

Effect of Water Potential on the Growth and Survival of *Macrophomina phaseolina*

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ABSTRACT

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A vapor-equilibration technique was developed to study the effect of growth-medium water potential on growth of *Macrophomina phaseolina*. Maximum growth at 30 C was attained at water potentials near -17 bars, with reduced growth above and below this water potential. Some growth was observed after 50 hr on media having water potentials as

low as -38 bars. Recovery of viable sclerotia from Edna fine sandy loam soil indicated that incubation for 2 wk at soil water potentials between -0.01 and -11.8 bars is deleterious to sclerotia survival only in soils maintained near saturation. The number of viable sclerotia recovered from saturated soils was reduced an average of 35%.

Macrophomina phaseolina (Tassi.) Goid [*Rhizoctonia bataticola* (Taub.) Butler] causes root and stem rots on a number of plant species (10). It is a soilborne fungus that survives in soil for long periods (11). Low soil moisture is reported to increase growth (8) and enhance survival (4) of *M. phaseolina* in soil. Several authors have suggested that low soil moisture levels favor the development of diseases caused by this fungus. It is apparent that soil water, and more particularly, soil water potential may be of great importance in the ecology of this fungus. In view of this, techniques were developed to study the effect of water potential on the growth of *M. phaseolina* and the survival of its sclerotia in soil.

MATERIALS AND METHODS

Growth experiments.—Sterile filter paper disks, 2 cm in diameter, were impregnated with potato-dextrose agar (PDA) containing 0.015% rose bengal. Five to seven disks were placed on van Tieghem cells which were embedded in equilibrating substrates (ES) in 25-ml petri dishes. The ES consisted of water agar amended with NaCl. Petri dishes were sealed with parafilm, covered with aluminum foil, and allowed to equilibrate in a chromatography jar partially immersed in a water bath at 30 ± 0.001 C for 1 wk. After equilibration, the disks received a standard 4-mm diameter plug of *M. phaseolina* grown on 1-mm-thick PDA. Mycelium plugs were cut with a cork borer from the periphery of a colony and transferred aseptically to the test medium. At the time of inoculation, two disks were removed from each petri dish for water potential determination. Dishes were resealed and returned to the

30-C water bath for 50 hr. After incubation, colony diameters were measured using a microscope with a calibrated eyepiece micrometer at $\times 15$. At the time of colony measurement, two additional disks were used for water potential measurement. Disk water potentials were determined with thermocouple psychrometers of the Spanner type (12).

Survival of sclerotia.—The soil used to evaluate survival of *M. phaseolina* in relation to soil water potential was an Edna fine sandy loam to which was added 65 viable sclerotia per gram of soil. Five soil samples (approximately 20 g each) were adjusted to -0.1, -0.5, -1.0, -3.0, -5.9, and -11.8 bars, respectively, using ceramic-plate soil moisture extractors. After equilibration to the adjusted water potentials, samples were sealed in moisture-proof cans with plastic electrical tape. A series of saturated samples (water potential taken to be -0.01 bar) were sealed in cans at the beginning of the moisture extraction process giving a total of seven water-potential treatments. All samples were incubated at 30 ± 1 C for 2 wk. Sample weights were taken before and after incubation to monitor water loss.

After incubation, samples were divided into two parts. One part was used for soil moisture determination (data not shown) and the other was used for recovery of sclerotia. Sclerotia were recovered via a modification of the method of Papavizas and Klag (9). It differed in that samples were not comminuted in a blender. The soil used in this study was coarse enough to disperse readily when added to the 0.5% NaOCl. The selective medium used to plate out aliquots of the sclerotia-soil suspension was a combination of that of Papavizas and Klag (9) and that of Meyer et al. (7) and consisted of PDA (27.5 mg/ml), Dexon (50 μ g/ml), chloroneb (300 μ g/ml), rose bengal (150 μ g/ml), HgCl (7 μ g/ml), streptomycin sulfate (40 μ g/ml), and sodium penicillin (60 μ g/ml). Plates were

incubated in the dark at 30 ± 1 C and colony counts were taken after 7 days for calculation of the number of viable sclerotia per gram of soil. Each soil sample was divided into five subsamples when plating so that there were at least 25 colony counts for each water potential treatment. This study was performed twice.

RESULTS AND DISCUSSION

The vapor-equilibration technique used to evaluate *M. phaseolina* growth provided low water potential substrates without any specific ionic effects of salts in the growth medium. The 2-cm PDA-disks also provided removable samples for water potential determinations. Water potentials of PDA-disks never reached that of the ES, but they decreased enough to provide a variety of water potentials for fungal growth evaluations (Fig. 1).

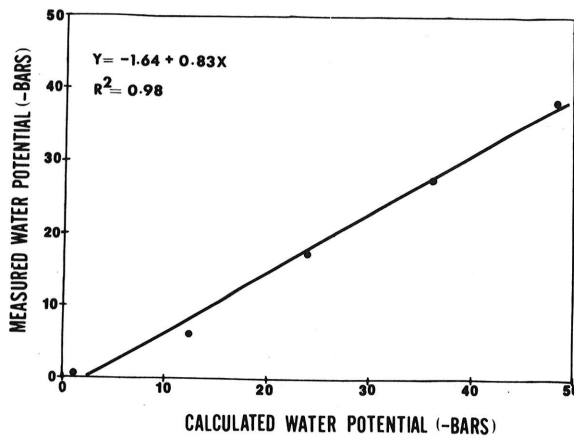


Fig. 1. A comparison of the calculated water potential of the equilibrating agar substrate to the measured water potential of agar-impregnated filter paper disks after 1 wk at 30 C. Equilibrating agar substrates consisted of water agar amended with NaCl.

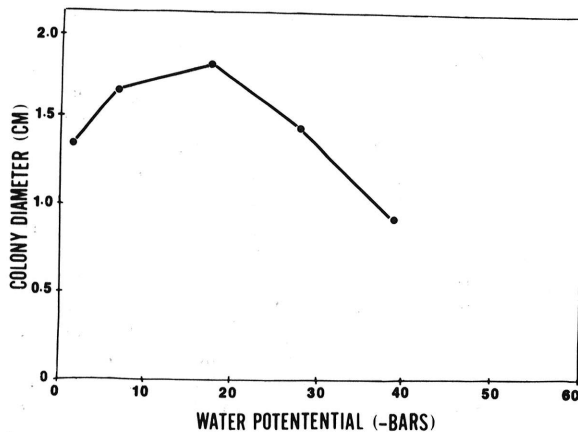


Fig. 2. Diameter of *Macrophomina phaseolina* colonies in relation to water potential after 50 hr of growth on PDA disks at 30 C. Water potential of disks were adjusted by equilibrating for 1 wk in petri dish chambers over water agar amended with NaCl. Inoculum plugs were initially 4 mm in diameter.

Data for *M. phaseolina* growth in relation to water potential of the agar medium (Fig. 2) depict what Adebayo et al. (1) describe as a typical fungal growth response to decreasing water potential. This is apparent as the optimum growth occurred between -15 and -20 bars with a gradual decrease in growth at lower water potentials. Growth equivalent to that for nonadjusted PDA was observed at a potential of -30 bars, with some growth at water potentials as low as -38 bars. Water potential values indicated here represent a mean of the preincubation and postincubation values.

Growth of *M. phaseolina* on artificial medium at low water potentials is not proof that it can grow in a soil or a plant under these conditions, but suggests that this could be the case. Under conditions of soil water stress, microflora are subjected to decreased water potentials which could favor the growth of *M. phaseolina* by decreasing the activity of competitive microorganisms or antagonists to this fungus.

The technique used to study sclerotial survival permitted soil samples to be adjusted to known water potentials and incubated without any appreciable change in water potential for 2 wk. The number of viable propagules recovered from soil incubated at water potentials from -0.1 to -11.8 bars were generally high (Fig. 3). Sclerotial survival was reduced 35% in saturated samples. Application of Duncan's multiple range test to the combined means for both experiments indicates that this is significantly less ($P = 0.05$) than drier samples. Some sclerotia apparently maintain viability up to 2 wk, even in saturated soils. This is supported by the observation that charcoal rot has occurred in south Texas fields that were alternately cropped to rice and soybeans.

Taken as a whole, the results of these experiments suggest that *M. phaseolina* can survive and grow in regions of the soil profile that become quite dry. If published estimates of the effect of soil water potential on bacterial activity are correct (2), this fungus should have a competitive advantage for substrates in the top few centimeters of soil during a substantial portion of the

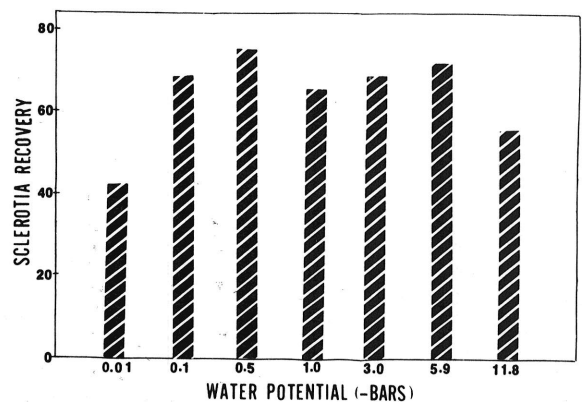


Fig. 3. Number of viable sclerotia of *Macrophomina phaseolina* recovered from Edna fine sandy loam inoculated with 65 sclerotia per gram of soil after incubation for 2 wk at seven different water potentials at 30 C. Recovery from the -0.01 bar treatment was reduced 35% and was the only treatment that decreased survival significantly, $P = 0.05$.

growing season in dryland areas. This could be a factor in the reported increased incidence of charcoal rot under hot, dry conditions (3, 5, 6).

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