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Effect of water potential on germination of *Verticillium dahliae* microsclerotia

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Summary. The effects of osmotic and matric potentials on the microsclerotial germination of *Verticillium dahliae* was examined at room temperature in 1% water agar amended with sodium chloride and polyethylene glycole. Treatments consisted of 6 levels of osmotic and matric potentials (0, -0.3, -0.6, -0.9, -1.2, and -1.5 MPa) laid out as factorial arrangement in a completely randomized design. Decreasing matric potential reduced germination, whereas the osmotic potential increased germination up to -0.6 MPa but any further increase caused it to decline. It was concluded that the matric potential is a more limiting factor than the osmotic potential for the germination of *V. dahliae* microsclerotia.

Key words: Verticillium wilt, salinity, water stress, matric potential, osmotic potential.

Introduction

Verticillium dahliae Kleb. causing Verticillium wilt is a plant pathogen of considerable importance with a wide host range and a worldwide distribution, under a variety of environmental conditions. It produces microsclerotia, which are the primary source of fungus survival, and from which new infections are initiated (Powelson, 1970). Microsclerotial germination is stimulated by root exudates in the rhizosphere; the germination mycelium penetrates the plant roots and invades the vascular tissues (Schnathorst, 1981). The survival and germination of microsclerotia are important factors in determining wilt management strategies.

Salinity and drought are major environmental factors affecting wilt development in both salt-sensitive and salt-tolerant plants. The water potential of a soil solution (Ψ) is the sum of all forces affecting its energy state and is the summation of its solute, matric, gravitational and pressure potentials (MacDonald, 1994). The solute or osmotic potential (Ψ_s) and the matric potential (Ψ_m) are the major forces affecting biological activity in the soil. In a non-saline soil, Ψ_m influences the uptake of water by plants and their growth more than the Ψ_s of soil water. However, in saline soils, the osmotic potential becomes important in controlling plant growth, particularly when the soil water is depleted.

In sandy soil when the water potential was adjusted osmotically, the mycelial growth rate of *Phytophthora cinnamomi* was highest at a potential of -1 to -1.5 MPa, and was one-half the optimum at

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-2 to -2.5 MPa. When comparing the effect of Ψ_m and Ψ_s on mycelial growth, it was found that *P. cinnamomi* was suppressed much more strongly by decreasing the Ψ_m than by decreasing the Ψ_s (Adebayo and Harris, 1971). The germination of chlamydospores of *P. cinnamomi* in the soil and their capacity to infect plant root are influenced much more by Ψ_m values in the 0 to -0.025 MPa range than by Ψ_s values in the -0.004 to -0.2 MPa range, a range that falls within the Ψ_s values of saline soil in California avocado groves (Sterne *et al.*, 1977). Considerations of solute diffusion to and from the hyphae, and the availability of an adequate amount of solute for osmotic adjustment suggest that low Ψ_m values are frequently more limiting to growth than the equivalent low Ψ_s values (Adebayo and Harris, 1971).

The influence of water potential on the mycelial growth and sclerotial production of *Sclerotium cepivorum*, as determined in potato dextrose agar (PDA) and potato dextrose broth adjusted to different water potentials with NaCl, potassium chloride (KCl), and sucrose or polyethylene glycole (PEG), showed similar patterns; however, mycelial growth was more severely depressed by PEG-amended media than by other osmotica amended media (Hoon *et al.*, 1997). Sclerotial production of *S. cepivorum* was more severely impaired with decreasing water potential than was mycelial growth. A 50% decrease of sclerotial production occurred between -450 and -810 J kg⁻¹ (Hoon *et al.*, 1997).

The objective of the present study was to determine the effects of the matric and osmotic potentials and their interaction on the germination of microsclerotia of *V. dahliae*.

Materials and methods

Inoculum production

Microsclerotia of the pistachio isolate of V. dahliae were produced on a liquid medium as described by Hall and Ly (1972). Cultures were inoculated in the dark at 25°C in sterile conditions, incubated for 12 wk and checked regularly for the formation of microsclerotia. The inoculum was separated from the media by vacuum filtration, rinsed with sterile distilled water, dried at 70°C for 72 h, weighed and passed through a 200 mesh screen to obtain smaller units of microsclerotia.

Adjustment of water potential

Water agar (0.1%) was adjusted to 0, -0.3, -0.6, -0.9, -1.2, and -1.5 MPa using either NaCl or PEG 6000. PEG was used to adjust the matric potential. The values of the matric potential exploited were those from the curve prepared by Zur (1966). For the different osmotic potentials the corresponding amount of NaCl was measured according to the US Salinity Laboratory Handbook (1954).

Germination

For the germination assay, microsclerotia of *V. dahliae* were added to each screw-cap bottle containing solution with different matric or osmotic potential, and incubated at room temperature for 21 d. Microsclerotium germination was monitored microscopically after 3 d and was continued until a stable condition was reached. The experiment was a factorial arrangement in a completely randomized design with two replications and was repeated twice. Analysis of variance was done using MSTATC software.

Results and discussion

The interactive effects of the osmotic and matric potentials on the germination of *V. dahliae* microsclerotia differed statistically (Table 1). Microsclerotial germination increased as a function of decreasing osmotic potential up to -0.6 MPa at all levels of matric potential. However, any further decrease in osmotic potential to levels below -0.6 MPa caused germination to decrease.

Microsclerotial germination also decreased as a function of decreasing matric potential at all levels of osmotic potential. The lowest amount of germination occurred with the lowest levels of osmotic and matric potential (Table 1). Sepaskhah and Boersma (1979) reported that the effect of the matric and osmotic potentials on growth was additive. Regression analysis detected that percent germination of microsclerotia decreased as a linear function of the decreasing potential (Table 2), indicating that the germination of microsclerotia was more strongly decreased by the matric than by the osmotic potential.

Germination increased with decreasing osmotic potential up to -0.6 MPa, but any further decreases in osmotic potential caused germination to decline (Fig. 1). However, a decrease in matric po-

Table 1. Effect of matric and osmotic potential and its interaction on microsclerotium germination (%) of *Verticillium dahliae*.

Matric potential	Osmotic potential (-MPa) ^a					
	0	0.3	0.6	0.9	1.2	1.5
0	74.0 ab	76.0 ab	82.5 a	63.0 bc	44.0 de	34.0 efgh
0.3	62.5 bc	65.5 bc	70.5 ab	53.5 cd	53.0 cd	36.0 efg
0.6	46.5 de	53.5 cd	62.5 bc	44.0 de	27.5 fghi	16.0 ijk
0.9	25.0 fghij	25.0 fghij	38.5 def	21.0 ghijk	13.0 ijk	11.5 ijk
1.2	19.0 hijk	17.0 ijk	17.0 ijk	14.5 ijk	13.5 ijk	6.0 k
1.5	12.5 ijk	16.0 ijk	19.5 hijk	12.0 ijk	10.0 jk	5.0 k

^a For each column, means followed by the same letters are not significantly different at $P \leq 0.01$.

Table 2. Parameters of the regression equations relating the microsclerotium germination (Y) of *Verticillium dahliae* to the means of the osmotic and matric potential (X).

Water potential	Regression equation	Correlation coefficient
Osmotic potential	$Y = -16.092 X + 47.084$	$R^2 = 0.6699$ *
Matric potential	$Y = -37.627 X + 63.233$	$R^2 = 0.9486$ **

Significance of differences is shown as *, $P \leq 0.05$ and **, $P \leq 0.01$

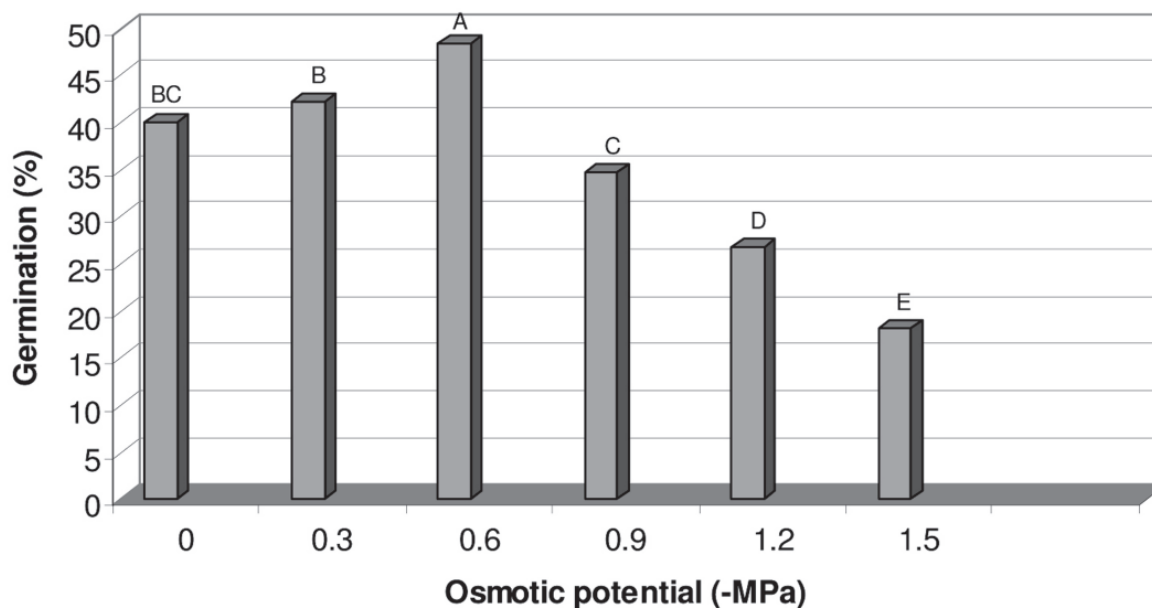


Fig. 1. Effect of osmotic potential on germination (%) of *Verticillium dahliae* microsclerotia. Significant differences between treatments are indicated by different letters at $P \leq 0.01$ by Duncan's Multiple Range Test.

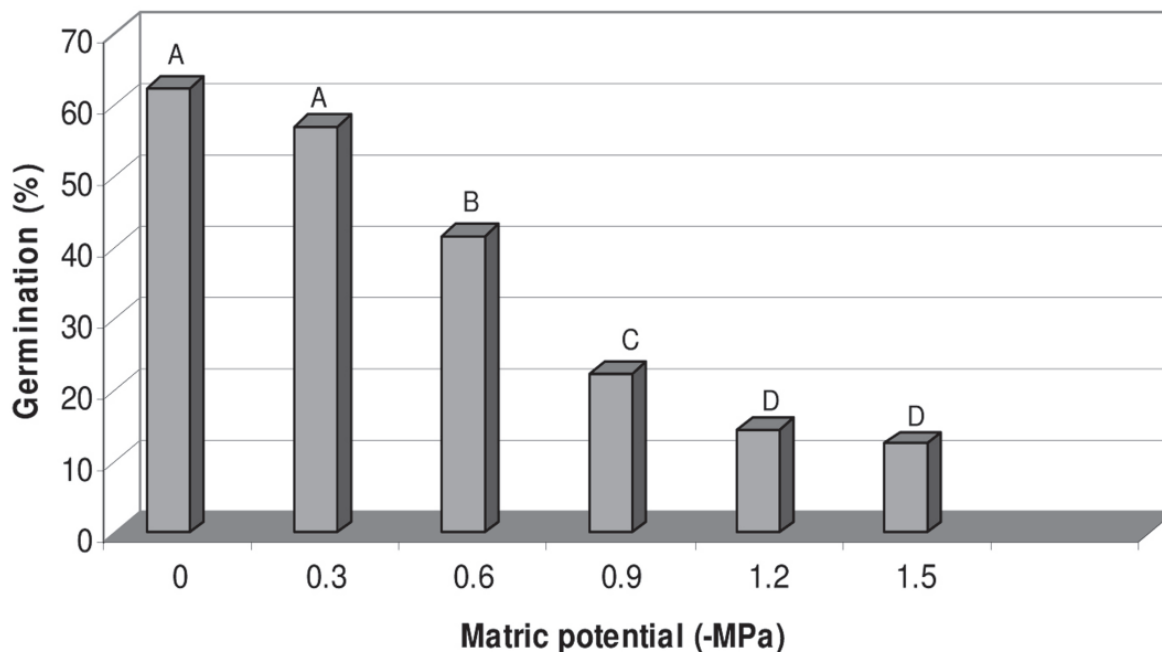


Fig. 2. Effect of matric potential on germination (%) of *Verticillium dahliae* microsclerotia. Significant differences between treatments are indicated by different letters at $P \leq 0.01$ by Duncan's Multiple Range Test.

tential decreased the germination rate of microsclerotia (Fig. 2). The lower sensitivity of microsclerotia to a decrease in water potential induced by the osmotic potential may be attributed to the adjustment of the turgor potential by osmoregulation. Sepaskhah and Boersma (1979) reported that salt stress was more effective in reducing the osmotic potential of the cell sap and resulted in stronger osmoregulation than stress induced by a water deficit. The effect of water potential among fungi differs. Sterne and McCarver (1978) in liquid and agar media found that growth of *Pythium ultimum* was always inhibited more by decreasing osmotic potential than was growth of either *V. dahliae* or *Rhizoctonia solani*. In a liquid medium at an osmotic potential of -3.2 MPa, *P. ultimum* barely increased its dry weight, whereas *V. dahliae* and *R. solani* maintained specific growth rates approaching one half their growth rate under optimal osmotic potential conditions (Duniway, 1979). The growth of several isolates of *Monosporascus cannonballus* *in vitro* increased as the Y_s was reduced to -0.8 MPa, with maximum growth occurring at Y_s values of -0.6 to -0.8 MPa. A fifty-percent growth reduction did not occur until the Y_s

was reduced to <-2.5 MPa (Ferrin and Stanghellini, 2006).

In our experiment maximum microsclerotium germination occurred at -0.6 MPa and a reduction of 50% was achieved with a Ψ_s level of -1.5 MPa. Generally the growth of the fungus was somewhat more strongly restricted by decreasing Ψ_m values in the soil than the effect of decreasing Ψ_s would have suggested (Griffin, 1978). The soil water potential is usually dominated by its Ψ_m component, which has a different effect on microsclerotial activity than the osmotic potential (MacDonald, 1994). The results of this study indicated that the germination of *V. dahliae* microsclerotia was suppressed by a lowering of the Ψ_m .

Microsclerotium germination was reduced to 50% at a matric potential of -0.9 MPa. At a matric potential of -1.5 MPa; germination was only 33% of optimal levels. In many zoosporic and non-zoosporic fungi, the osmotic potential at some levels usually leads to more disease development (MacDonald, 1994). *Pistacia vera* is generally considered a salt-tolerant plant (Parsa and Karimian, 1975). Species of *Phytophthora* (Banihashemi and Tabatabaee, 2004) and *V. dahliae* (Banihashe-

mi and Mohammadi, 2001) caused more root infection and vascular invasion of *P. vera* respectively, by predisposing the plants to salinity. The germination of *V. dahliae* microsclerotia increased when NaCl levels were increased up to 25 g NaCl l⁻¹, NaCl levels were tolerated well up to 60 g NaCl l⁻¹ (Mohammadi, 2000).

Although there are some reports concerning the additive effect of the osmotic potential in certain crops on infection with *V. dahliae* (Kaufman *et al.*, 1990; Nachmias *et al.*, 1993; Howell *et al.*, 1994; Banihashemi and Mohammadi, 2000), no information is available on the effect of Ψ_m and its interaction with Ψ_s on microsclerotium germination and subsequent infection of the host plant with *V. dahliae*. However, there is scant information on the soil water content and the population increase of *V. dahliae* microsclerotia in the root zone of *P. vera* (MacDonald *et al.*, 1992). Water stress reduced pistachio growth but also caused lower levels of infection with *V. dahliae*, and lower levels of Na and Cl (Banihashemi and Saadatmand, 2006). The present study showed that water stress which reduced Ψ_m also resulted in lower microsclerotium germination, and hence that water management is an important factor in disease control. It is also interesting to note that lower Ψ_s and Ψ_m potentials had a cumulative effect on the germination of *V. dahliae* microsclerotia, but that germination was always maximum at the optimum Ψ_s of -0.6 MPa, irrespective of the Ψ_m .

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