# The Effect of Matric and Osmotic Potential of Soil on Phytophthora Root Disease of Persea indica

R. E. Sterne, G. A. Zentmyer, and M. R. Kaufmann

Graduate Research Assistant and Professor, Department of Plant Pathology, and Associate Professor, Department of Plant Sciences, respectively, University of California, Riverside, CA 92521. Present address of senior author, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

We thank C. W. Asbell for assistance in construction of the ceramic tension plates used in the experiments.

Supported in part by National Science Foundation Grant PCM 74-19982, and by a California Avocado Advisory Board Grant to G. A. Zentmyer.

Accepted for publication 24 May 1977.

## ABSTRACT

STERNE, R. E., G. A. ZENTMYER, and M. R. KAUFMANN. 1977. The effect of matric and osmotic potential of soil on Phytophthora root disease of Persea indica. Phytopathology 67:1491-1494.

The influence of the matric and osmotic potential of soil on root disease caused by chlamydospores of *Phytophthora cinnamomi* was examined by growing seedlings of *Persea indica* (a species related to commercial avocado) in infested soil with different combinations of matric and osmotic potential. Matric potential in a range from 0 to −0.25 bar was controlled by ceramic tension plates. Osmotic potential of soil was adjusted with NaCl or $CaCl_2$ and was in a range (−0.37 to −2.37 bar) found in the saline soils in avocado groves in California where root rot caused by *P. cinnamomi* is a problem. Disease was rated by determining the portion of the root system of *P. indica* seedlings with black lesions, and infection was verified by plating roots on $P_{10}VP$ medium. Over the range of potentials tested, disease severity was a function of matric potential but not of osmotic potential. The percentage of diseased roots averaged 80 to 90%, 50 to 90%, and 10 to 50% at matric potentials of zero, −0.05, and −0.10 bar, respectively. At −0.25 bar, only a few lesions occurred. Disease was not significantly different over the range of osmotic potentials imposed with these respective matric potentials.

In avocado (*Persea americana* Mill.) plantings in California, the severity of root rot caused by *Phytophthora cinnamomi* Rand. increases when the water content of soil remains near saturation (zero water potential) because of overirrigation or poor drainage (15). Yet, it is reported that the fungus grows rapidly at quite low water potentials (1, 6, 12). In sandy soil with water potential adjusted osmotically, mycelial growth rate of *P. cinnamomi* was highest at −10 to −15 bars and was one-half optimum at −20 to −25 bars (1). With matric-controlled water potential, optimum and one-half optimum growth rate occurred at −5 to −10 bars and −15 to −20 bars, respectively (1). The results indicated that *P. cinnamomi* tolerated low water potentials and was less tolerant to stress associated with low matric potential than with low osmotic potential.

Since damage to avocado roots by *P. cinnamomi* is greatest in the field when the water potential of soil is considerably higher than the potentials reported to reduce mycelial growth rate (1), we felt that an evaluation of matric and osmotic effects on Phytophthora root disease at high soil water potentials was in order. This paper describes a system in which plants were grown in soil containing *P. cinnamomi*; the matric potential of the soil was controlled over a range from zero to approximately −0.3 bar. We used the system to examine the influence of matric and osmotic components of water potential on root disease in soil infested with chlamydospores of *P. cinnamomi*.



## MATERIALS AND METHODS

**Inoculum.**—Isolate Pc 40, ATCC 32992, (Phytophthora Culture Collection, Department of Plant Pathology, University of California, Riverside) of *P. cinnamomi* was used in all experiments. Suspensions of chlamydospores were obtained from mycelial mats of 30- to 40-day-old cultures grown in the dark at 24 C in 250-ml bottles containing 25 ml of V-8 juice broth (100 ml V-8 juice, 2 g $CaCO_3$, and 900 ml demineralized water). The mats were washed thoroughly with sterile deionized water, and the chlamydospores were separated from mycelium by comminuting the mats on a Vortex mixer at speed 7 for 30 sec. Chlamydospores were separated from hyphae and damaged spores, and the germinability and viability of the spores were tested as described (10). The concentration of spores in a stock suspension was determined with a 1-ml capacity eelworm slide (German Hawksley Ltd., Lancing, England). The stock chlamydospore suspension was stored at 8 ± 1 C but never longer than 16 hr prior to use.

**Control of matric and osmotic potential.**—The soil matric potential was controlled by applying tension to a layer of soil on a tension plate (8, 9). Soil was placed as a slurry on the unglazed upper surface of a hollow, porous ceramic plate. The lower surface and sides of the plate had been sealed by glazing. A plexiglass cylinder was sealed to the edges of the unglazed surface and provided an inner surface diameter of 8.5 cm. The cylinder was 8.5 cm tall and allowed adequate room for a layer of soil 1.5 cm in depth and air space for shoots of seedlings used in the tests. The tops of the cylinders were covered with

polyethylene film to reduce evaporation.

When the hollow plate was filled with water, a tube connected to the side allowed readjustment of the distance between the upper surface, which functioned as a tension plate, and a water reservoir at the bottom of the tube. The height of the water column from the plate to the reservoir controlled the hydrostatic head supported by matric forces in the plate and soil. For example, at −0.3 bar matric potential, the height of the column from plate to reservoir was 300 cm. The apparatus functioned as the Büchner funnel tension plates described by Duniway (7), and changes in the water content of soil layers indicated that the adjustment of soil matric potential was essentially complete within 30 min. The accuracy of control by matric potential was confirmed by comparing a drying curve of water content vs. matric potential of soil on tension plates with a similar curve for water content of soil equilibrated at similar matric potentials in a pressure plate apparatus (9, and Fig. 1).

Osmotic potentials were chosen to represent a range that occurs in the saline soils of avocado groves in southern California (3). Solutions of NaCl and $CaCl_2$ were used to adjust the osmotic potential of soil placed on the tension plates; for disease assay tests, osmotic solutions were added with chlamydospores suspended in them. In tension plates where osmotic potentials were adjusted with salt solutions, the water column joining the plate to the reservoir also contained the appropriate solution.

A coarse sandy loam soil from an avocado grove on the University of California, Riverside, campus was used in all the experiments. Before use, the soil was sieved (1.5-mm openings) and autoclaved at 120 C for 50 min on 2 successive days. The electrical conductivity of seven saturation extract samples of the soil varied from 0.8 to 1.1 millimhos/cm. These conductivity values corresponded to osmotic potentials ranging from −0.28 to −0.39 bars (14). An average of −0.37 bars agreed with psychrometric determinations of the osmotic potential of saturation extracts (4) and −0.37 bars was used for the osmotic potential of the soil solution in calculating the total water potential of soil on tension plates.

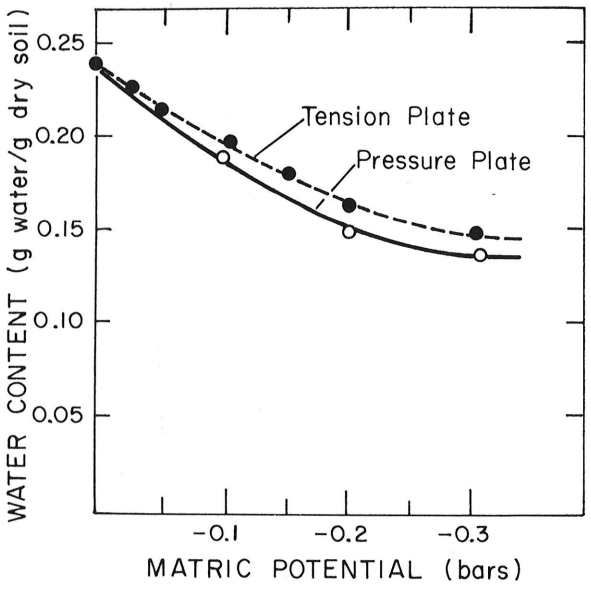


**Fig. 1.** Relationship between water content and matric potential for autoclaved sandy loam soil. Solid line is for samples taken from the ceramic tension plates used to control matric potential (7, 9). Dashed line is for samples brought to equilibrium in a pressure plate apparatus (9).

The water potential of soil on a tension plate was calculated by the equation: $\psi_T = \psi_{se} + \psi_\pi + \psi_m$ where $\psi_T$ is the total soil water potential and $\psi_{se}$, $\psi_m$, and $\psi_\pi$ are, respectively, the average osmotic potential of saturation extracts of nonamended soil, the osmotic potential of the salt solution added to the soil, and matric potential (11). For example, a combination of $\psi_\pi = 1.00$ bar solution of NaCl, $\psi_\pi = 0.05$ bar on the tension plate, and $\psi_{se} = 0.37$ bar (the same in all treatments) resulted in a total water potential of −1.42 bar.

The soil temperature on the plates ranged from 24 to 26 C, but varied only 1 C among plates at one time. The pH of saturation extracts of the sandy loam soil with or without NaCl solutions at −1.00 or −2.00 bar varied from 4.9 to 6.3 and was unrelated to the salt amendment. Photosynthetically active radiation (400-700 nm wavelengths) averaged 4.1 nanoeinsteins · $cm^{-2}$ · $sec^{-1}$ for 10 hr/day at the soil surface.

**Root disease assay.**—In a preliminary experiment, dilutions of a stock chlamydospore suspension were used to saturate 200 g of soil, and the dilutions provided inoculum densities of 0.1, 1, 2, 4, 10, and 20 chlamydospores/g of dry soil. The soil was placed in styrofoam cups with holes in the bottom and a *Persea indica* seedling was planted in each cup. The cups were placed on trays, and the soil was maintained at saturation ($\psi_m = 0$) by adding water to the trays. *Persea indica* seedlings, which are sensitive indicator plants for *P. cinnamomi* in soil (Zentmyer, *unpublished*), were removed from the cups after 2 wk and the roots were washed and inspected for lesions caused by *P. cinnamomi*. Few lesions occurred at inoculum levels lower than 10 spores/g dry soil.

For disease assay tests at different water potentials, a stock chlamydospore suspension was diluted to provide 15 spores/g of dry soil when 25 ml was added to 100 g of dry sterilized soil. The spore suspension was diluted with either deionized water, or solutions of NaCl or $CaCl_2$ at −1.0 or −2.0 bars. Infested soil was placed on a tension plate and two *P. indica* seedlings were planted in the soil. Water columns were adjusted to provide soil matric potentials of 0, −0.05, −0.10, and −0.25 bar. With polyethylene film enclosing the seedlings in the cylinder, transpiration was minimal and plant water stress induced by matric effects was negligible.

After 10 days, the seedlings were removed from the soil, the roots were washed, and the percentage of diseased roots was determined from the portion of the root system with black lesions. Diseased roots were plated on $P_{10}VP$ agar (13) to verify that *P. cinnamomi* was present in the root.

## RESULTS AND DISCUSSION

A controlled experiment confirmed that damage to roots growing in soil on the tension plates was caused by

*P. cinnamomi* rather than by effects of the osmotic and matric potentials of the soil. Seedlings were planted in noninfested soil on tension plates adjusted with either water alone or with NaCl solutions providing $\psi_\pi = -1.00$ and $-2.00$ bar; matric potentials were adjusted to give all combinations of $\psi_m$ and $\psi_\pi$ that were used later with infested soil. After 12 days, when seedlings were removed from the soil and the roots inspected, the root systems of all the seedlings were approximately the same size and color as roots of seedlings grown in sand for 12 days. There was no visual difference in the appearance of roots grown at the lowest soil water potential ($-2.62$ bar; i.e., the sum of $\psi_{se}$ at $-0.37$ bar, $\psi_\pi$ at $-2.00$ bar, and $\psi_m$ at $-0.25$ bar) and those at the highest potential ($-0.37$ bar; i.e., $\psi_{se}$ at $-0.37$, $\psi_\pi$ at 0.00 bar, and $\psi_m$ at 0.00 bar). In subsequent disease assays roots with lesions were collected from infested soil and plated on $P_{10}VP$ agar; *P. cinnamomi* grew into the medium from 70 to 100% of the roots and was easily identified.

Figures 2-a and 2-b show the results of two experiments measuring the effect of different combinations of $\psi_m$, $\psi_\pi$, and salt used to adjust $\psi_\pi$ on root disease ratings in soil infested with chlamydospores. In soil in which $\psi_m$ was 0 and no salt was added to the soil solution ($\psi_{se} = -0.37$ bar), 80-90% of the roots were diseased. Following reductions in matric potential to $-0.05$ and $-0.10$, respectively, the percentage of diseased roots decreased to 60-85% and 10-50%. More importantly, at $-0.25$ bar matric potential, the percentage of diseased roots was only 3-5%. Even when soil was amended with solutions of NaCl or $CaCl_2$ at $-1.00$ or $-2.00$ bar, the amount of root damage was affected only by lowering the matric potential. As $\psi_m$ decreased in soil amended with salt, the average disease ratings followed the same pattern as in soil with no salt amendment. For different osmotic potentials at one matric potential, the ranges of disease ratings overlapped (Fig. 2-a and -b), and no consistent osmotic effect was observed.

Surprisingly, the $-2.0$ bar salt solution did not affect the indicator plants or alter disease ratings. Avocado (*P. americana*) is extremely sensitive to sodium and chloride ions at osmotic potentials between $-0.5$ and $-1.5$ bars (3, 14). Most other plants are not affected by sodium and chloride at these potentials (14). Infection of pineapple by *P. cinnamomi* was reduced more than 10 times by a three-fold increase in the potassium concentration in solution cultures (2). Unless *P. indica* plants respond differently than commercial avocado plants, it is not clear why the disease ratings in the present study were not influenced by the osmotic potential of the soil solution or the salt species used to adjust osmotic potential. Osmotic or salt effects might have occurred with longer exposure of roots to treatments with osmotic potential more negative than $-2.0$ bars.

The results reported here express quantitatively the relationship between high soil water status and Phytophthora root disease; under our experimental conditions, disease was favored by matric potentials higher than $-0.25$ bar. In our experiments, the water potential in soil was reduced by matric forces (matric potential) and by dissolved salts (osmotic potential). If the potential energy of water in soil was the factor that influenced disease ratings, theoretically there would have been an additive effect of osmotic and matric potential on disease. However, we found that the matric component of water potential dominated the effect of the total soil water potential on disease ratings. Therefore, it is unlikely that the free energy of water expressed as soil water potential was the direct influencing factor on disease.

In terms of water relations factors the study of an

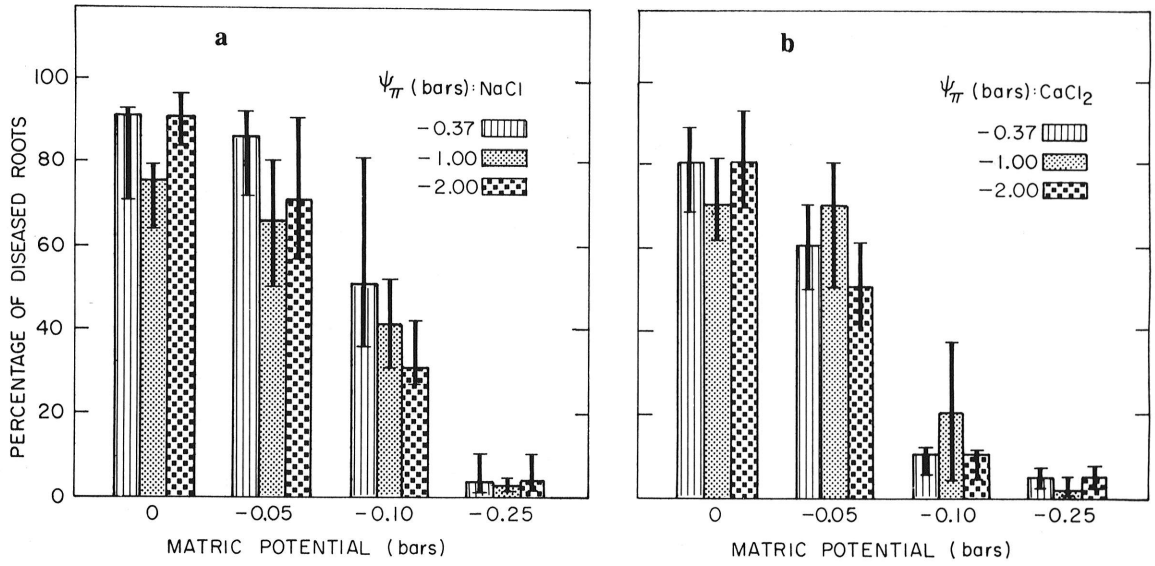


**Fig. 2-(a, b).** The effect of matric and osmotic potential of soil on infection of *Persea indica* seedling roots by *Phytophthora cinnamomi*. **a)** Osmotic potential adjusted with NaCl solutions. **b)** Osmotic potential adjusted with $CaCl_2$ solutions. Soil was infested with 15 chlamydospores/g dry soil, and root disease was rated by estimating the portion of the root system with black lesions. The percentages are means of eight samples (four replications in two experiments). Osmotic potential in nonamended soil (no salt added) = $-$ 0.37 bar. Ranges are bracketed at the heights of the bars.

organism in soil is complex because the matric component of water potential influences not only the free energy of water but also, (i) mechanical strength of the soil matrix, (ii) pore structure, (iii) soil aeration, and (iv) hydraulic conductivity (5). The relationship of matric potential to a soil-borne disease is even more complex than its relation to a single organism in the soil matrix. For example, in Phytophthora root disease, factors related to matric potential might affect any one or combinations of several components of disease; e.g., sporangium germination, zoospore movement, germ tube growth and penetration, and susceptibility of the root, as well as development of disease after penetration by the fungus.

Observations on movement of zoospores of *Phytophthora* in soil indicate why severe root disease in our experiments was related more to high matric potential than to increasing the total soil water potential (i.e., increasing osmotic and matric potential). Duniway (7) found that zoospores of *Phytophthora cryptogea* infected safflower seedlings when the matric potential of soil was between zero and −0.10 bars. Apparently, zoospore movement was restricted to high matric potentials because soil pores large enough to accommodate swimming zoospores were filled with water at those potentials; the same pores drained at lower matric potentials. In our experiments, the matric potentials that favored disease probably reflected suitable water relations in the soil matrix for germination of chlamydospores and for infection by zoospores released after indirect germination.

## LITERATURE CITED

1. ADEBAYO, A. A., and R. F. HARRIS. 1971. Fungal growth responses to osmotic as compared to matric water potentials. Soil Sci. Soc. Am. Proc. 35:465-469.
2. ANDERSON, E. J. 1951. Effect of nutrient salt concentrations on infection of pineapple roots by Phytophthora cinnamomi. Phytopathology 41:2 (Abstr.).
3. AYERS, A. D. 1950. Salt tolerance of avocado trees grown in culture solutions. Calif. Avocado Soc. Yrbk. 1950:139-148.
4. BOYER, J. S. 1966. Isopiestic technique: measurements of accurate leaf water potentials. Science 154:1459-1460.
5. COLLIS-GEORGE, N., and J. WILLIAMS. 1968. Comparison of the effects of soil matric potential and isotropic effective stress on the germination of Lactuca sativa. Aust. J. Soil. Res. 6:179-192.
6. COOK, R. J., and R. I. PAPENDICK. 1972. Influence of water potential on root disease. Annu. Rev. Phytopathol. 10:349-374.
7. DUNIWAY, J. M. 1976. Movement of zoospores of Phytophthora cryptogea in soils of various textures and matric potentials. Phytopathology 66:877-882.
8. GARDNER, W. R. 1968. Availability and measurement of soil water. Pages 107-135 *in* T. T. Kozlowski, ed. Water deficits and plant growth, Vol. I. Academic Press, New York. 390 p.
9. GRIFFIN, D. M. 1972. Ecology of soil fungi. Syracuse Univ. Press, Syracuse, New York. 193 p.
10. MIRCETICH, S. M., G. A. ZENTMYER, and J. B. KENDRICK, JR. 1968. Physiology of germination of chlamydospores of Phytophthora cinnamomi. Phytopathology 58:666-671
11. SOIL SCIENCE SOCIETY OF AMERICA. Committee on terminology. 1965. Glossary of soil science terms. Soil Sci. Soc. Am. Proc. 29:330-351.
12. SOMMERS, L. E., R. F. HARRIS, F. N. DALTON, and W. R. GARDNER. 1970. Water potential relations of three root-infecting Phytophthora species. Phytopathology 60:932-934.
13. TSAO, P. H., and G. OCANA. 1969. Selective isolation of species of Phytophthora from natural soils on an improved antibiotic medium. Nature 223:636-638.
14. U. S. SALINITY LABORATORY STAFF. 1954. Diagnosis and improvement of saline and alkali soils. U. S. Dep. Agric., Agric. Handb. No. 60. 160 p.
15. ZENTMYER, G. A., and A. O. PAULUS. 1957. Avocado root rot. Calif. Agric. Exp. Stn. Circ. 465. 16 p.