Effect of Water Potential on Mycelial Growth and on Production and Germination of Sclerotia of *Macrophomina phaseolina*

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ABSTRACT

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The influence of osmotic water potential (Ψs) on growth parameters of *Macrophomina phaseolina* was determined at 30°C on potato dextrose agar (PDA) and potato dextrose broth (PDB) adjusted to different water potentials with KCl, NaCl, or sucrose. Radial growth of *M. phaseolina* on PDA was maximum at Ψs values between –1,220 and –1,880 J/kg, but growth was reduced at lower Ψs values. Biomass of *M. phaseolina* grown on PDB was maximum at Ψs values between –2,030 and –3340 J/kg. Production of sclerotia of *M. phaseolina* on PDB was not affected by Ψs of –670 to –3,920 J/kg, but was completely inhibited at Ψs between –8,270 and –12,020 J/kg. After 2 days of incubation, the germination of sclerotia of *M. phaseolina* on PDA was not significantly affected at Ψs of –320 to –4,760 J/kg, but germination was drastically reduced with further reductions in Ψs. After 10 days of incubation, the percentage of germinating sclerotia was higher than at earlier sample times, particularly at Ψs between –7,250 and –10,220 J/kg. The growth of colonies formed by germinating sclerotia at Ψs greater than –3,060 J/kg was reduced. The influence of Ψs adjusted by KCl, NaCl, or sucrose on *M. phaseolina* followed a similar pattern, but growth of *M. phaseolina* was much greater in media adjusted with sucrose. Results of this study showed that *M. phaseolina* can grow and produce large quantities of sclerotia under relatively low water potentials. These findings may partially explain why diseases caused by *M. phaseolina* have been recognized previously as being favored by drought.

Additional keywords: beans, charcoal rot, *Phaseolus vulgaris*

Charcoal rot caused by the fungus *Macrophomina phaseolina* (Tassi) Goidanich (syn. *Rhizoctonia bataticola* (Taubenhaus) E. J. Butler) (17,27) causes considerable damage to beans (*Phaseolus vulgaris* L.) in many production areas. The overall impact of charcoal rot on bean production is highly variable as disease incidence and severity are influenced by many physical and biological factors. Disease development is generally considered to be enhanced by some combinations of heat stress, soil water deficit, coarse texture soil, or physiological stress associated with seed set and development (11).

This pathogen survives in soil mainly in the form of sclerotia, the primary source of inoculum. The sclerotia are formed in host tissues and are released into the soil as infected tissues decay. Viable microsclerotia have been shown to persist in soil for 3 months to 3 years (10). The pathogen is also seedborne (1), and thus infected seeds can serve as a source of inoculum and an efficient method for the dissemination of the pathogen.

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Water potential is recognized as an important parameter in the ecology and growth of plant pathogenic fungi (31). Soil fungi respond to fluctuations of water potential and temperature by changes in metabolic activities, vegetative growth, and reproductive strategies (19). The effect of water potential on the development of charcoal rot in beans is still unclear, as are its effects on the growth and ecology of the fungus. Few reports dealing with the effect of water potential on growth of *M. phaseolina* are available (21,28); none exists on the effect of water potential on production and germination of sclerotia. In one of the studies on the effect of water potential on growth of *M. phaseolina* (28), only a limited range of water potentials and one osmoticum were used. The objective of this study was to determine the effect of water potential on mycelial growth, and on sclerotial production and germination of *M. phaseolina* under controlled conditions. Preliminary reports of this study have been published (22,23).

MATERIALS AND METHODS

Effect of water potential on mycelial growth of *M. phaseolina*. The influence of osmotic water potential (Ψs) on mycelial growth of *M. phaseolina* was determined at 30°C on potato dextrose agar (PDA) and potato dextrose broth (PDB) adjusted to different water potentials with KCl, NaCl, or sucrose. An isolate of *M. phaseolina* obtained from naturally infected beans in Colombia was used throughout this study. The isolate was maintained on beet seeds at 5°C and was transferred to PDA as needed.

Osmotic potential of unamended PDA and PDB was determined psychrometrically with a thermocouple psychrometer and HR-33 dew point microvoltmeter (Wescor Inc., Logan, UT). The osmotic potential of the amended media was calculated as the sum of the derived water potential of unamended PDA or PDB and the theoretical water potential provided by the osmoticum at a particular concentration in pure water. Water potential was expressed in J/kg. One J/kg is equivalent to 0.01 bar (5,18–20,25,26).

Mycelial disks (5 mm in diameter) from the margin of 4-day-old colonies of *M. phaseolina* growing on unamended PDA were transferred to the center of PDA or PDB dishes adjusted to different water potentials. There were four replications of each water potential treatment in each test. Plates were stored in closed plastic boxes to avoid evaporation and changes in osmotic pressure. After 72 h of incubation at 30°C, the radial growth of the fungus on PDA was determined by measuring the radius of the colony. Growth of *M. phaseolina* colonies on PDB was measured after 10 days of incubation and recorded as the increase in colony dry weight (15). Mycelial mats were recovered, washed several times to remove the growth medium and osmoticum, dried at 60°C for 24 h, and weighed.

Effect of water potential on production and germination of sclerotia. Production of sclerotia was determined by growing *M. phaseolina* in PDB adjusted to different water potentials at 30°C for 15 days. Sclerotia were collected and dried in an oven at 30 to 35°C. Dried sclerotial masses were gently ground in a mortar to separate sclerotia. Production of sclerotia was determined by suspending a subsample of the dried sclerotia into 200 ml of distilled water. The sclerotial suspension was then homogenized by blending for 30 s. One-millimeter samples of the homogenized suspension were placed in nematode-counting chambers and the number of sclerotia in the samples was counted under a dissecting microscope (15). Production of sclerotia was expressed as total number of sclerotia per colony.

The effect of water potential on sclerotial germination was determined by placing sclerotia of *M. phaseolina* in petri dishes containing the osmotically modified PDA. These sclerotia were obtained by growing *M. phaseolina* on toothpicks. Sterilized toothpicks were placed on colonies of *M. phaseolina* growing on PDA. After 10 to 14 days of incubation at 30°C, toothpicks with sclerotia were surface sterilized for 1 to 2 min in 1% sodium hypochlorite, rinsed with sterile distilled water, and dried for at least 1 to 2 days. Twenty sclerotia were used per each water potential treatment. Germination of sclerotia was recorded after 2 and 10 days of incubation at 30°C.

RESULTS

Effect of water potential on mycelial growth of *M. phaseolina*. After 72 h of incubation, the radial growth of *M. phaseolina* increased as Ψs decreased and was maximum at Ψs values between –1,220 and –1,880 J/kg, basal PDA –320 J/kg. Radial growth was reduced for Ψs values lower than –1,880 J/kg (Fig. 1). Radial growth of *M. phaseolina* on sucrose-adjusted PDA declined more slowly with decreasing Ψs than on KCl- or NaCl-adjusted PDA. About 50% reduction in radial growth occurred at –3,060, –4,760, and –9,870 J/kg on PDA adjusted with NaCl, KCl, and sucrose, respectively. Cultural differences, such as color, of *M. phaseolina* colonies grown on osmotically adjusted PDA were observed. Colonies of *M. phaseolina* were usually dark black or black in color at Ψs higher than –3,990 J/kg, whereas colony color was gray at Ψs between –3,990 and –4,550 J/kg and whitish at Ψs lower than –6,040 J/kg.

Similarly, dry weight of *M. phaseolina* colonies after 10 days of incubation in PDB increased as Ψs decreased to Ψs values between –2,030 and –3,340 J/kg, basal PDB –670 J/kg. However, the dry weight of colonies was reduced with further reduction in the Ψs of PDB (Fig. 2). Growth of *M. phaseolina* expressed as mycelial dry weight was considerably higher in the sucrose-adjusted PDB than in PDB adjusted with KCl or NaCl. Mycelial dry weight also declined more slowly when the Ψs of PDB was adjusted with sucrose. Reduction in mycelial dry weight was about 50% at –3,060, –4,760, and –9,870 J/kg in PDB adjusted with NaCl, KCl, and sucrose, respectively. The minimum Ψs value that allowed growth on PDA and PDB was between approximately –9,000 and –12,000 J/kg. The Ψs experiments on PDA and PDB were conducted twice and similar results were obtained.

Effect of water potential on production and germination of sclerotia. Production of sclerotia of *M. phaseolina* was not adversely affected by Ψs between –670 and –3,920 J/kg, but was completely inhibited at Ψs between –8,270 and –10,220 J/kg (Fig. 3). Production of sclerotia was stimulated when Ψs was decreased from –670 J/kg, especially in PDB adjusted with KCl or sucrose. Production of sclerotia was highest at Ψs between –2,010 and –2,500 J/kg, and steadily decreased at lower Ψs values (Fig. 3). Greater production of sclerotia was obtained when the osmoticum in PDB was sucrose.

The influence of the different Ψs of the PDA adjusted with KCl, NaCl, or sucrose on germination of sclerotia followed a similar pattern. After 2 days of incubation, germination of sclerotia on PDA was not significantly affected at Ψs values of –320 to –4,760 J/kg, but germination was drastically reduced with further reductions in Ψs. After 10 days of incubation, germination of sclerotia increased, especially at Ψs between –7,250 and –10,220 J/kg (Fig. 4). The growth of colonies formed by germinated sclerotia on PDA with Ψs lower than –3,060 J/kg was reduced. Sclerotial production and germination experiments were conducted twice and the results obtained showed a similar pattern.

DISCUSSION

Macrophomina phaseolina has been recognized previously as a drought-favored pathogen (24,30). Results of this study showed that this fungus can grow vegetatively and produce large quantities of sclerotia, and sclerotia will germinate, under relatively low water potentials. Radial growth and dry weight of mycelia were increased when the Ψs was reduced to values of approximately –1,880 and –3,340 J/kg, respectively. In 1977, Shokes et al. (28) reported similar growth stimulation by Ψs for *M. phaseolina*. In addition, increased radial growth or dry weight of the mycelium as a result of decreasing Ψs of conventional media has been observed in other fungi such as *Verticillium dahliae* Kleb. (15), *Sclerotinia sclerotiorum* (Lib.) de Bary (12), *Fusarium oxysporum* (Schlechtend.:Fr.) (4), *Sclerotinia minor* Jagger (14), *Botrytis squamosa* J. C. Walker (2), *Fusarium culmorum* (W. G. Sm.) Sacc., *Fusarium graminearum* Schwabe, and *Gaeumannomyces graminis* (Sacc.) Arx & D. Olivier var. *tritici* J. Walker (9), and *Phytophthora cinnamomi* Rands, *Phytophthora megasperma* Drechs., and *Phytophthora parasitica* Dastur (29).

The amount of stimulation and the exact optimal water potential depends on the fungus, and in some cases the osmoticum, temperature, or other factors in the environment (7). Stimulation of growth may result from uptake of the solute, which may lower the water potential of the protoplasm to a value more ideal for cellular processes or may increase turgor and hence acceleration of growth (7). Growth of *M. phaseolina* at different soil matric potentials (Ψm) needs to be determined. The growth of various soilborne plant pathogenic fungi has usually been more affected at low Ψm than at low Ψs. The ability of some fungi to grow at lower osmotic than matric potentials may be due to ion uptake by the fungal cells, resulting in cell osmotic potentials more ideal for cell functions and maintenance of turgor (7,8).

The lack of black pigment exhibited by colonies of *M. phaseolina* growing on PDA at Ψs lower than –6,040 J/kg suggests that the synthesis of melanin is repressed.

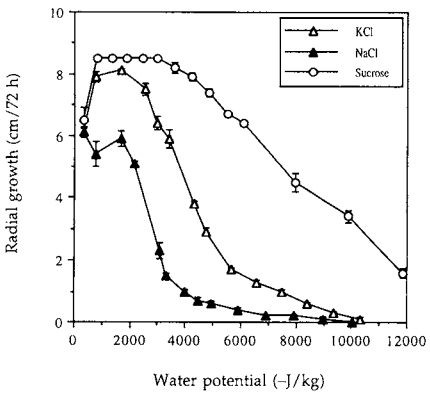

Fig. 1. Radial growth of *Macrophomina phaseolina* on potato dextrose agar adjusted to different osmotic water potentials with KCl, NaCl, or sucrose. Colony radius was recorded after 72 h of incubation.

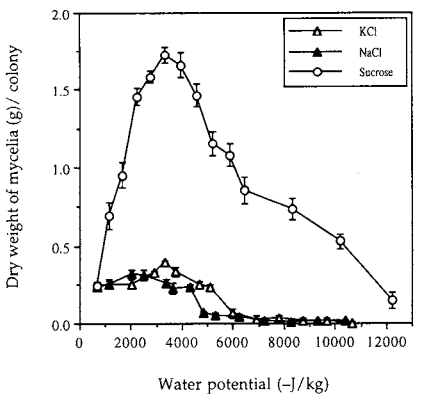

Fig. 2. Biomass of *Macrophomina phaseolina* on potato dextrose broth at various water potentials adjusted with KCl, NaCl, or sucrose.

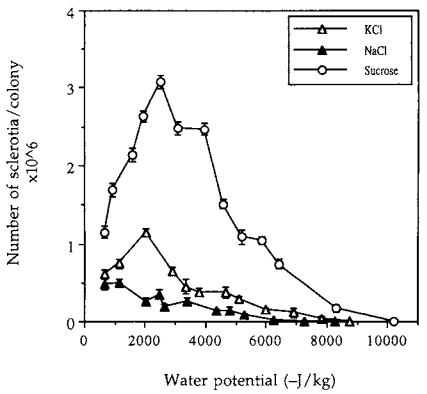

Fig. 3. Sclerotial production of *Macrophomina phaseolina* on potato dextrose broth adjusted to different osmotic water potentials with KCl, NaCl, or sucrose after 15 days of incubation.

Production of melanin of microsclerotia of *Verticillium dahliae* also was inhibited at Ψs values between –7,000 and –8,000 J/kg (15).

The growth curves obtained in this study with KCl, NaCl, or sucrose followed a similar pattern, indicating that the effect on growth is due mainly to differences in water potential. Growth of *M. phaseolina* was greater in media when the Ψs was adjusted with sucrose. The use of sucrose to control the water potential resulted in higher growth rates of different *Phytophthora* spp., which were reported to be due principally to sucrose utilization as a carbon and energy source (29,31). It is unlikely that the lower growth of *M. phaseolina* in media adjusted with KCl or NaCl was due to toxicity or deprivation of selected nutrients in the media by these salts. Both KCl and NaCl have been used in several water potential studies involving various plant-pathogenic fungi, with similar results (2, 15,29,31). In addition, it has been observed that potassium ions are easily accumulated by microbial cells and can serve as a compatible cytoplasmic osmoticum of low toxicity (13). NaCl is considered to be an inducible, compatible solute. Solutes in this category are known to be less inhibitory to metabolic processes (13). A large number of yeasts and fungi are known to grow in the presence of high concentrations of sugars or salts (16). It has been observed that the production of polyols increased in mycelium and cells in response to a decrease in the osmotic potential of the medium. It has been suggested that polyols play an important role in maintaining a suitable environment for enzyme activity within the cytoplasm in the face of a potentially unfavorable external osmotic potential (16). Thus, it is possible that the higher growth of *M. phaseolina* in the medium adjusted with sucrose may be due to the higher production of polyols by the fungus when the water potential of the medium was adjusted with this osmoticum and not with KCl or NaCl.

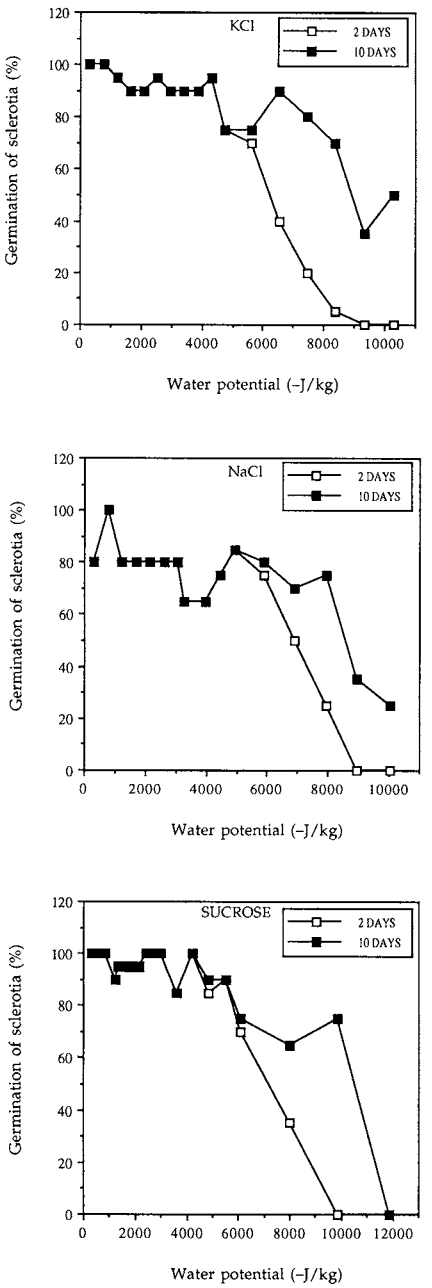

Fig. 4. Germination of sclerotia of *Macrophomina phaseolina* on potato dextrose agar adjusted to different osmotic water potentials with KCl, NaCl, or sucrose.

Production of sclerotia of *M. phaseolina* was not affected significantly at Ψs of –670 to –3,920 J/kg, but production was inhibited at Ψs between –8,270 and –12,020 J/kg. These results indicate that production of sclerotia by *M. phaseolina* may be enhanced at moderate water deficits. The observed effects of reduced Ψs on *M. phaseolina* reported here suggest that this fungus is able to maintain positive turgor for hyphal tip growth as documented by the increase in mycelial growth and sclerotial production. Fungal growth at low Ψs values requires effective mechanisms of osmoregulation (3,31). Sclerotial formation facilitates maintenance of a quiescent viable state in the absence of either a suitable host or conditions that favor saprophytic growth (6). Ability of sclerotia to germinate at low Ψs is perhaps attributable to solute uptake by the sclerotium bringing about a reduction in its internal osmotic potential and so allowing maintenance of germination processes (6).

The tolerance of *M. phaseolina* to reduced water potentials documented in this study may have an important role in the epidemic development of charcoal rot on beans in production areas in the tropics characterized by high temperatures and drought conditions. Knowledge gained from studying the effects of water potential on *M. phaseolina* may also be used to modify certain cultural practices for the effective management of damage inflicted by this pathogenic fungus.

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