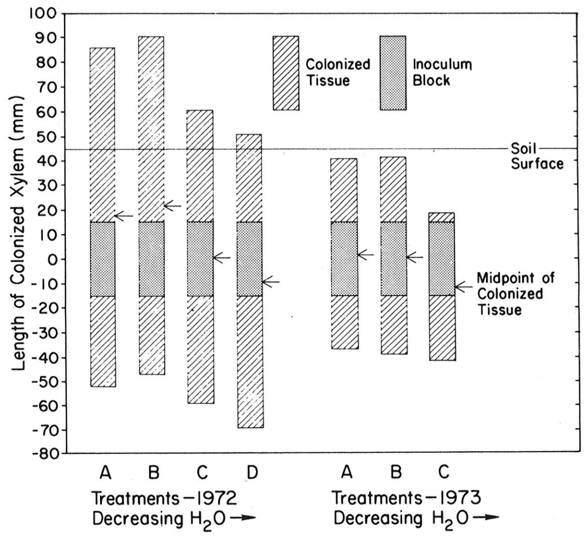

Fig. 2. Mean values for vertical colonization of ponderosa pine seedlings by *Verticicladiella wagenerii* under different soil moisture treatments. Treatments are: 1972 A above 1/3 bar tension, B near 1/3 bar tension, C 1/3 bar to 15 bars tension, D 1/3 bar to 15 bars tension plus 10 days; 1973 A near 1/3 bar tension, B 1/3 bar to 5 bars tension, C 1/3 bar to 15 bars tension.

TABLE 1. Mean percentage circumference of inoculated ponderosa pine seedlings stained by *Verticicladiella wagenerii* under different soil moisture treatments

Treatment	Mean % of stem circumference stained
1A. Above 1/3 bar tension	85
1B. Near 1/3 bar tension	73
1C. 1/3 bar to 15 bars tension	66
1D. 1/3 bar to 15 bars tension + 10 days[a]	62
2A. Near 1/3 bar tension	29
2B. 1/3 bar to 5 bars tension	29
2C. 1/3 bar to 15 bars tension	20
2D. 1/3 bar to 15 bars tension, inoculated[b]	0
2E. 1/3 bar to 15 bars tension + 10 days, inoculated at 15 bars[c]	0

[a]Moisture allowed to remain below 15 bars tension for 10 days before watering.

[b]Seedlings inoculated when moisture had decreased to 15 bars; seedlings were rewatered immediately after inoculation.

[c]Seedlings inoculated as in 2D but not rewatered until 10 days later.

DISCUSSION

Although the extent of *V. wagenerii* colonization differed very little between infected trees in wet and dry treatments, the significant differences in numbers of infections suggest strongly that the infectivity of *V. wagenerii* is favored by high soil moisture. In this respect, the fungus appears to be similar to wilt pathogens such as *Fusarium oxysporum* and *Verticillium* spp. that are favored by wet soil conditions (5). It is also similar to the root pathogens *Thielaviopsis basicola*, *Pythium ultimum*, and *Pythium irregulare*, for which high soil moisture favors infection (2, 17) but dissimilar to many other soil fungi for which growth and infectivity occur over a wide range of soil moisture conditions (3, 4, 6, 7, 10, 12, 15, 19, 20). Occasionally preference for dry conditions among this latter group has been shown.

The number of seedlings that became infected in the 1972 and 1973 experiments of this study differed considerably. This difference may be the result of differences in temperatures, the average in 1973 being about 3 C higher than in 1972. Smith (18) has shown that temperatures influence infectivity of *V. wagenerii.*

Whether soil moisture is influencing infection through its effects on the pathogen, the host, or both cannot be determined from this study. However, when seedlings were stressed prior to and after inoculation (Treatment 2D), no infection occurred compared to 10-40% infection when seedlings were stressed only after inoculation (Treatment 2C, also 1C). Hence, host resistance may not be weakened as a result of moisture stress. Nor does near saturation appear to increase infection over 1/3 bar tension. It has been suggested (P. J. Zinke, *personal communication*) that the fungus may be favored in wet soils as a result of decreased reduction-oxidation potential and associated increase in soluble manganese levels.

Implications of a relationship between high soil moisture and successful infection by *V. wagenerii* may be far-reaching to forest managers. Possibly, the disease may be limited or controlled by manipulating forest stands on poorly drained sites. Conversion of pure stands of host species and establishment and maintenance of mixed species stands, particularly on wet sites, could contribute greatly to reducing loss caused by *V. wagenerii.*

LITERATURE CITED

1. ARMSEN, P. 1955. Tables for significance tests of 2 × 2 contingency tables. Biometrika 42:494-511.
2. BATEMAN, D. F. 1961. The effect of soil moisture upon development of Poinsettia root rots. Phytopathology 51:445-451.
3. CHEN, A. W. -C., and D. M. GRIFFIN. 1966. Soil physical factors and the ecology of fungi. V. Further studies in relatively dry soils. Trans. Br. Mycol. Soc. 49:551-561.
4. COOK, R. J., and R. I. PAPENDICK. 1970. Effect of soil water on microbial growth, antagonism, and nutrient availability in relation to soil borne fungal diseases of plants. Pages 81-88 *in* T. A. Tousson, R. V. Bega, and P. E. Nelson, eds. Root diseases and soil borne pathogens.

Univ. of Calif. Press, Berkeley. 252 p.

5. COOK, R. J., and R. I. PAPENDICK. 1972. Influence of water potential of soils and plants on root disease. Annu. Rev. Phytopathol. 10:349-374.
6. COOK, R. J., R. I. PAPENDICK, and D. M. GRIFFIN. 1972. Growth of two root-rot fungi as affected by osmotic and matric water potentials. Soil Sci. Soc. Am. Proc. 36:78-82.
7. COUCH, H. B., and E. R. BEDFORD. 1966. Fusarium blight of turfgrasses. Phytopathology 56:781-786.
8. COUCH, H. B., and J. R. BLOOM. 1958. Influence of soil moisture, pH, and nutrition on the alternation of disease proneness in plants. Trans. N.Y. Acad. Sci. 20:432-437.
9. GOHEEN, D. J., and F. W. COBB, JR. 1974. Soil moisture as a factor affecting infection of ponderosa pine by Verticicladiella wagenerii. Proc. Am. Phytopathol. Soc. 1:111.
10. GRIFFIN, D. M. 1969. Soil water in the ecology of fungi. Annu. Rev. Phytopathol. 7:289-310.
11. JOHNSON, L. F., and S. B. CURL. 1972. Methods for Research on the ecology of soil-borne plant pathogens. Burgess Publishing Co., Minneapolis, MN. 247 p.
12. KOUYEAS, V. 1964. An approach to the study of moisture relations of soil fungi. Plant Soil 20:351-363.
13. KRAMER, P. J. 1969. Plant and soil water relationships: a modern synthesis. McGraw-Hill, New York, N.Y. 482 p.
14. LATSCHA, R. 1953. Tests of significance in a 2×2 contingency table: extension of Finney's table. Biometrika 40:74-85.
15. MOORE, L. D., H. B. COUCH, and J. R. BLOOM. 1963. Influence of environment on diseases of turfgrasses. III. Effect of nutrition, pH, soil temperature, air temperature, and soil moisture on Pythium blight of Highland bentgrass. Phytopathology 53:53-57.
16. RICHARDS, L. A., and L. R. WEAVER. 1944. Moisture retention by some irrigated soils as related to soil-moisture tension. J. Agric. Res. 69:215-235.
17. ROTH, L. F., and A. J. RIKER. 1943. Influence of temperature, moisture, and soil reaction on the damping off of red pine seedlings by Pythium and Rhizoctonia. J. Agric. Res. 67:273-293.
18. SMITH, R. S., JR. 1967. Verticicladiella root disease of pines. Phytopathology 57:935-938.
19. STOVER, R. H. 1953. The effect of soil moisture on the growth and survival of Fusarium oxysporum f. cubense in the laboratory. Phytopathology 43:499-504.
20. TOWERS, B., and W. J. STAMBAUGH. 1968. The influence of induced soil moisture stress upon Fomes annosus root rot of loblolly pine. Phytopathology 58:269-272.