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EFFECT OF OSMOTIC AND MATRIC POTENTIALS ON *SCLEROTINIA MINOR* AND *SCLEROTINIA SCLEROTIUM* VIRULENCE ON PEANUT

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ABSTRACT

The effect of osmotic and matric potentials on mycelial growth, sclerotia production, germination, and virulence of two isolates of *Sclerotinia sclerotiorum*, and one isolate of *S. minor* were studied on potato dextrose agar (PDA) media adjusted with KCl, glycerol, or agar. Osmotic potentials created by KCl and glycerol significantly reduced vegetative growth of the three isolates. On matrically adjusted PDA, vegetative growth of the three isolates was not negatively affected by matric stress up to -3.5 MPa. When KCl was the osmoticum, sclerotia number did not follow a consistent pattern. However, sclerotia number decreased when osmotic stress created by glycerol was increased. Matric stress was not a consistent factor affecting sclerotia production by both species. However, the highest levels of matric stress -3.0 and -3.5 MPa significantly reduced sclerotia production by both species. In general, there was a trend toward lower sclerotial germination with increasing osmotic and matric stress. Pathogenicity of *S. minor* and *S. sclerotiorum* on the peanut cultivar (Okrun) was reduced by high concentrations of KCl. Mycelia of both species produced at high matric potential -3.5 MPa did not differ in pathogenicity on Okrun compared with mycelia grown on non-amended PDA. In water-stressed-Okrun, induced by polyethylene glycol 8000, the Area under Disease Progress Curve (AUDPC) was significantly decreased. The relevance of these results to the behavior of *S. minor* and *S. sclerotiorum*, and their pathogenicity on peanut is discussed.

Keywords: Water potential; Peanut; Sclerotia; *Sclerotinia* species.

INTRODUCTION

Sclerotinia blight of peanut (*Arachis hypogaea* L.) caused by the soilborne fungi *Sclerotinia minor* Jagger and *S. sclerotiorum* (Lib.) de Bary was first reported in the United States in Virginia in 1971 (Kokalis-Burelle *et al.* 1997). Sclerotinia blight has become a widespread problem in Virginia, North Carolina, Oklahoma and Texas (Smith *et al.* 2006). *S. minor* and *S. sclerotiorum* survive in soil mainly by producing sclerotia on infected plants (Wu *et al.* 2008). Infection occurs primarily through eruptive germination of sclerotia that gives rise to white, fluffy mycelia that infect stems and pegs of peanut. Many factors affect survival and germination of sclerotia of the two species in the field (Wu *et al.* 2008).

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Constant soil temperature for 3 weeks or more at 35°C reduces survival of sclerotia. Other factors such as sclerotial position and duration in soil, sclerotial shape, soil gases or chemicals, activities of other microorganisms and nutrition affect survival of sclerotia (Adams, 1975; Abwai and Grogan, 1975, 1979; Huang and Kozub, 1994; Imolehin *et al.*, 1980; Burgess & Hepworth, 1996). Temperature and moisture are significant factors affecting development of diseases caused by species of *Sclerotinia* spp. (Willets and Wong, 1980). Viability of sclerotia also declines rapidly over time in moist soil (i.e., low water stress) (Abwai and Grogan, 1979). Almost 100% of the sclerotia of *S. sclerotiorum* were totally decayed when soil was flooded with water for 24 to 45 days. In general, sclerotia of *S. minor* survive better in dry soil than in moist soil, and better in shallow rather than at a deeper depth in soil

where higher moisture usually exists (Imolehin *et al.*, 1980). Lower soil moisture (i.e., high water stress) in lettuce fields increased sclerotia survival, and sclerotia only survive short periods in saturated soils at 0 MPa (Hao *et al.*, 2003). Sclerotia viability decreased in soil with water potentials equal to or higher than -0.02 Mpa as soil temperature increased from 15°C to 40°C. Sclerotia of *S. minor* can germinate directly at soil moisture levels between -0.03 and -1.5 Mpa (Imolehin *et al.* 1980), while sclerotia of *S. sclerotiorum* germinate between -0.6 and 0 Mpa (Duniway *et al.* 1977). Optimum radial growth occurred on basal medium with osmotic potential of -1.2 Mpa and at -10 Mpa there was no growth (Imolehin *et al.* 1980). Sclerotia of *S. minor* produced over the range of -0.1 to -4.35 Mpa did not differ significantly in its ability to germinate eruptively when moistened (Imolehin *et al.* 1980). Sclerotia of *S. minor* and *S. sclerotiorum* maintained within wet soil (≥ 0.02 Mpa) for four weeks at 40°C did not germinate, while sclerotia maintained within dry soil ≤ -10 Mpa for 4 weeks at 40°C were viable (Matheron & Porchas, 2005). Most research on the effect of water potential on *S. minor* and *S. sclerotiorum* was performed on isolates infecting lettuce under environmental factors significantly different from those found in peanut fields. Our research was performed with *Sclerotinia* isolates pathogenic on peanut. Development of more effective integrated disease management strategies for control of Sclerotinia blight peanut could be improved benefit from new knowledge of the factors that affect the biology of the host, the fungi, and their interaction. Therefore, the objectives of this study were to: 1) study the effect of water potential on the vegetative growth and sclerotia production of *S. minor* and *S. sclerotiorum*, 2) determine germination success of sclerotia produced on nutrient media at various water potentials, 3) study the pathogenicity of *S. minor* and *S. sclerotiorum* produced on media at various matric and osmotic potentials, and 4) determine the impact of water stress on the infection rate of peanut with *S. minor* and *S. sclerotiorum*.

MATERIALS AND METHODS

Plant materials and fungal cultures: The cultivar Okrun, a sclerotinia blight-susceptible runner type peanut, was used in this study. Seeds were germinated on wet filter paper at 30°C in an incubator for two days, and then planted in pots (10 cm dia) containing a 2:1:1 (sand: soil: shredded peat moss). Plants were grown in a climate-controlled greenhouse, watered daily, and

fertilized with 0.45% ammonium nitrate solution weekly, starting on the third week after planting, to promote the production of highly succulent stems. Three *Sclerotinia* isolates were used, including one isolate of *S. minor* from peanut and two *S. sclerotiorum* isolates, one from peanut grown in Nebraska, and the other was isolated from pumpkin fruit that was bought from a supermarket in Stillwater, OK. Isolates were maintained at 25±2°C, in darkness, on potato dextrose agar (Difco Laboratories, Detroit, MI) containing 100 ppm of streptomycin sulfate (SPDA).

Preparation of media at various water potentials: SPDA containing 100 ppm of streptomycin sulfate was used as a basal medium. SPDA medium was osmotically modified over the range of -0.5 to -4 MPa with potassium chloride (Ritchie *et al.* 2006) or glycerol (Dallyn & Fox 1980) and sterilized by autoclaving for 20 minutes. Total water potential was the sum of the water potential of the SPDA -0.34 MPa and the osmotic potential of the added osmotica (potassium chloride or glycerol) (Campbell & Gardner, 1971; Dallyn & Fox, 1980). Osmotic potential was calculated according to (Liddell, 1993). The actual osmotic potential of all media was checked by Vapor Pressure Osmometer (VAPRO 5520, Wescor, Utah, USA). Various matric potentials of SPDA were adjusted by granulated agar (Fisher Scientific, Fair Lawn, New Jersey). Matric potentials of media equivalent to -1, -1.5, -2.0, -2.5, -3.0, and -3.5 MPa at 25°C were determined using a Vapor Pressure Osmometer (Wescor). The total matric potential was the sum of the water potential of SPDA and the matric potential of the added agar.

Mycelial growth and sclerotia production on nutrient medium: Petri dishes containing 15 ml of nutrient medium were each inoculated in the center with a 3-mm -dia mycelial disc taken from the periphery of 2-day-old cultures of *S. minor* and *S. sclerotiorum* grown on SPDA. Inoculated plates were incubated at 25±2°C in darkness. Radial growth (mm) of colony was measured up to four days after inoculation. Sclerotia were harvested from 21-days old cultures with the aid of camel hair brush. Harvested sclerotia were dried at 22°C for two weeks in a desiccator containing anhydrous CaSO₄. Sclerotia from each 9.0-cm plate were counted. This experiment was conducted twice with five plates as replications in each treatment.

Viability of sclerotia: Sclerotia produced under different osmotic and matric potentials were tested for viability by plating on SPDA medium. Before plating,

sclerotia were surface sanitized with a sodium hypochlorite solution 1.0% (Melouk *et al.*, 1999). For each treatment, five sclerotia were plated on each of five plates, and incubated at a room temperature of $25 \pm 2^\circ\text{C}$ in darkness. Percentage of sclerotial germination was determined after 7 days of incubation.

Pathogenicity of mycelia produced on media at various water potentials: Plant inoculations were performed on six-to-eight-weeks old peanut plants (Faske *et al.*, 2006). A total of eight pots (4 replicates) were used for each of the osmotic and matric potentials. Plants were then placed in humidity chambers (150 x 60 x 60 cm) built from PVC pipe and clear plastic. Temperature was maintained at $19 \pm 2^\circ\text{C}$ at night and $26 \pm 2^\circ\text{C}$ during the day, and relative humidity was maintained at 95 to 100%. Light in the incubation chamber was adequate ($13.5 \mu\text{mol}/\text{m}^2/\text{sec}$) to sustain healthy plants. Inoculated plants were watered as needed for the duration of the experiments. Starting three days after inoculation, lesion length on infected stems was measured and recorded, and continued every 24 hours until day 7 post inoculation. The plants were then left to dry for 1 week in the chambers to facilitate production of sclerotia on infected tissue. To facilitate further drying, the infected stems were clipped at soil level and placed in brown paper bags for one week more. Sclerotia were collected from both the stem surface and from within the pith cavity of the stem, and quantified based on number and weight. Experimental design was a random complete block design (RCBD) with 4 replicates.

The effect of water stress on the infection of peanut by *S. minor* and *S. sclerotiorum*: Total number of plants in the experiment was 72, representing 9 treatments and 8 replicates. Each treatment had 8 plants that were placed in a humidity chamber (58.7 x 42.9 x 40 cm). Six-to-eight-week old Okrun plants that received PEG 8000 solutions were prepared for inoculation as described by Faske *et al.* (2006). Water stress was applied to plants using polyethylene glycol 8000 (Union Carbide Chemicals and Plastics). PEG solutions of various water potentials were prepared according to (Michel and Kaufmann, 1973) (Table 1). PEG 8000 was applied by pouring each solution into the bottom of its assigned plastic chamber on the fourth week after planting. In the non-inoculated control group water was used to keep seedlings well

irrigated. Six-to-eight week old plants were prepared for inoculation as described by Faske *et al.* (2006).

Table 1. Required concentrations of polyethylene glycol (PEG 8000) solutions to attain corresponding water stress on peanut plants at 25°C .

% PEG 8000	Osmotic stress in MPa
0	<-0.05
5	-0.05
10	-0.15
15	-0.30
20	-0.49
25	-0.73
30	-1.03
35	-1.37
40	-1.76

Statistical analysis: The experiment was performed using the same methods with each of the three isolates. Lesion length was taken at the fourth day post inoculation. This experiment was repeated once. Statistical analyses were done using SAS 9.3 (SAS Institute). Analysis of variance procedures (PROC MIXED) were conducted to determine the effects of the factors in question. Simple effects of factors were compared with planned comparisons with a SLICE option in an LSMEANS statement. Pairwise comparisons of least square means were made when overall significance was attained at a 0.05 level.

RESULTS

Mycelial growth of *Sclerotinia* isolates on SPDA with various water potentials: In osmotic potential (ψ_s) experiments, mycelial growth response of *Sclerotinia* isolates to (ψ_s) was similar for the two osmotica (Table 2). On both KCl and glycerol amended SPDA, the vegetative growth of *S. sclerotiorum* (peanut isolate) was consistently reduced at osmotic stress values below -1.5 MPa (Table 2). On both KCl and glycerol amended SPDA, the vegetative growth of *S. sclerotiorum* (pumpkin isolate) was consistently reduced at osmotic stress values below -2.5 MPa (Table 2). On both KCl and glycerol amended SPDA, the vegetative growth of *S. minor* was significantly reduced at osmotic stress values below -1.5 MPa (Table 2). This suppression of vegetative growth suggests that *S. sclerotiorum* (pumpkin isolate) may tolerate higher levels of osmotic stress and survive better than *S. sclerotiorum* (peanut isolate) and *S. minor* under similar conditions. In matric potential (ψ_m) studies,

vegetative growth of *S. minor*, *S. sclerotiorum* (peanut isolate), and *S. sclerotiorum* (pumpkin isolate) was not negatively affected by matric stress of up to -3.5 MPa (Table 3).

Table 2. Mean area under mycelial growth progress curve (AUMGC) for *S. sclerotiorum* (peanut isolate), *S. sclerotiorum* (pumpkin isolate) and *S. minor* grown on SPDA with different osmotic potentials (ψ_s) using KCl and Glycerol.

Isolates	Osmotic potentials (MPa)	AUMGC ¹ (KCl)	AUMGC ² (Glycerol)
SS ³	-0.34	19.190 b ⁴	21.580 b ⁵
SS	-0.50	18.480 b	25.010 a
SS	-1.00	20.440 a	23.310 ab
SS	-1.50	19.120 b	18.630 c
SS	-2.00	19.090 b	15.495 d
SS	-2.50	14.925 c	14.826 d
SS	-3.00	13.980 c	10.560 ef
SS	-3.50	9.830 d	12.092 e
SS	-4.00	10.390 d	10.160 f
SSP	-0.34	16.910 c	28.560 a
SSP	-0.50	19.040 b	26.810 a
SSP	-1.50	21.325 a	17.886 b
SSP	-2.00	22.255 a	13.490 c
SSP	-2.50	18.750 b	13.270 c
SSP	-3.00	16.980 c	8.380 d
SSP	-3.50	13.500 d	7.745 d
SSP	-4.00	12.905 d	5.220 d
SM	-0.34	16.155 a	29.710 a
SM	-0.50	16.514 a	29.050 a
SM	-1.00	16.670 a	26.240 b
SM	-1.50	13.015 b	22.328 c
SM	-2.00	9.945 c	20.102 d
SM	-2.50	10.545 c	20.090 d
SM	-3.00	7.795 d	14.121 e
SM	-3.50	7.895 d	11.972 f
SM	-4.00	5.425 e	09.630 g

¹ Means of area under mycelial growth progress curve values on KCl amended SPDA.

² Means of area under growth progress curve values on glycerol amended SPDA.

³ SS, *S. sclerotiorum* (peanut isolate), SSP, *S. sclerotiorum* (pumpkin isolate), and SM, *S. minor*.

^{4,5} Means with the same letter in the same column for each isolate were not significantly different at $P \geq 0.05$ level of significance.

Table 3. Mean area under mycelial growth progress curve (AUMGC) for *S. sclerotiorum* (peanut isolate), *S. sclerotiorum* (pumpkin isolate) and *S. minor* grown on SPDA with different matric potentials (ψ_m).

Isolates	Matric potentials in MPa	AUMGC ¹
SS ²	-0.34	21.95 b ³
SS	-1.00	22.81 a
SS	-1.50	21.91 b
SS	-2.00	22.24 ab
SS	-2.50	22.88 a
SS	-3.00	22.46 ab
SS	-3.50	22.89 a
SSP	-0.34	21.62 bc
SSP	-1.00	24.49 a
SSP	-1.50	22.03 bc
SSP	-2.00	22.75 ab
SSP	-2.50	21.58 bc
SSP	-3.00	21.14 bc
SSP	-3.50	20.34 c
SM	-0.34	26.93 bc
SM	-1.00	28.62 a
SM	-1.50	26.85 bc
SM	-2.00	26.25 cd
SM	-2.50	25.49 d
SM	-3.00	25.39 d
SM	-3.50	27.52 b

¹ Means of area under mycelial growth progress curve values on matrically amended SPDA.

² SS, *S. sclerotiorum* (peanut isolate), SSP, *S. sclerotiorum* (pumpkin isolate), and SM, *S. minor*

³ Means with the same letter in the same column for each isolate were not significantly different at $P \geq 0.05$.

Sclerotial production on nutrient media: Different levels of osmotic potentials (ψ_s) created by KCl and glycerol significantly ($P=0.05$) affected sclerotia number produced by the three *Sclerotinia* isolates (Table 4). In general, when KCl was the osmoticum, sclerotia number did not follow a consistent pattern. However, when glycerol was the osmoticum, sclerotia number decreased when osmotic stress increased (Table 4). In matric potential (ψ_m) studies, different levels of (ψ_m) significantly affected the mean sclerotia number produced by the three isolates (Table 5).

Sclerotial production on nutrient media: Different levels of osmotic potentials (ψ_s) created by KCl and glycerol significantly ($P=0.05$) affected sclerotia number produced by the three *Sclerotinia* isolates (Table 4). In general, when

KCl was the osmoticum, sclerotia number did not follow a consistent pattern. However, when glycerol was the osmoticum, sclerotia number decreased when osmotic stress increased (Table 4). In matric potential (ψ_m) studies, different levels of (ψ_m) significantly affected the mean sclerotia number produced by the three isolates (Table 5).

Table 4. Mean of sclerotia number of *S. sclerotiorum* (peanut isolate), *S. sclerotiorum* (pumpkin isolate) and *S. minor* produced on SPDA amended to various osmotic potentials using KCl and glycerol.

Isolates	Osmotic potentials in MPa	Sclerotia number ¹ KCl	Sclerotia number ² Glycerol
SS ³	-0.34	9.8 bcd ⁴	20.40 a ⁵
SS	-0.50	8.6 cd	19.20 ab
SS	-1.00	9.6 bcd	17.80 abc
SS	-1.50	15.2 a	15.40 bc
SS	-2.00	11.2 bcd	15.20 bc
SS	-2.50	10.8 bcd	14.00 cd
SS	-3.00	8.2 d	13.80 cd
SS	-3.50	11.6 bc	10.00 ed
SS	-4.00	11.8 b	9.60 e
SSP	-0.34	13.8 de	70.80 a
SSP	-0.50	17.0 cde	48.20 b
SSP	-1.00	20.2 bcd	39.60 bc
SSP	-1.50	22.0 abc	36.80 c
SSP	-2.00	28.4 a	35.20 c
SSP	-2.50	22.8 abc	33.00 cd
SSP	-3.00	25.2 ab	36.00 cd
SSP	-3.50	20.4 bcd	25.00 d
SSP	-4.00	13.0 e	24.60 d
SM	-0.34	460.2 d	1018.80 a
SM	-0.50	546.0 d	992.20 a
SM	-1.00	492.4 d	948.00 a
SM	-1.50	735.2 bc	924.40 a
SM	-2.00	776.8 b	731.20 b
SM	-2.50	760.8 b	682.80 b
SM	-3.00	788.0 b	644.00 b
SM	-3.50	1069.6 a	535.20 c
SM	-4.00	593.6 cd	520.00 c

¹Means of sclerotia number produced/plate on KCl amended SPDA.

²Means of sclerotia number produced/plate on glycerol amended SPDA.

³SS, *S. sclerotiorum* (peanut isolate), SSP, *S. sclerotiorum* (pumpkin isolate), and SM, *S. minor*

^{4,5}Means with the same letter in the same column for each isolate were not significantly different at $P \geq 0.05$.

Table 6. Percentage of sclerotia germination of *S. sclerotiorum* (peanut isolate), *S. sclerotiorum* (pumpkin isolate) and *S. minor*, produced on SPDA with various osmotic potentials of 0.0 to -4.0 MPa.

Isolates	Osmotic Potentials in MPa	% Sclerotia germination ¹
SS ²	-0.34	100.0 a ³
SS	-0.50	100.0 a
SS	-1.00	100.0 a
SS	-1.50	100.0 a
SS	-2.00	100.0 a
SS	-2.50	95.0 a
SS	-3.00	95.0 a
SS	-3.50	90.0 a
SS	-4.00	90.0 a
SSP	-0.34	100.0 a
SSP	-0.50	100.0 a
SSP	-1.00	100.0 a
SSP	-1.50	100.0 a
SSP	-2.00	100.0 a
SSP	-2.50	100.0 a
SSP	-3.00	95.0 ab
SSP	-3.50	85.0 bc
SSP	-4.00	80.0 c
SM	-0.34	100.0 a
SM	-0.50	100.0 a
SM	-1.00	100.0 a
SM	-1.50	95.0 ab
SM	-2.00	95.0 ab
SM	-2.50	95.0 ab
SM	-3.00	90.0 ab
SM	-3.50	90.0 ab
SM	-4.00	80.0 b

¹Percentage of sclerotia germination on SPDA amended to different osmotic potentials.

²SS, *S. sclerotiorum*, SSP, *S. sclerotiorum* (pumpkin), and SM, *S. minor*

³Means with the same letter in the same column for each isolate were not significantly different at $P \geq 0.05$.

Sclerotial germination: Germination of sclerotia of *S. sclerotiorum* (pumpkin isolate) and *S. minor* produced at different levels of osmotic potentials (ψ_s) created by KCl was significantly ($P=0.05$) affected (Table 6). However, germination of the sclerotia of *S. sclerotiorum* (peanut isolate) produced at various levels of (ψ_s) was not affected (Table 6).

Virulence of mycelia produced on media at various water potentials:

In osmotic potential (ψ_s) studies, mycelia of *S. sclerotiorum* (peanut isolate), and *S. minor* produced on different (ψ_s) were inconsistent in its virulence against the runner peanut cv. Okrun (Table 8). Only mycelia of *S. sclerotiorum* (pumpkin isolate) produced at osmotic stress at -2.0 MPa and lower were statistically less virulent (Table 8). In matric potential studies (ψ_m), mycelia of *S. minor* and *S. sclerotiorum* produced at different matric levels were inconsistent in its virulence against peanut cv. Okrun (Table 9).

Table 7. Percentage of sclerotia germination of *S. sclerotiorum* (peanut isolate), *S. sclerotiorum* (pumpkin isolate) and *S. minor*, produced on SPDA with various matric potentials of 0.0 to -3.5 MPa.

Isolates	Matric potentials in MPa	% Sclerotia germination ¹
SS ²	-0.34	100.0 a ³
SS	-1.00	100.0 a
SS	-1.50	100.0 a
SS	-2.00	100.0 a
SS	-2.50	100.0 a
SS	-3.00	85.0 b
SS	-3.50	80.0 b
SSP	-0.34	100.0 a
SSP	-1.00	100.0 a
SSP	-1.50	100.0 a
SSP	-2.00	100.0 a
SSP	-2.50	95.0 a
SSP	-3.00	90.0 a
SSP	-3.50	55.0 b
SM	-0.34	100.0 a
SM	-1.00	99.0 a
SM	-1.50	99.0 a
SM	-2.00	99.0 a
SM	-2.50	99.0 a
SM	-3.00	98.0 a
SM	-3.50	98.0 a

¹Percentage of sclerotia germination on SPDA amended to different matric potentials.

²SS, *S. sclerotiorum* (peanut); SSP, *S. sclerotiorum* (pumpkin); SM, *S. minor*

³Means with the same letter in the same column for each isolate were not significantly different at $P \geq 0.05$.

Effect of plant water stress on infection of peanut:

Water stressed seedlings of cultivar Okrun, as measured by determining the relative water content (RWC) (Teulat

et al. 1997), differed significantly ($P=0.05$) in their susceptibility to infection by each of the three *Sclerotinia* isolates (Table 10). Stressed plants exhibited less disease when inoculated with *S. minor* or *S. sclerotiorum* (peanut isolate). AUDPC produced by both isolates decreased as the water stress level increased (Table 10).

Table 8. Mean area under disease progress curve (AUDPC) on "Okrun" inoculated with mycelia of *S. sclerotiorum* (peanut isolate), *S. sclerotiorum* (pumpkin isolate) and *S. minor* produced on osmotic amended SPDA.

Isolates	Osmotic Potentials in MPa	AUDPC ¹
SS ²	-0.34	16.108 ab ³
SS	-0.50	15.731 abc
SS	-1.00	12.956 cbd
SS	-1.50	17.781 a
SS	-2.00	9.844 d
SS	-2.50	12.200 cbd
SS	-3.00	11.000 d
SS	-3.50	10.419 d
SSP	-0.34	40.375 ab
SSP	-0.50	47.032 a
SSP	-1.00	18.000 c
SSP	-1.50	30.875 b
SSP	-2.00	18.813 c
SSP	-2.50	19.281 c
SSP	-3.00	14.656 c
SSP	-3.50	15.094 c
SM	-0.34	33.500 a
SM	-0.50	33.815 a
SM	-1.00	30.313 ab
SM	-1.50	30.719 ab
SM	-2.00	31.219 ab
SM	-2.50	24.813 b
SM	-3.00	26.406 b
SM	-3.50	29.438 ab

¹Means of area under disease progress curve values caused by mycelia produced on KCl amended SPDA.

²SS, *S. sclerotiorum* (peanut isolate), SSP, *S. sclerotiorum* (pumpkin isolate), and SM, *S. minor*

³Means with the same letter in the same column for each isolate were not significantly different at $P \geq 0.05$.

In case of *S. sclerotiorum* (pumpkin isolate), AUDPC decreased significantly ($P=0.05$) as the water stress applied on plants increased but there was an eruption in the amount of the disease observed on plants when water stressed to -1.76 MPa (Table 10).

Our results have shown that stressed peanut plants exhibited less disease when inoculated with the peanut isolate of *S. minor* or *S. sclerotiorum*. Our search of the literature has found no previous research that examined the effect of the status of water hydration on peanut and its infection by *S. minor* and *S. sclerotiorum*. Short-term droughts for days or weeks during the growing season may predispose plants to diseases (Schoeneweiss, 1975). For example, larger cankers were induced by *Lasiodiplodia theobromae* on water stressed *Cornus florida* L. (Mullen *et al.* 1991), by *Hypoxyton prunatum* on water stressed *Populus tremuloides* (Bagga and Smalley, 1969), and drought stress increased the severity of *Botryosphaeria* blight of *Pistacia vera* caused by *Botryosphaeria dothidea* (Ma *et al.* 2001).

Table 9. Mean area under disease progress curve (AUDPC) on "Okrun" inoculated with mycelia of *S. sclerotiorum* (peanut isolate), *S. sclerotiorum* (pumpkin isolate) and *S. minor* produced on metrically amended SPDA.

Isolates	Matric Potentials in MPa	AUDPC ¹
SS ²	-0.34	19.925 a ³
SS	-1.00	21.743 a
SS	-1.50	23.893 a
SS	-2.00	28.050 a
SS	-2.50	20.225 a
SS	-3.00	28.431 a
SS	-3.50	25.225 a
SSP	-0.34	15.937 a
SSP	-1.00	15.543 ab
SSP	-1.50	15.718 ab
SSP	-2.00	15.293 ab
SSP	-2.50	13.718 b
SSP	-3.00	15.469 ab
SSP	-3.50	15.398 ab
SM	-0.34	20.093 a
SM	-1.00	16.587 a
SM	-1.50	15.550 a
SM	-2.00	17.293 a
SM	-2.50	16.931 a
SM	-3.00	16.906 a
SM	-3.50	13.468 a

¹Means of area under disease progress curve values caused by mycelia produced on metrically amended SPDA using agar.

²SS, *S. sclerotiorum* (peanut isolate), SSP, *S. sclerotiorum* (pumpkin isolate), and SM, *S. minor*.

³Means with the same letter in the same column for each isolate were not significantly different at P≥0.05.

Table 10. Mean area under disease progress curve (AUDPC) on Okrun cultivar under water stress and infected by *S. sclerotiorum* (peanut isolate), *S. sclerotiorum* (pumpkin isolate) and *S. minor*

Isolates	Water stress in MPa	AUDPC ¹
SS ²	0.00	35.406 a ³
SS	-0.05	35.953 a
SS	-0.15	32.891 ab
SS	-0.30	26.563 abc
SS	-0.49	26.734 abc
SS	-0.73	24.844 bc
SS	-1.03	22.078 c
SS	-1.37	21.031 c
SS	-1.76	21.016 c
SSP	0.00	34.109 a
SSP	-0.05	32.344 a
SSP	-0.15	33.047 a
SSP	-0.30	30.588 ab
SSP	-0.49	30.438 ab
SSP	-0.73	27.766 bc
SSP	-1.03	25.844 cd
SSP	-1.37	23.828 d
SSP	-1.76	38.625 e
SM	0.00	33.463 a
SM	-0.05	30.225 ab
SM	-0.15	29.038 ab
SM	-0.30	24.797 bc
SM	-0.49	19.984 cd
SM	-0.73	19.234 cd
SM	-1.03	18.784 cd
SM	-1.37	13.531 d
SM	-1.76	14.703 d

¹Means of area under disease progress curve values on Okrun under water stress created by PEG8000.

²SS, *S. sclerotiorum* (peanut isolate), SSP, *S. sclerotiorum* (pumpkin isolate), and SM, *S. minor*

³Means with the same letter in the same column for each isolate were not significantly different at P≥0.05.

Our results contradict these observations. Our data indicate that water stressed plants had smaller lesions compared with non-water stressed plants or plants that were under less water stress. This information can be used in disease management by applying less irrigation to infected peanut plants or on plants grown in infested soils. Reduction in mycelial growth of *S. minor* and *S. sclerotiorum* under increased osmotic stress suggests that the reduced growth of both species may partly explain the reduction in AUDPC on plants under high

level of water stress. *Sclerotinia sclerotiorum* (pumpkin isolate), caused larger lesions when the water stress level increased. There was no published data, to our knowledge, concerning effects of water potential on mycelial growth, sclerotial number and germination of *Sclerotinia sclerotiorum* and *Sclerotinia minor* collected from peanut fields. Therefore, this study is the first to show the negative effects of osmotic and matric stress on mycelial growth and sclerotial formation of the two *Sclerotinia* species. Furthermore, this study stated for the first time the effect of water stress on the infection of peanut by *S. sclerotiorum* and *S. minor*.

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DISCUSSION

Sudden changes in the external conditions can disrupt the homeostasis and normal physiology of all living organisms. Therefore, cells have developed complex systems to identify the adverse changes of their environment, and rapidly generate defense mechanism to survive environmental stresses (Lushchak, 2011). The pattern of mycelial growth of *S. minor* and *S. sclerotiorum*, presented in the results section, was similar to that observed by Ferrin and Stanghellini (2006) with *Monosporascus cannonballus*, which indicates that the observed responses were caused by changes in osmotic stress rather than by toxicity of the osmotica. Also, the mycelial growth responses of *S. minor* and *S.*

sclerotiorum to different osmotic stress in this study are similar to those previously observed for other soil borne pathogens (Ritchie *et al.* 2006). For instance, mycelial growth of *Rhizoctonia solani* (Kumar *et al.* 1999), *Gaeumannomyces graminis* (Grose *et al.* 1984), *Typhula idanoensis* and *Typhula incarnata* (Bruehl and Cunfer 1971), *Macrophomina phaseolina* (Cervantes-Garcia *et al.* 2003), and *Aspergillus niger* and *Fusarium moniliforme* (Subbarao *et al.* 1993) was reduced when osmotic stress increased.

Solutes present in agar medium trap water molecules, therefore water will not be available to *S. minor* and *S. sclerotiorum*. The energy spent by the fungi to obtain water molecules from the medium is increased as the solute concentrations in the agar medium increase, and therefore reduction of fungal growth occurs. Ionic solutes such as KCl and NaCl and non-ionic solutes such glycerol and sucrose have been used in several water potential studies involving various plant pathogenic fungi like *F. solani* (Palacios *et al.*, 2014), *Phytophthora cryptogea* and *Fusarium moniliforme* (Woods and Duniway, 1986), *Verticillium dahlia* (Ioannou *et al.* 1977) and the biocontrol agent *Coniothyrium minitans* (Jones *et al.*, 2011). In this research, *S. minor* and *S. sclerotiorum* isolates grew on KCl and glycerol adjusted PDA over all levels of the osmotica tested (Table 2). The ability of a fungus to grow under osmotic stress and the exact optimal water potential depends on the fungal species and in some cases on the osmoticum, temperature, or other factors in the environment (Cook, 1981). Mycelial growth under KCl osmotic stress may result from uptake of potassium ions and its accumulation by microbial cells, which lower the water potential of the protoplasm to a value more ideal for cellular processes, or may increase turgor and hence acceleration of growth (Olaya *et al.* 1996). On matrically modified SPDA, *S. sclerotiorum* (peanut isolate) had the highest mycelial growth at -3.5 MPa, however, there were no significant differences over the range -2.0 to -3.5 MPa. *S. sclerotiorum* (pumpkin isolate) and *S. minor* grew best at -1.0 MPa. However, area under mycelial growth curve (AUMGC) values produced by the three isolates at the lowest matric potential were greater than those recorded at the lowest osmotic potential used in this study. Moreover, the mycelial growth of the three isolates have not been inhibited at the lowest matric potential used in this study, -3.5 MPa, which is lower than the permanent wilting point of mesophytic higher plants -1.5 MPa; (Slayter, 1967).

In general, matric stress has not shown to be a consistent factor affecting the number of sclerotia produced by the three *Sclerotinia* isolates. However, there appears to be a statistical trend to support that the highest level of matric stress, -3.5 MPa, favorably affected the number of sclerotia produced by *S. sclerotiorum* and *S. minor* (Table 5). Total sclerotia production by any test isolate was greater on glycerol amended PDA than on KCl amended PDA (Table 4). This may be due to the utilization of the glycerol as a carbon source by *S. sclerotiorum* and *S. minor* (Sommers *et al.* 1970). On matrically amended SPDA, the three isolates of *S. sclerotiorum* and *S. minor* produced the biggest numbers of sclerotia on -3 and -3.5 Mpa (Table 5). This indicates these isolates of *S. sclerotiorum* and *S. minor* were well adapted to wider ranges of soil water potentials well beyond the limits of their peanut host, provided that other environmental factors are conducive. Also, osmotic stress forces the isolates of *S. sclerotiorum* and *S. minor* to produce sclerotia as survival structure. This could be one of the factors involved in its fitness as a soil-borne plant pathogen (Ritchie *et al.* 2006).

The difference in sclerotial germination between the two isolates of *S. sclerotiorum* in response to osmotic stress (ψ_s) suggests that within each species there may exist ecotypes with variability in their response to environmental factors. This needs future research. Evaluating matric potential (ψ_m), significant differences were observed between treatments for *S. sclerotiorum* isolates but not for *S. minor*. At the lowest (ψ_m) -3.5 Mpa, the percentage of sclerotia germination was 80% for *S. sclerotiorum* (peanut isolates), 55% for *S. sclerotiorum* (pumpkin isolate) and 98% for *S. minor*. Ability of sclerotia to germinate at low (ψ_s) is perhaps due to solute uptake by the sclerotium causing a reduction in its internal osmotic potential, and so allowing maintenance of germination processes (Cook and Al-Hamdani, 1986). In this study, sclerotial formation and germination of *S. minor* and *S. sclerotiorum* occurred at (ψ_s) and (ψ_m) lower than those at which most crops seed germination and root development are curtailed (-1.4 to -2.0 MPa); (Tommerup, 1984). This could be of importance to understand the ecological factors that could affect pathogenicity as well as saprophytic behavior.

Different matric potentials did not significantly affect AUDPCs produced by the three isolates (Table 9). No

research has been done before to investigate the effect of osmotic and matric potentials on the virulence of *S. minor* and *S. sclerotiorum*. Few studies in the literature investigated the effect of water potential on the virulence of plant pathogenic fungi. Cervantes-Garcia *et al.* (2003) observed a reduction in the pathogenicity of *Macrophomina phaseolina* on seeds of common beans, as NaCl concentrations increased in potato-glucose-agar medium. The results reported herein show that *S. minor* and *S. sclerotiorum* can grow vegetatively under relatively low water potentials. The ability of *S. minor* and *S. sclerotiorum* to grow in a wide range of water potentials indicates the presence of adaptive mechanisms for life under variable environmental conditions. Adapting to a wide range of water potentials may be a strategy to survive as a saprophyte.

Our data have shown that stressed peanut plants exhibited less disease when inoculated with the peanut isolate of *S. minor* or *S. sclerotiorum*. Our search of the literature has found no previous research that examined the effect of the status of water hydration on peanut and its infection by *S. minor* and *S. sclerotiorum*. However, Schoeneweiss (1975) has found that short-term droughts for days or weeks during the growing season may predispose plants to diseases. Other examples, larger cankers were induced by *Lasiodiplodia theobromae* on water stressed *Cornus florida* L. (Mullen *et al.* 1991), by *Hypoxyylon prunatum* on water stressed *Populus tremuloides* (Bagga and Smalley 1969), and drought stress increased the severity of *Botryosphaeria* blight of *Pistacia vera* caused by *Botryosphaeria dothidea* (Ma *et al.* 2001). Our results contradict these observations. Our data indicate that water stressed plants had smaller lesions compared with non-water stressed plants grown under near optimal conditions of peanut growth. This information can be used in disease management by applying less irrigation to infected peanut plants or on plants grown in infested soils. Reduction in mycelial growth of *S. minor* and *S. sclerotiorum* under increased osmotic stress suggests that the reduced growth of both species may partly explain the reduction in AUDPC on plants under high level of water stress.

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