

Water Potential Relations of Three Root-infecting *Phytophthora* Species

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ABSTRACT

The effect of nutritional status and mode of water potential control on the susceptibility to water stress of three soil-borne *Phytophthora* species was investigated. The osmotic water potential relations of *Phytophthora cinnamomi*, *P. megasperma* var. *sojae*, and *P. parasitica* were relatively independent of the inorganic solute (KCl or salts mixture) used for water potential control of a low organic nutrient agar medium: Optimum growth of *P. cinnamomi* occurred at -10 to -15 bars; *P. megasperma* var. *sojae* growth rate declined steeply with decreasing potential, and growth ceased at about -30 bars; although *P. parasitica* growth also declined with decreasing potential, some growth occurred at -50

bars. Substitution of sucrose to control the water potential of the basal medium or the use of nutrient-rich media increased the growth rates at all water potential levels. The nutrient-water potential interaction was particularly pronounced for *P. parasitica*; on V-8 juice media, no appreciable water stress occurred before -15 bars, and vigorous growth was evident at -40 bars. *Phytophthora cinnamomi* showed a marked growth rate reduction and susceptibility to water stress on vapor pressure-controlled as compared to osmotic-controlled agar media; it is probable that this growth response reflected solute transport as well as water potential properties of the media. *Phytopathology* 60: 932-934.

As reviewed recently by Griffin (4, 5), although water is a major soil environmental variable affecting fungal growth, unambiguous information is limited on the water relations of fungi in soil. This paper examines the effect of nutritional status and mode of water potential control on the susceptibility to water stress of three soil-borne pathogens, *Phytophthora cinnamomi* Rands, *P. parasitica* Dast., and *P. megasperma* (Drechs.) var. *sojae* Hild. The work is part of a broader study to characterize and delineate the factors affecting the response of root-infecting pathogens to fluctuations in soil water status.

MATERIALS AND METHODS.—*P. cinnamomi*, *P. megasperma* var. *sojae*, and *P. parasitica* were obtained from J. E. Mitchell (Department of Plant Pathology, Univ. Wis., Madison).

Growth on media of osmotic-controlled water potential.—The effect of osmotic water potential on *Phytophthora* growth was investigated using substrates osmotically maintained at various water potentials by (i) addition of different solutes to the same basal medium, and (ii) addition of the same solute to media of different composition. For the first experiment, a relatively low water potential basal medium comparable to that of Scott (10) and Roncadori (9) was adjusted to water potential levels ranging from -1.2 bars (nonamended basal medium) to -50 bars. The composition of the basal medium was as follows: Na₂HPO₄ (0.75 g), KH₂PO₄ (0.75 g), MgSO₄ (0.12 g), NaCl (0.10 g), NH₄NO₃ (0.40 g), glucose (1.80 g), yeast extract (0.10 g), malt extract (1.00 g), agar (15 g), H₂O (1 liter); pH 6.4. Water potential control involved addition of appropriate concentrations of KCl, sucrose (8), or a NaCl:KCl:Na₂SO₄ mixture (10). In the second experiment, KCl was used to control the water potentials of the basal medium (described above), potato-dextrose agar (PDA), V-8 juice agar (V-8), and malt extract agar (ME) (6). The water potentials of the nonamended media were determined psychrometrically

(1) to be -1.2, -3.4, -2.5, and -4.8 bars for the basal, PDA, V-8, and ME media, respectively. The potential of the solute-amended media was calculated as the sum of the media plus solute water potentials. The three *Phytophthora* species were grown in petri dishes on basal medium agar (2 mm thick) until the colony covered approximately three-fourths of the agar surface; then agar discs (4 mm diam) were cut from the periphery of the colony with a sterile cork borer and used to inoculate plates of agar media maintained osmotically at various water potentials. The inoculated dishes were secured with rubber bands, placed in plastic bags, and incubated at 23 ± 1.5 C. The plates were examined periodically during the incubation period, and the colony was measured with the aid of calipers. Triplicate plates were used in all experiments.

Comparative growth on media of vapor pressure- and osmotic-controlled water potential.—Petri dishes of dried basal medium agar were equilibrated isopiesticly for 2 weeks with appropriate vapor pressure-controlling KCl agar gels (A. A. Adebayo et al., unpublished data) in an attempt to obtain matric control of agar media water potential (13). The experimental approach was basically the same as is used commonly for isopiestic (equal vapor pressure) equilibration and maintenance of substrates at known water vapor pressures (8, 11). Because agar films in the -5 to -50 bar range have approximately a 1:1 water:agar ratio (unpublished data), the initial basal medium (50:1, water:agar) was diluted 50-fold to minimize osmotic contribution to the total water potential of the equilibrated agar films and to minimize nutrient discrepancies between the agar films and the undried basal agar medium. As will be presented in detail elsewhere, however, precise control of the nutrient status of isopiestic-equilibrated agar films is questionable because of ill-defined water gradients, salt-sieving phenomena, and the presence of substantial amounts of uncharacterized solutes in the films. To compare *Phytophthora* growth in media of

vapor pressure- and osmotic-controlled water potential, a set of basal medium plates amended with appropriate amounts of KCl were inoculated with the *Phytophthora* at the same time that the isopiestic equilibration plates were inoculated. Inoculation and growth analysis were comparable to the preceding experiment.

RESULTS AND DISCUSSION.—Fungal responses to water potential differences were expressed in terms of colony diam growth rates to facilitate direct comparison of slow- and fast-growing fungi. As is true for many other fungi (2), after an initial short lag period, linear growth rates of the *Phytophthora* species were generally constant with time. Staling did not occur except, as discussed later, in the isopiestic (vapor pressure-controlled) agar systems. The major problem encountered was that of achieving inoculum plugs of the same physiological state (12) to ensure reproducible data between experiments. Although replication within experiments using the same fungal plates as inoculum source was consistently good, fungal growth rates obtained for a specific organism growing under the same experimental conditions but at different times (e.g., 6 months apart and therefore using a different source plate of inoculum) sometimes varied. But even when this occurred, growth trends related to media water potential differences were usually comparable. Another limitation of the agar plate method is the problem of fungal growth density evaluation. For most experimental systems, density differences were not marked; but for some, namely, the osmotic as compared to the isopiestic systems, growth density differences were not reflected in the growth rate-water potential relationships.

Water potential relations on media of osmotic-controlled water potential.—The three species of *Phytophthora* exhibited different responses and tolerances to increasing substrate water potential caused by addition of inorganic salts (Fig. 1-A). Optimum growth of *P.*

cinnamomi occurred at -10 to -15 bars. This was a consistent characteristic of this fungus on relatively low organic nutrient media. The phenomenon of optimum growth at water potentials appreciably less than 0 bars is not uncommon, especially for fungi (11), but is largely neglected as a possible factor affecting microbial growth in nutritional studies involving additions of different nutrient levels. The other two *Phytophthora* species growing on the inorganic salts-amended media (Fig. 1-A) grew best at the highest water potential (-1 to -2 bars) and declined thereafter. *Phytophthora megasperma* var. *sojae* growth rate declined relatively steeply, so that growth cessation occurred at about -30 bars. In contrast, although *P. parasitica* growth declined with decreasing water potential, growth still occurred at < -50 bars. The water potential relations of the three *Phytophthora* species appeared to be relatively independent of the salt used for adjusting media water potential (Fig. 1-A), although growth rates and water stress tolerances were in general higher for the KCl than the NaCl:KCl:Na₂SO₄ solute-amended media.

The use of sucrose as the water potential-controlling solute resulted in higher growth rates for all species, presumably due to sucrose utilization as a carbon and energy source by the *Phytophthora* species (3). Thus, the increased growth of *P. parasitica* and *P. megasperma* var. *sojae* at -8 bars was more likely a response to increased available carbon (sucrose) than to decreased water potential. Apart from this particular nutrient interaction, the growth-water potential curves obtained using different solutes were similar (Fig. 1-A), indicating that, as for other fungi such as *Aspergillus amstelodami* (11), the *Phytophthora* growth trends on media of increasing solute concentration were related more to water stress than to specific ion toxicity.

From a practical standpoint, the effect of sucrose in displacing the growth-water potential curve upward and increasing the growth rate at all water potential levels was to increase the water potential growth range of the *Phytophthora* species, particularly *P. cinnamomi* and *P. megasperma* var. *sojae* (Fig. 1-A). This nutrition-water potential interaction has also been observed for other microorganisms (11), and may influence competitive growth of pathogens in soils. Accordingly, the effect of nutrition on *Phytophthora* growth response at different water potential levels was evaluated in more detail using three rich media known to be more favorable to *Phytophthora* than the basal medium. The water potential was adjusted with KCl, since this solute appeared to have less adverse physiological effects than NaCl or the salts mixture (Fig. 1-A). The effect of nutrient status on growth-water potential relations was much more pronounced for *P. parasitica* than for *P. cinnamomi* (Fig. 1-B). Whereas water stress was apparent at > -10 bars on the basal medium (Fig. 1-A), no appreciable growth reduction of *P. parasitica* occurred on the richer media until < -15 bars. With adequate nutrition, *P. parasitica* grows well at water potentials below -40 bars (Fig. 1-B). This emphasizes the importance of considering nutrient-water potential interactions, since Kouyeas (7) reported that *P. para-*

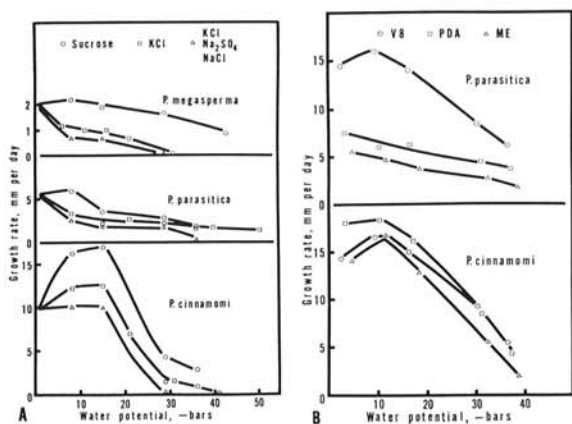


Fig. 1. A) Relationship between growth rate and substrate water potential for three *Phytophthora* species grown on a low nutrient basal medium in which the water potential was controlled by addition of KCl, sucrose, or a mixture (5:3:2 molal ratio) of KCl, Na₂SO₄, and NaCl. B) Relationship between growth rate and substrate water potential for two *Phytophthora* species grown on media of different nutritional composition in which the water potential was controlled by KCl addition.

sitica ceased to grow onto glass slides devoid of nutrients at a water potential equivalent to about -30 bars.

Comparative growth on media of vapor pressure-controlled and osmotic-controlled water potential.—This experiment was designed initially to evaluate the growth of the *Phytophthora* on agar media of osmotic-controlled as compared to matric-controlled water potential. Recent evidence (Dalton et al., unpublished data) indicates that on the basis of present knowledge it is not possible to partition the potential energy between a matric and an osmotic component for agar films. But there is no doubt that agar gels exert a matric force on water, as do other porous and adsorptive systems such as soil, and that the relative importance of matric as compared to osmotic forces in agar gels increases with decreasing water content.

Phytophthora parasitica and *P. megasperma* var. *sojae*, in contrast to *P. cinnamomi*, showed similar growth trends and tolerances to substrate water potential whether the water potential was controlled osmotically or isopiesticly. The typical growth-water potential curve shown by *P. cinnamomi* growing on osmotic-controlled basal medium, i.e., a definite growth optimum at -10 to -15 bars, was not evident on the plates of vapor pressure-controlled water potential (Fig. 2). Rather, growth rates of *P. cinnamomi* on the vapor pressure-controlled plates declined rapidly with decreasing water potential, and growth cessation occurred at about -20 bars.

Even when the *Phytophthora* growth rates were similar, mycelia density was always visibly much less on the isopiestic plates. This is most probably related to

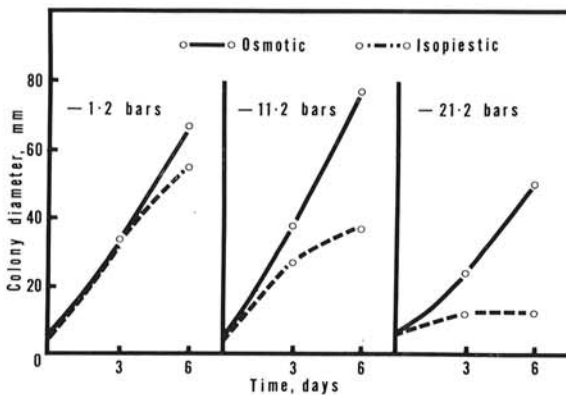


Fig. 2. Relative growth of *Phytophthora cinnamomi* on low nutrient basal medium maintained at different water potentials by osmotic (KCl addition to the medium) and isopiestic (equilibration of medium at controlled vapor pressure) mechanisms.

differences in solute transport properties of the osmotic and isopiestic plates; considerable reduction in the rate of nutrient diffusion to and metabolic by-products diffusion away from the fungal colonies might be expected in the relatively dry agar films of the isopiestic plates as compared to the high water content (2% agar) osmotic media. This is exemplified by the increasing staling effect shown by *P. cinnamomi*, with decreasing water potential in the isopiestic but not the osmotic system (Fig. 2).

The pure culture *Phytophthora* data, combined with similar data on other microorganisms (10), and the fact that fungi tend to be more susceptible to matric than osmotic water stress in soil systems (A. A. Adebayo et al., unpublished data), indicate that the effect of soil water on fungal growth in soil should not be interpreted solely from a simple potential energy basis, but that the nutrient status and the dynamics of ion and water uptake must also be considered. A complete understanding of the relative effects of the different components of water potential on microbial growth in soil awaits elucidation of the osmoregulation mechanisms involved in the simultaneous uptake of ions and water by microorganisms in response to water stress.

LITERATURE CITED

- BARRS, H. D. 1968. Determination of water deficits in plant tissues, p. 235-368. In T. T. Kozłowski [ed.] Water deficits and plant growth, Vol. I. Academic Press, N.Y. 390 p.
- BUTLER, G. M. 1961. Growth of hyphal branching systems in *Coprinus disseminatus*. Anal. Bot. N.S. 25: 341-352.
- CAMERON, H. R., & G. M. MILBRATH. 1965. Variability in the genus *Phytophthora*. Phytopathology 55:653-657.
- GRIFFIN, D. M. 1963. Soil moisture and the ecology of soil fungi. Biol. Rev. 38:141-166.
- GRIFFIN, D. M. 1969. Soil water in the ecology of fungi. Annu. Rev. Phytopathol. 7:289-310.
- JOHNSON, L. F., E. A. CURL, J. H. BOND, & H. A. FRIBOURG. 1959. Methods of studying soil microflora-plant disease relationships. Burgess Publishing Co., Minneapolis, Minn. 178 p.
- KOUYEAS, V. 1964. An approach to the study of moisture relations of soil fungi. Plant Soil 20:351-363.
- ROBINSON, R. A., & R. H. STOKES. 1955. Electrolyte solutions. Academic Press, N.Y. 571 p.
- RONCADORI, R. W. 1965. A nutritional comparison of some species of *Phytophthora*. Phytopathology 55: 595-599.
- SCOTT, W. J. 1953. Water relations of *Staphylococcus aureus* at 30 C. Australian J. Biol. Sci. 6:549-564.
- SCOTT, W. J. 1957. Water relations of food spoilage microorganisms. Advances Food Res. 7:83-127.
- WARD, E. W. B., & A. W. HENRY. 1961. Comparative response of two saprophytic and two plant parasitic soil fungi to temperature, hydrogen ion concentration, and nutritional factors. Can. J. Bot. 39:65-79.
- WIEBE, H. H. 1966. Matric potential of several plant tissues and biocolloids. Plant Physiol. 41:1439-1442.