

## Effect of water potential on sclerotial germination and mycelial growth of *Macrophomina phaseolina*

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**Summary.** The effect of the osmotic ( $\Psi_s$ ) and matric ( $\Psi_m$ ) potential on the sclerotial germination and mycelial growth of *Macrophomina phaseolina* was examined at room temperature. Sclerotial germination was determined in 0.1% water agar and mycelial growth on potato dextrose agar (PDA) and potato dextrose broth (PDB) amended with sodium chloride and polyethylene glycol (PEG 6000). Treatments consisted of 6 levels of osmotic and matric potentials (0, -0.3, -0.6, -0.9, -1.2, and -1.5 MPa) arranged in a factorial manner in a completely randomized design. Decreasing the matric and the osmotic potentials to -1.2 and -0.6 MPa, respectively, increased sclerotial germination and mycelial growth, but any further decrease caused both sclerotial germination and mycelial growth to decline again. It was concluded that the matric potential was more important as a factor than the osmotic potential in promoting the vegetative growth of *M. phaseolina*.

**Key words:** *Macrophomina phaseolina*, salinity, water stress, matric potential, osmotic potential.

### Introduction

*Macrophomina phaseolina* is a soilborne plant pathogen with a wide host range and a worldwide distribution (Dhingra and Sinclair, 1974). It causes charcoal rot and ashy stem blight of several important crops and is especially prevalent in subtropical and tropical arid climates. The pathogen survives in the soil mainly in the form of sclerotia, which are the primary source of inoculum (Olaya and Abawi, 1996).

Water potential is considered an important factor in the ecology and growth of soilborne plant pathogenic fungi (Woods and Duniway, 1986). Low soil water potential is the result of low soil moisture and low soil matric potentials (Richardson and McCree, 1985). All micro-organisms have optimal and minimal water potentials for growth (Cook and Papendick, 1972). Soil

fungi respond to fluctuation in the water potential and temperature by changes in their metabolic activity, vegetative growth, and reproductive strategy (Olaya and Abawi, 1996, Whiting and Rizzo, 1999, Whiting *et al.*, 2001). The matric potential is generally more important than the osmotic potential for the growth of soil borne plant pathogens. The ability of some fungi to grow better at lower osmotic than at lower matric potentials may be due to these fungi having a better ion uptake by the fungal cells. This results in cell osmotic potentials being better for cell functions and for the maintenance of turgor (Cook and Christen, 1976; Ma *et al.*, 2001).

Charcoal rot of different herbaceous and woody plants is a good example of a disease that is influenced by stress factors. High temperatures and low soil water potentials are important factors in causing this disease (Olaya *et al.*, 1996). Shokes *et al.* (1977) reported that low soil moisture increased growth and enhanced survival of *M. phaseolina* in the soil. Several authors have suggested that low soil moisture favors *M. phaseolina* (Odvody and Dunkle, 1979; Cook and

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Papendick, 1972; Diourt *et al.*, 1995). In culture, this fungus grows at high temperatures (35°C) and at low osmotic potentials, especially if nutrients are available. Sclerotia germinate readily over a wide range of water (osmotic) potentials (Odvody and Dunkle, 1979). It is clear that soil humidity and, more particularly, the soil water potential are of great importance in the ecology of *M. phaseolina* (Shokes *et al.*, 1977).

The objective of the present study was to determine how the matric and osmotic potentials affect sclerotial germination and mycelial growth of *M. phaseolina* and how these potentials interact with each other in producing their effect on the fungus.

## Materials and methods

### Inoculum production

Sclerotia of the melon isolate of *M. phaseolina* were cultured on potato dextrose broth (PDB) (Short and Wyllie, 1978). Cultures were incubated in the dark at 25°C for 12 wk and checked regularly for sclerotia. The inoculum was separated from the medium by vacuum filtration, rinsed with sterile distilled water, dried at 70°C for 72 h, and passed through a 325 mesh screen to obtain smaller units of sclerotia (Saadatmand *et al.*, 2006).

### Germination

The influence of osmotic and matric potential on the germination of sclerotia was studied at 25°C on a water medium adjusted to 0, -0.3, -0.6, -0.9, -1.2, and -1.5 MPa with sodium chloride (NaCl) for the osmotic and polyethylene glycol 6000 for the matric potential. The matric potential was estimated from the curve proposed by Zur (1966). The osmotic potential was adjusted using different rates of NaCl according to the US Salinity Laboratory Handbook (1954). A 0.1% aliquot of agar was added to increase medium viscosity (Saadatmand *et al.*, 2006).

For the germination assay, sclerotia of *M. phaseolina* were added to screw-cap bottles containing a 0.1% water agar solution with different matric or osmotic potentials, and incubated for 14 days at room temperature. Sclerotia germination was monitored by removing one drop from each bottle with a sterile pipet and inspecting the drop microscopically after 1 d to assess germination; inspection of sclerotia continued until a stable condition was reached and no further germination occurred. To prevent overlapping of the hyphae, a growth retardant, NPX (100 ppm) (Steiner and Watson, 1965),

was added to the medium. Two replications were used for each water potential treatment and the experiment was repeated twice.

### Mycelial growth

Mycelial growth, adjusted to different water potentials as described above, was determined at 25°C on potato dextrose agar (PDA) and potato dextrose broth (PDB). Depending on the results of the germination test, 4 levels of matric potential (0, -0.3, -0.9, and -1.2 MPa) were used in this experiment. Mycelial discs (5 mm in diameter) from the margin of 1-day-old colonies of *M. phaseolina* growing on PDA were transferred to the center of PDA plates or to PDB flasks. Four replications were used for each treatment. Plates were covered with parafilm and stored in closed plastic containers to avoid evaporation and changes in the water pressure. After incubating for 72 hours at 25°C, the radial growth of the fungus was determined. The mycelial weight of *M. phaseolina* in PDB was determined after incubating for 10 days at room temperature. Mycelial mats were separated using a Buchner funnel with Whatman filter paper No. 1, washed several times to remove the medium, dried for 25 h at 60°C, and weighed. The experiment was a factorial arrangement in a completely randomized design. Three replications were used for each water potential treatment. Analysis of variance was done using MSTATC software.

## Results and discussion

The influence of the different osmotic and matric potentials on sclerotial germination and mycelial growth of *M. phaseolina* followed nearly similar patterns. Sclerotial germination increased as osmotic potential decreased up to -0.6 MPa, but decreased again at lower osmotic potentials (Fig. 1). Optimum radial growth of the fungus occurred after 72 h, at an osmotic potential of -0.6 MPa, but radial growth gradually decreased at lower osmotic potentials. A similar trend was observed with the mycelial weight in liquid medium (Fig. 2).

Sclerotial germination increased with decreasing matric potential up to -1.2 MPa but then started to decrease again (Fig. 1). The interaction of matric and osmotic potentials caused the highest germination to occur at a matric potential of -1.2 MPa and an osmotic potential of -0.6 MPa (Table 1). Similarly, mycelial growth increased with decreasing matric potentials, with maximum growth at -1.2 MPa (Fig. 3).

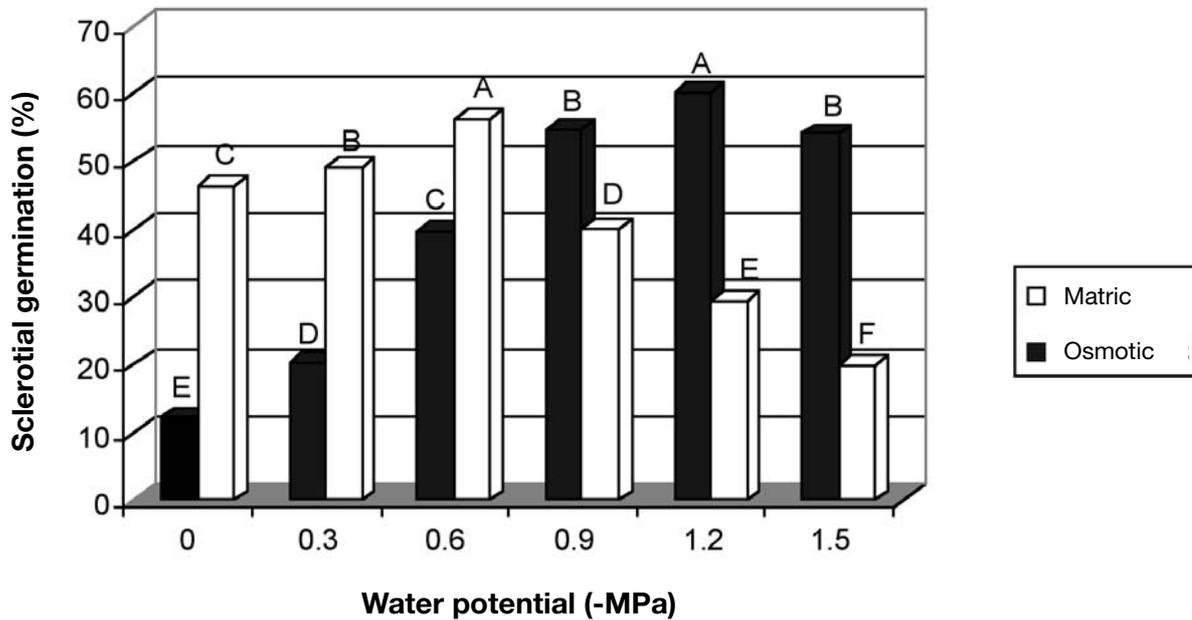


Fig. 1. Effect of matrix and osmotic potential on sclerotial germination of *Macrophomina phaseolina*. In the matrix and osmotic potential columns, values followed by the same letters are not significantly different at  $P \leq 0.05$  according to Duncan's Multiple Range Test.

Table 1. Effect of the matrix and the osmotic potential (from 0 to 1,5 -MPa) and their interaction on sclerotia germination of *Macrophomina phaseolina*.

Matrix potential (-MPa)	Sclerotia germination (%)					
	0	0.3	0.6	0.9	1.2	1.5
0	12.5 opq*	15 no	18 mn	12.5 opq	11 pq	4 r
0.3	23 l	23 l	36.5 j	19 m	11.5 opq	9.5 q
0.6	44.5 i	51.5 gh	60.5 f	42 i	25.5 l	14 op
0.9	60.5 f	63.5 ef	68.5 cd	51.5 gh	51 h	34 jk
1.2	72 bc	74 b	80.5 a	6 1f	42 i	32 k
1.5	66 de	68 d	74 b	55 g	36 j	26 l

\* Means followed by the same letters are not significantly different at  $P < 0.05$  according to Duncan's Multiple Range Test.

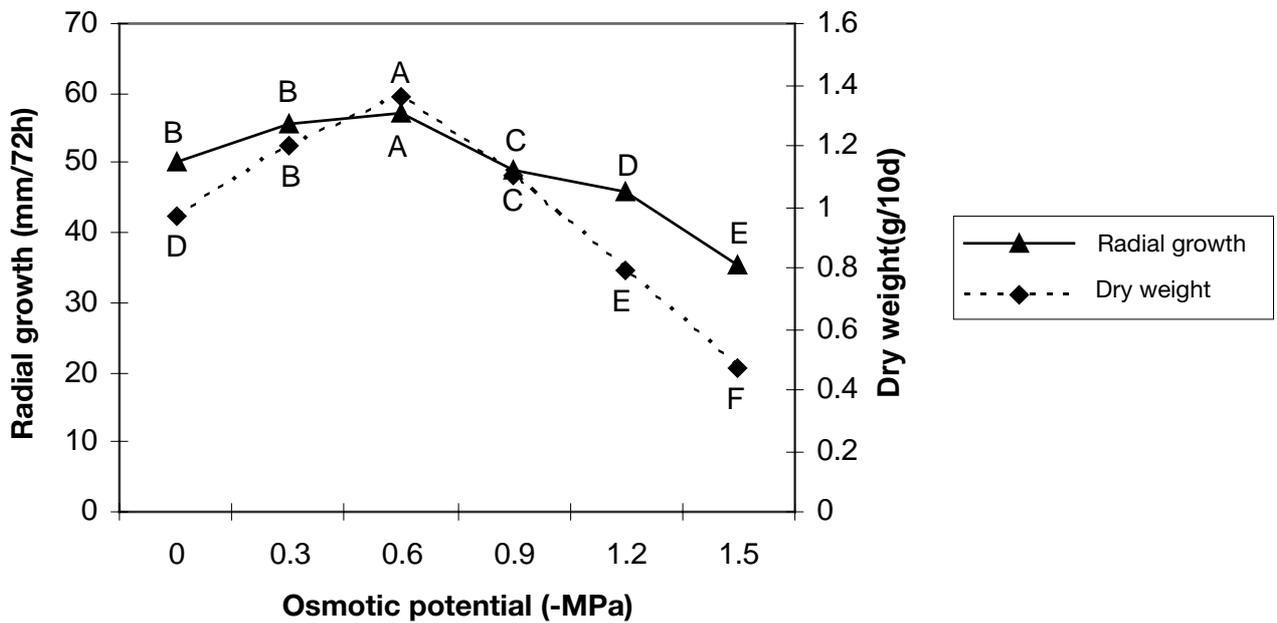


Fig. 2. Effect of osmotic potential on the radial growth and dry weight of *Macrophomina phaseolina* mycelia. Significant differences between treatments are indicated by different capital letters (Duncan,  $P \leq 0.05$ ).

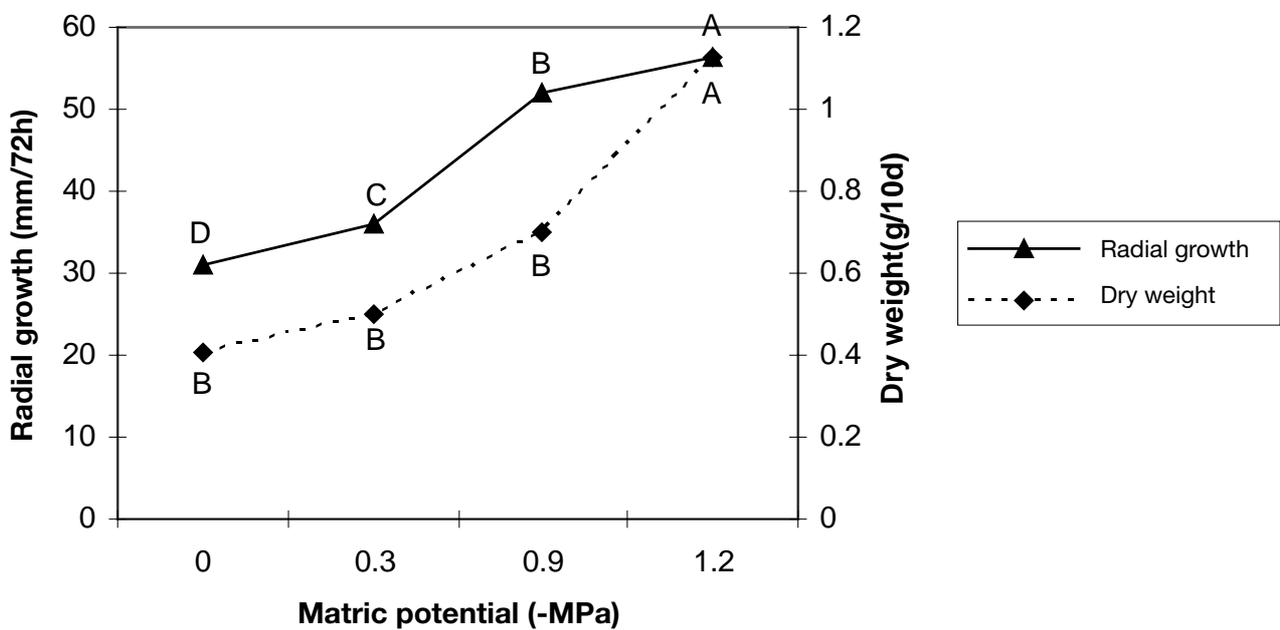


Fig. 3. Effect of matric potential on the radial growth and dry weight of *Macrophomina phaseolina* mycelia. Significant differences between treatments are indicated by different capital letters (Duncan,  $P \leq 0.05$ ).

The results were similar to those reported by Olaya and Abawi (1996) and by Shokes *et al.*, (1977) with sorghum isolate of *M. phaseolina*. This fungus produced large quantities of sclerotia, which germinated under relatively dry conditions (Olaya and Abawi, 1993). Shokes *et al.*, (1977) reported that at 30°C, *M. phaseolina* grew best at a water potential of -1.7 MPa and that sclerotial survival was drastically reduced at a water potential of about -0.001 MPa. The observed effects of the lower  $\Psi$ s on *M. phaseolina* suggest that this fungus maintains a positive turgor for hyphal tip growth as shown by an increase in mycelial growth and sclerotial production (Olaya and Abawi, 1996). Increased radial growth or dry weight of the mycelium due to decreasing  $\Psi$ s of the conventional medium has also been observed in other fungi such as *Verticillium dahliae* (Ioannou *et al.*, 1977), *Sclerotinia sclerotiorum* (Grogan and Abawi, 1975) *Fusarium oxysporum* (Brownell and Schneider, 1985), and *Phytophthora cinnamomi* (Sommers *et al.*, 1970).

Sepaskhah and Boersma (1979) stated that the effects of the matric potentials on growth was added to that of the osmotic potentials. The same cumulative trend was also found on microsclerotial germination in *Verticillium dahliae* (Saadatmand *et al.*, 2006). The water potential that was optimal for growth stimulation depended on the fungus, and in some cases on the osmoticum, the temperature, or other environmental factors. Growth stimulation may result from uptake of the solute, which lowers the water potential of the protoplasm to a value better adapted for cellular processes, or increases turgor and hence acceleration of growth (Cook, 1981).

*Macrophomina phaseolina* has previously been recognized to be a drought-favored pathogen (Waller, 1986). Olaya *et al.*, (1996) reported that *M. phaseolina* survived in the soil and colonized bean stem segments at low water potentials. Colonization increased as the osmotic potential was decreased to about -3.99 MPa. Survival of *M. phaseolina* sclerotia was drastically reduced at high matric potential, was less affected at low matric potential, and was not affected in the air-dry soils (natural and pasteurized). Cervantes-Garcia *et al.*, (2006) stated that charcoal rot grew vegetatively at relatively low water potentials. The growth of *M. phaseolina* in host tissues at a wide range of water potentials indicated that it had developed adaptative mechanisms to survive under varied environmental and host conditions

(Diourt *et al.*, 1995; Olaya *et al.*, 1996). Adaptation to a wide range of water potentials may be a strategy to enable the saprophyte or latent pathogen to survive in host tissue. The fungus may be able to continue vegetative development and grow under conditions of low moisture that would affect the host (Cervantes-Garcia *et al.*, 2006).

Germination of sclerotia and mycelial growth increased with decreasing osmotic potential up to -0.6 MPa. The percentage of sclerotial germination was inversely correlated with osmotic potential (Table 2). Sclerotial germination with an osmotic potential of -1.2 MPa was generally only half that achieved with the optimum osmotic potential.

Cervantes-Garcia *et al.*, (2006) reported that NaCl reduced growth of *M. phaseolina* *in vitro*, particularly in isolates MF-02, ICTA-2 and Mochis, while isolate MF-06 was tolerant to low osmotic potentials.

Sodium chloride is an inducible, compatible solute. Solutes in this category have fewer inhibitory effects on metabolic processes (Harris, 1981). Compatible solutes in the agar medium trap water molecules, so that no water is available to *M. phaseolina*. The energy spent by the fungus to obtain water molecules from the medium is increased as the solute concentration in the agar medium increases. Thus, the fungus reduces its growth rate under *in vitro* conditions (Cervantes-Garcia *et al.*, 2006).  $K^+$  is found in high concentrations within fungal cells, and is an anion compatible with cellular development.  $Na^+$  is found in high concentrations outside fungal cells, and an increase in  $Na^+$  concentrations modifies the biochemical pathways involved in water transport to the cells (Harris, 1981).

Fungal growth at low  $\Psi$ s values requires effective osmoregulation (Woods and Duniway, 1986; Amir *et al.*, 1992). The capacity of sclerotia to germinate at low  $\Psi$ s could be attributable to solute uptake by the sclerotium, in which reduces its osmotic potential and thus maintains the germination processes (Cook and Al-Hamdani, 1986).

In the present study, sclerotial germination and mycelial growth increased with decreasing matric potential. Germination and radial growth were reduced to 50% at matric potentials of about -0.3 MPa. Analysis of variance showed that sclerotial germination had a linear pattern with decreasing matric potential (Table 2). Only the relations between radial growth of *M. phaseolina*

and matric potential was significant and it is expressed by the following regression equation:

$$Y = 39.533X + 53.93 \quad R^2 = 0.99^{**}$$

where Y = radial growth (mm) and X = matric potential (-MPa).

Saadatmand *et al.*, (2006) reported that sclerotial germination of *V. dahliae* increased with decreasing osmotic potential and peaked at -0.6 MPa. Decreasing matric potential suppressed sclerotial germination. These researchers also found that decreasing osmotic and matric potential decreased germination of *Sclerotium rolfsii* sclerotia (Saadatmand *et al.*, 2007), so that the lowest germination occurred at the lowest osmotic and matric potential levels. The mycelial growth rate of *Phytium aphanidermatum* increased slightly with increasing EC levels up to 7.1 ds m<sup>-1</sup>. Zoospore production of the fungus decreased with increasing salinity levels up to 7.1 ds m<sup>-1</sup> and production was completely inhibited at 14.2 ds m<sup>-1</sup> (Rasmussen and Stanghellini, 1988).

Turco *et al.*, (2005) evaluated growth of *Phytophthora cambivora*, *P. cinnamomi*, *P. citricola* and *P. quercina* on basal media supplemented with polyethylene glycol 3350 and 6000. Optimum growth occurred at about -0.35 MPa, and declined when the osmotic potential was reduced to between -1.18 and -1.75 MPa. Mycelium growth was severely reduced at -3.00 MPa, and was completely inhibited at -3.98 MPa. That study suggested that minimum amounts of water are essential for the mycelial growth of *Phytophthora* spp.

*Phytophthora parasitica* was stimulated by salinity, forming maximum numbers of sporangia at 5-37 ds m<sup>-1</sup> (Blaker and MacDonald, 1986). It is known that many yeasts and fungi grow in the presence of high concentrations of sugars or salts, and that the production of polyols in mycelium and

cells goes up in response to a lowering of the osmotic potential of the medium. Polyols play an important role in maintaining a suitable environment for enzyme activity within the cytoplasm, where an unfavorable external osmotic potential prevails (Jennings, 1984).

Ma *et al.*, (2001) found that the spore germination, germ tube elongation, and mycelial growth of *Botryosphaeria dothidea* increased as the  $\Psi_m$  decreased from 0 to -2.0 MPa, but then declined again as the  $\Psi_m$  decreased further to below -2.0 MPa.

Three isolates of *Phaeoconiella chlamydospora* were similarly unresponsive to changes in the water potential, but there was some variation in how isolates of both *P. inflatipes* and *P. aleophilum* responded to changes in water potential (Whiting *et al.*, 2001). Adapting to a wide range of water potentials may also be a strategy for *Phaeoacremonium* spp. and particularly for *Phaeoconiella chlamydospora*, to survive as an endophyte or as a latent pathogen in grapevine xylem tissue. The fungus may continue its vegetative development and grow even under conditions of low moisture that would stress the grapevine host.

Root rot caused by *Phytophthora cinnamomi* is more severe when the water content of soil remains near saturation (zero water potential). In sandy soil with a varying osmotic water potential, the mycelial growth rate of *P. cinnamomi* was highest at -1 to -1.5 MPa (Sterne *et al.*, 1977). Thus, the fungus tolerated low water potentials and was less tolerant to stress associated with a low matric potential than to stress associated with a low osmotic potential.

Although no attempt was made in our experiment to measure melanin formation at the various water potential, the pigmentation of sclerotia declined with decreasing  $\Psi_s$  and was entirely inhibited at  $\Psi_m$  lower than -0.9 MPa. Cervantes-Garcia *et al.*, (2006) found that high NaCl concentrations redu-

Table 2. Regression equations describing the relation between sclerotial germination (Y) of *Macrophomina phaseolina* and osmotic and matric potential (X).

Water potential	Regression equation	Coefficient of determination
Osmotic potential	$Y = -35.109X + 97.771$	$R^2 = 0.6804^*$
Matric potential	$Y = 54.453X + 25.957$	$R^2 = 0.8531^{**}$

\*, \*\* significantly different at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively.

ced the synthesis of mycelial pigments and the size and shape of *M. phaseolina* microsclerotia. Lower amounts of melanin were synthesized from sclerotia following an increase in the solute concentration of agar medium. Olaya and Abawi (1996) reported a lack of black pigment in colonies of *M. phaseolina* growing on PDA at a  $\Psi$ s lower than -6.04 MPa suggesting that at these water potentials the synthesis of melanin was repressed. A similar result was found with *V. dahliae* at  $\Psi$ s values between -7 and -8 MPa (Ioannou *et al.*, 1977). Sodium chloride could be toxic and modify the synthesis of melanin or the expression of genes related to the synthesis of this pigment (Ioannou *et al.*, 1977; Olaya and Abawi, 1996).

The tolerance of *M. phaseolina* to reduced water potentials may be an important factor in the epidemic development of charcoal rot in the tropics characterized by high temperatures and droughts (Olaya and Abawi, 1996). An understanding of the effect of water potential on pathogen and disease development is critical to control soilborne plant diseases (Olaya *et al.*, 1996). Of the cultural practices that reduce damage by the fungus, applying irrigation water seems to be the most effective. Water management has a significant effect on root colonization by *M. phaseolina* (Kendig *et al.*, 2000).

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