Influence of Water Potential on Survival of Sclerotia in Soil and on Colonization of Bean Stem Segments by *Macrophomina phaseolina*

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ABSTRACT

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Survival of sclerotia of *Macrophomina phaseolina* was studied in both nonpasteurized (natural) and pasteurized very fine, sandy loam soils. The soil matric water potentials (Ψm) tested were 0, –10, –30, –100, –300, –500, and –1,500 J/kg. An air-dried soil treatment also was included in this study (about –40,000 J/kg). At a Ψm of 0 J/kg, viability of sclerotia was 40 and 0% after 2 and 4 weeks of incubation at 30°C, respectively. Survival of sclerotia decreased with time in the soil samples adjusted from –10 to –1,500 J/kg and remained about 100% viable in the air-dry soil treatment. After 20 weeks of incubation, viability of sclerotia was reduced to 10% in the Ψm treatment of –10 J/kg and was more than 50% at Ψm of –500 and –1,500 J/kg. Survival of sclerotia followed a similar trend in the pasteurized soil. However, the rate of decrease in survival of sclerotia in the pasteurized soil (Ψm = –10, –30, –100, –300, –500, and –1,500 J/kg) was less than that in the natural soil. The influence of water potential on colonization of bean stem tissues by *M. phaseolina* was studied in small constant-humidity chambers controlled with KCl solutions. Segments of bean stems inoculated with sclerotia of *M. phaseolina* were incubated in the chambers for 10 days at 30°C. The extent of colonization was increased as osmotic water potential (Ψs) decreased and was most severe at Ψs = –3,990 J/kg. However, further decreases in the Ψs resulted in a decrease in the extent of colonization, which was significantly diminished at Ψs lower than –7,150 J/kg. The number of sclerotia of *M. phaseolina* produced per square millimeter of colonized tissues was also increased as the Ψs in the incubation chamber was decreased from 0 to –5,330 J/kg. Maximal number of sclerotia was produced at Ψs = –3,990 J/kg and sclerotial production was still relatively high at Ψs = –8,080 J/kg. These results show that *M. phaseolina* can survive and colonize beans under relatively dry conditions.

Additional keywords: ashy stem blight, charcoal rot, *Phaseolus vulgaris*, water stress

Charcoal rot, also known as ashy stem blight, is an economically important disease of beans (*Phaseolus vulgaris* L.) in many parts of the world, especially in production areas with high temperatures and water stress (26). It has been considered a major problem in the southern United States, the Caribbean, and Central and South America (11). Charcoal rot is caused by the fungus *Macrophomina phaseolina* (Tassi) Goidanich (syn. *Rhizoctonia bataticola* (Taubenhaus) E. J. Butler) (14,26,28). Damage to beans can be in the form of poor seedling establishment, pre-emergence and post-emergence damping-off, and reduced vigor and productivity of older plants. Severely infected plants may show wilting, chlorosis, premature defoliation, and early maturity or death (23).

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A few days after infection, *M. phaseolina* produces small, smooth, spherical, black sclerotia (50 to 150 µm in diameter) in infected tissues (11,16,26). *M. phaseolina* survives mainly in the form of sclerotia, the primary source of inoculum. Sclerotia in host tissues are released into the soil as diseased tissues decay. Viable microsclerotia have been reported to persist in soil or plant residues from 3 months to 3 years. However, sclerotia rapidly lose viability in wet soils (6,9,28).

Charcoal rot of different herbaceous and woody plants is a good example of a disease that is influenced by stress factors. The association of charcoal rot development with flowering and maturation has been demonstrated for many crops (6,8,9, 21). High temperature and low soil water potential also are important factors in disease development. *M. phaseolina* is favored by high temperature, with optimal growth in culture at 30 to 37°C. The fungus possesses a relative advantage at high temperatures over the host, which develops better at lower temperatures. Low soil water potential also increases the susceptibility of the host, and reduces activity of other microorganisms antagonistic to *M. phaseolina* (21). Water potential and temperature play a fundamental role in plant disease epidemiology, as well as in the ecology and growth of plant pathogenic or saprophytic fungi (15,30).

Detailed information on the effect of water potential on charcoal rot incidence and development in beans, as well as on its effects on the growth and ecology of the pathogen, is limited. Olaya and Abawi (19, 20) and Olaya (18) recently reported that *M. phaseolina* can grow and produce large quantities of sclerotia under relatively dry, controlled conditions. The objective of this study was to determine the influence of water potential on the colonization of bean stems by *Macrophomina phaseolina* and survival of sclerotia in soil adjusted to different soil matric potentials.

MATERIALS AND METHODS

Influence of soil water potential on survival of sclerotia of *M. phaseolina*. An isolate of *M. phaseolina* highly pathogenic to beans, obtained from naturally infected beans in Colombia, was used throughout this study. This isolate was maintained on beet seeds at 5°C and transferred to potato dextrose agar (PDA) as needed. Sclerotia of *M. phaseolina* were produced by placing autoclaved toothpicks on *M. phaseolina* colonies growing on PDA; then the plates were incubated at 30°C for 10 to 14 days. Toothpicks with sclerotia were surface sterilized in 1% sodium hypochlorite for 1 to 2 min, rinsed with distilled water, and dried for at least 1 to 2 days (18,20).

The effect of water potential on survival of sclerotia was determined in soil pasteurized (60°C for 30 min) and nonpasteurized (natural). The soil was obtained from a bean field near Geneva, NY. The soil was classified as very fine, sandy loam, and consisted of 71.8% sand, 23.6% silt, and 4.6% clay, and a pH value of 6.6 (Cornell Nutrient Analysis Laboratories, 804 Bradfield Hall, Cornell University, Ithaca, NY). Soil matric water potentials (Ψm) were adjusted to –10, –30, –100, –300, –500, and –1,500 J/kg by placing soil samples on pressure plate extractors (Soil Moisture Equipment Corporation, Santa Barbara, CA). Water potential was expressed in J/kg units. One J/kg is equivalent to 0.01 bar. Ψm of –30 and –1,500 J/kg generally correspond to soil water contents of field capacity and per-

manent wilting point, respectively (15,18, 22). After equilibration to the appropriate Ψm, toothpicks with sclerotia of *M. phaseolina* were placed in nylon mesh and buried in about 40 cm^3 of each adjusted soil sample in 100-cm^3 plastic cups. Saturated (Ψm = 0 J/kg) and air-dried (about –40,000 J/kg) soil samples also were included in this study. Plastic cups were sealed and stored in plastic boxes to avoid evaporation, temperature fluctuations, and changes in soil matric potential. Toothpicks with sclerotia were recovered, washed with running tap water for 1 to 2 min, surface sterilized in 1% sodium hypochlorite for 1 to 2 min, rinsed with distilled water, and dried for at least 3 to 4 h in sterilized petri dishes with filter papers. Viability of 20 sclerotia per each soil water potential treatment was determined after 2, 4, 8, 12, 16, and 20 weeks of incubation at 30°C by placing sclerotia on acidified PDA. This experiment was conducted twice and viability of sclerotia was expressed as a percentage. Data also were submitted to logistic regression. An analysis of deviance was performed, regression coefficients were determined, and odds factors (ratios) for survival of sclerotia were calculated (12).

Influence of water potential on colonization of excised bean stems by *M. phaseolina*. The effect of water potential on colonization by *M. phaseolina* on bean stem segments was determined by inoculating stem segments with sclerotia. Small constant-humidity chambers were prepared by placing 100 ml of KCl solutions adjusted to different water potentials in 300-ml glass jars (17,25). The appropriate KCl solutions and bean stem segments that were placed on a platform were allowed to equilibrate for 48 h at 30°C. Bean stem segments (7 to 8 cm in length and 0.5 cm in diameter) were inoculated with 3 to 4 sclerotia of *M. phaseolina*. Jars were sealed with Parafilm and placed in plastic boxes to reduce temperature fluctuations and evaporation. Plastic boxes with jars were incubated at 30°C for 10 days. Five bean stems of the cv. Labrador (highly susceptible to *M. phaseolina*) were used per each water potential treatment. Extent of colonization was rated according to a 1 to 9 scale in which 1 refers to no visible symptoms and no formation of sclerotia, whereas 9 indicates all tissues of the stem segment are colonized and densely covered by sclerotia. In addition, the number of sclerotia per square millimeter of tissue also was determined by counting them directly on the colonized tissue.

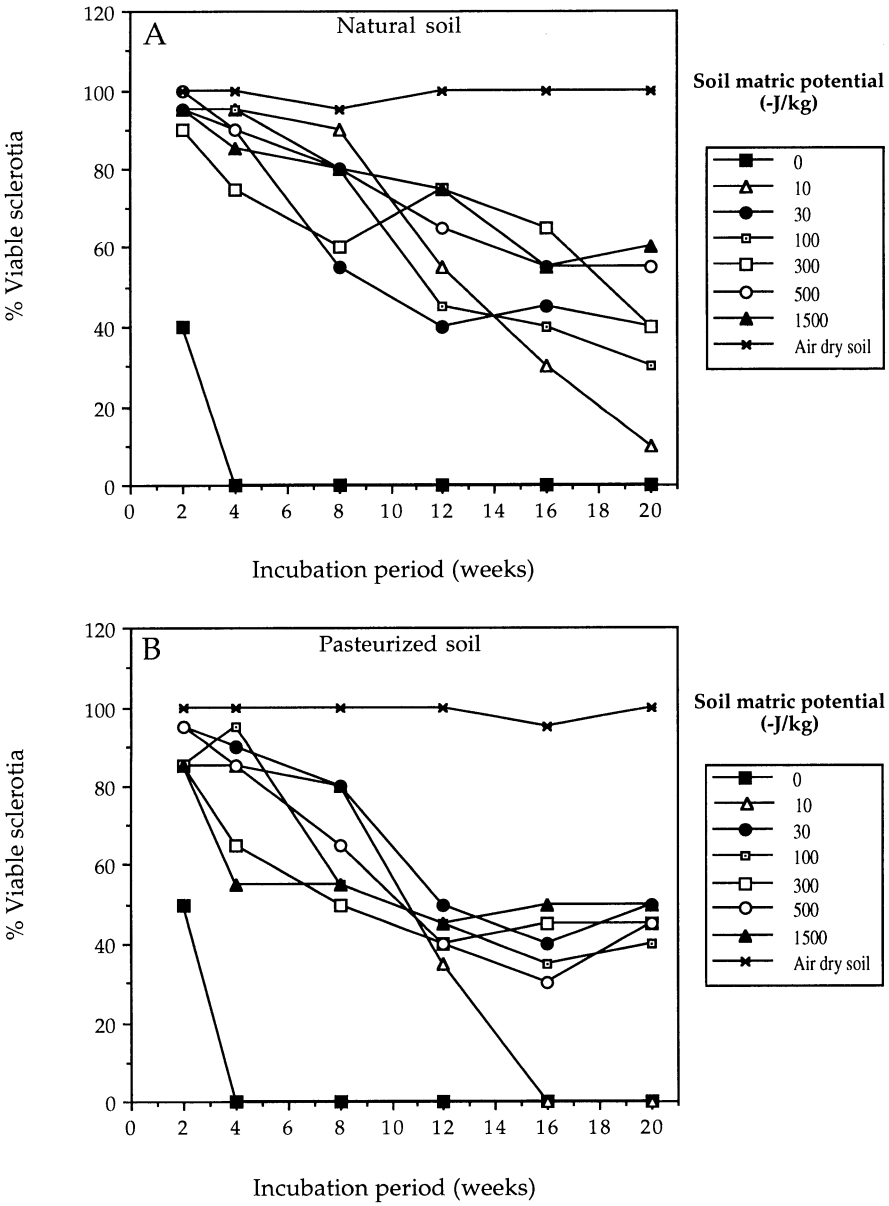

Fig. 1. Survival of sclerotia of *Macrophomina phaseolina* at different matric potentials under controlled conditions in a very fine, sandy loam soil: **(A)** nonpasteurized (natural), **(B)** pasteurized.

RESULTS

Influence of soil water potential on survival of sclerotia of *M. phaseolina*. Viability of sclerotia was 40 and 0% at a Ψm of 0 J/kg after 2 and 4 weeks of incubation, respectively (Fig. 1A). Survival of sclerotia decreased steadily over time in the soil samples adjusted from –10 to –1,500 J/kg. After 20 weeks of incubation, viability of sclerotia was 10, >30, and >50% at Ψm of –10, –30 to –300, and –500 to –1,500 J/kg, respectively (Fig. 1A). Sclerotia of *M. phaseolina* remained 100% viable in the air-dry soil treatment. Survival of sclerotia followed a similar pattern in the pasteurized soil (Fig. 1B). Viability of sclerotia in the saturated soil (Ψm = 0 J/kg) was reduced to 50 and 0% after 2 and 4 weeks of incubation, respectively. All sclerotia were nonviable after 16 weeks of incubation at Ψm = –10 J/kg. Survival of sclerotia was between 40 and 50% at Ψm of –500 and –1,500 J/kg, and remained at 100% in the air-dry soil (Fig. 1B).

Logistic regressions were fitted to the binomial time of incubation and soil water potential data. The 0 J/kg and air-dry soil treatments were omitted from the statistical analysis since for all intents and purposes their viabilities for almost all the incubation times were 0 and 100%, respectively (Fig. 1A and B). The analysis of deviance (Table 1) showed that there was a significant difference between survival of sclerotia in the natural and pasteurized soils ($P < 0.002$). Time of incubation and the interaction between soil water potential and time of incubation were also significant ($P < 0.001$) (Table 1). Effects were examined in terms of odds ratios, i.e., how the odds of viability of sclerotia change with Ψm treatment. Overall, the odds of viability of sclerotia showed that the decrease in survival of sclerotia in the pasteurized soil was less than that in the natural soil. Regression coefficients for the soil + Ψm + time + Ψm × time model were calculated. According to these coefficients, the probability factor of weekly decrease of viable

sclerotia in both soils is 0.83 at Ψm = –10 J/kg, whereas the probability factor is 0.95 at Ψm = –1,500 J/kg (Fig. 2). For all the soil water potential treatments, the effects have been plotted and show a pattern of decreasing viability of sclerotia when the Ψm increased (Fig. 2). The experiment was conducted twice with the same soil and water potential treatments and the survival of sclerotia followed a similar pattern both times.

Influence of water potential on colonization of excised bean stems by *M. phaseolina*. After 10 days of incubation, *M. phaseolina* was able to colonize bean stem segments that were placed at a range of water potentials. Extent of colonization increased as osmotic water potential (Ψs) was initially decreased and was highest at a Ψs of –3,990 J/kg, compared with other water potentials (Fig. 3). However extent of colonization was decreased with further reductions of Ψs and was significantly diminished at Ψs lower than –7,150 J/kg (Fig. 3). The number of sclerotia per square millimeter also increased as water potential decreased and was highest at Ψs values of –3990 J/kg. Number of sclerotia produced per square millimeter was reduced with further reductions in Ψs values, but was still relatively high at –8,080 J/kg (Fig. 4). The experiment was conducted twice and similar results were obtained both times.

DISCUSSION

Results from this study showed that *M. phaseolina* can survive and colonize excised bean stems under relatively dry conditions. Survival of sclerotia of *M. phaseolina* was greatly influenced by the water status of the soil. Survival of sclerotia was drastically reduced at high Ψm, was less affected at low Ψm, and was not affected in the air-dry soils (natural and pasteurized). Dhingra and Sinclair (5) and Shokes et al. (27) also reported a rapid loss of viability of *M. phaseolina* sclerotia at high soil water levels. Saturated soil conditions also affect the survival of sclerotia of other plant-pathogenic fungi, including *Sclerotinia minor* Jagger (1), *Verticillium dahliae* Kleb. (13), and *Rhizoctonia solani* Kühn AG-4 (24). Reduction of the survival of sclerotia at higher soil water potentials is related to the inability of sclerotia to regulate their water content and to the absence of a state of constitutive dormancy (3). Reduced availability of O_2 or increased levels of CO_2, volatiles, alcohols, or other compounds found in very moist or flooded soils also may have a negative effect on the survival of sclerotia. Death of sclerotia of *M. phaseolina* in infested fields has been achieved by flooding with or without paddy cultivation (31). The survival of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary and *S. minor* under flooded conditions is about 8 weeks. This knowledge has been used to control *Sclerotinia* diseases, using flooding as a control measure (1). Flooding is an important component in the integrated management strategy against onion white rot caused by *Sclerotium cepivorum* Berk. (2).

In this study, survival of sclerotia in natural and pasteurized soils was assessed for a 20-week incubation period at 30°C. Previous studies on survival of sclerotia of *M. phaseolina* covered shorter periods, such as 2 weeks (27) or 7 weeks of incubation (5). Detailed information on the longevity of sclerotia of plant-pathogenic fungi in soil is essential in order to forecast the potential for disease occurrence and recommend appropriate control measures (1,15).

Survival of sclerotia of *M. phaseolina* followed a similar pattern in both natural and pasteurized soils. However, the probability of decrease in survival of sclerotia in the pasteurized soil (Ψm = –10, –30, –100, –300, –500, and –1,500 J/kg) was less than that in the natural soil, which may be due to microbial activity. Low soil water potentials may adversely affect the development of antagonistic microflora in the soil, allowing *M. phaseolina* propagules (sclerotia, etc.) to survive longer in dry soils. Knowledge of the effect of water potential on the pathogen, disease development, and antagonistic microorganisms is critical for enhancing the biological control of soilborne plant diseases (7,15). Results suggest that reductions in the survival of sclerotia of *M. phaseolina,* especially in the pasteurized soil, were produced by the direct effect of the soil water potential.

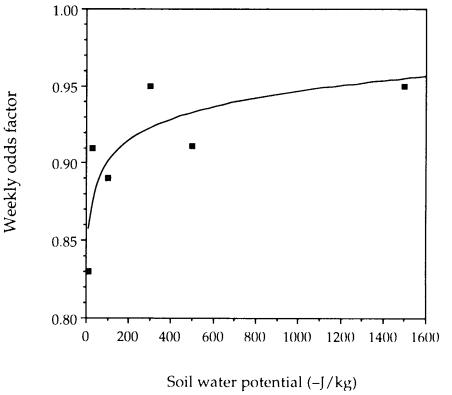

Fig. 2. Odds factors showing the effect of different matric potentials (–10, –30, –100, –300, –500, and –1,500 J/kg) on the survival of sclerotia of *Macrophomina phaseolina* over time, in a very fine, sandy loam soil under controlled conditions.

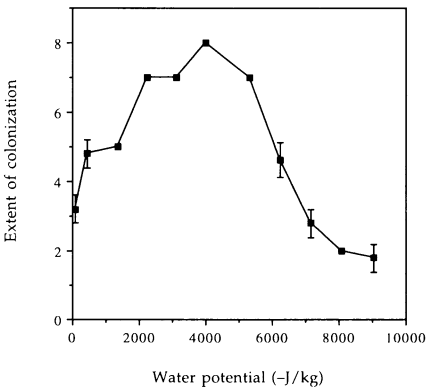

Fig. 3. Influence of water potential on colonization of bean stem segments by *Macrophomina phaseolina* rated on a scale of 1 (no visible colonization and no formation of sclerotia) to 9 (100% tissue colonized and densely covered by sclerotia).

Table 1. Analysis of deviance of the data to study the effect of different matric potentials on the survival of sclerotia of *Macrophomina phaseolina* in a very fine sandy loam soil[a]

Source of variation	df	Deviance	Mean deviance	Deviance ratio
Soil	1	14.55	14.55	10.79 **[b]
Ψm	5	9.60	1.92	1.42 NS
Time (incubation)[c]	1	264.76	264.76	192.29 ***
Soil × Ψm	5	13.39	2.67	1.99 NS
Time × soil	1	0.97	0.97	0.72 NS
Time × Ψm	5	49.47	9.89	7.34 ***
Soil × time × Ψm	5	2.98	0.59	0.44 NS
Residual	48	64.74	1.34	
Total	72	420.48		

[a] Data were submitted to logistic regression. Soil matrix water potential (Ψm) = 0 and air-dry soil treatments were omitted from the statistical analysis since sclerotial viabilities for almost all the incubation times were 0 and 100%, respectively.

[b] ** and *** refer to statistical significance at $P < 0.002$ and $P < 0.001$, respectively. NS = not significant.

[c] Time of incubation was treated as a continuous variable.

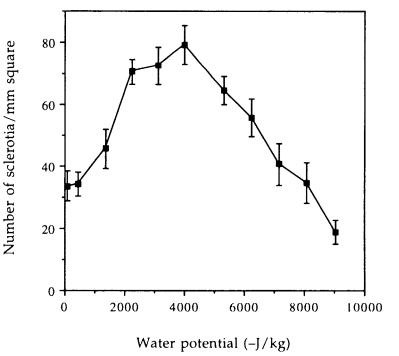

Fig. 4. Influence of water potential on colonization of bean stem segments by *Macrophomina phaseolina* measured as the number of sclerotia per square millimeter of infected tissue.

Extent of colonization of bean stem segments by *M. phaseolina* was also influenced by water potential. Colonization increased as Ψs was decreased to about –3,990 J/kg. However, colonization was significantly diminished at Ψs lower than –7,150 J/kg. These data support field observations. High soil temperatures and low soil moisture were the most important factors in predisposing sorghum to infection by *M. phaseolina* (9). Water stress can predispose the plant to *M. phaseolina* when the defense mechanism of the plant is impaired (29). Root rot damage caused by *M. phaseolina* in cotton occurred regardless of temperature or the addition of nutrients, but developed only in plants subjected to water stress before or after inoculation (10). In sorghum, the combined stress of blooming and lack of soil water predispose the plant to charcoal stalk rot. The pathogen apparently infects early, but remains latent until the host is stressed, at which time the disease progresses rapidly (4,8).

M. phaseolina has been recognized previously as a drought-favored pathogen (21, 29). *M. phaseolina* can grow, and produce large quantities of sclerotia, under relatively dry, controlled conditions (18,19, 20). Results of this study have also shown that *M. phaseolina* can survive in soils and colonize bean stem segments at low water potentials. These attributes of *M. phaseolina* may explain the epidemic development of charcoal rot of beans in areas characterized by high temperatures and drought conditions. Knowledge of the predisposition factors for charcoal rot development is also essential for the elaboration of accurate screening methods to be employed in genetic studies.

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