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EFFECTS OF OSMOTIC AND MATRIC POTENTIALS ON *Pyrenophora tritici-repentis*

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ABSTRACT

The effects of osmotic and matric water potential on mycelial growth, production and germination of conidia, and production and maturation of pseudothecia of *Pyrenophora tritici-repentis* (PTR), the cause of tan spot of wheat, were examined on clarified V8 juice agar amended with KCl or polyethylene glycol 8000 (PEG8000). Patterns of the growth responses of three isolates to decreasing osmotic and matric potentials were similar for KCl and PEG8000, respectively. Compared with growth on non-amended CV8 agar (-0.24 MPa), growth of all isolates significantly decreased as osmotic and matric potentials reduced to -4.0 MPa and -2.0 MPa, respectively. Conidia production and germination decreased in response to the reduction in osmotic and matric potentials. All isolates produced pseudothecia on wheat straw at all water potentials created by PEG8000 over the range of -0.29 to -2.0 MPa. Mycelial growth, conidia production, germination and pseudothecia production were not completely inhibited at any of the osmotic and matric potentials used in this study. In additional experiments, treatment with various concentrations of PEG8000 was used to simulate water stress on the wheat cultivar TAM105 in the second week after planting, followed by inoculation with PTR when three leaves were fully expanded. Increasing water stress on TAM105 was associated with a greater susceptibility to tan spot.

Keywords: water potential, *Pyrenophora tritici-repentis,* mycelial growth, conidia.

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INTRODUCTION

Tan spot or yellow leaf spot is an economically important foliar disease of wheat. It occurs worldwide in most major wheat growing areas (Hunger and Brown, 1987). Tan spot was detected in the United States in New York in 1940 and in Kansas in 1947 (Watkins et al., 1978) and in Canada, the first serious outbreak occurred in 1974 (Ciuffetti and Tuori, 1999). By the end of the 1970's tan spot was detected and became a major disease on wheat in Oklahoma and the southern plains of the United States (Hunger and Brown, 1987). The first foliar symptoms of tan spot appear as small, light brown blotches that develop into oval–shaped, necrotic lesions bordered with a chlorotic yellow halo (Necrosis typically begins near the tip and progresses towards the base of the leaf. As lesions age, they merge and cause senescence of the entire leaf (Schilder and Bergstrom, 1993).

Pyrenophora tritici-repentis (Died.) Drechs. (synonym *P. trichostoma* (Fr.) Fuckel), anamorph: *Drechslera tritici-repentis* (Died.) Shoemaker (Synonym *Helminthosporium tritici-repentis* Died.), is a homothallic ascomycete that is the causal agent of tan spot on bread wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L. var. *durum*) (Pfender et al., 1991). This fungus has a multi-nucleated (haploid) mycelium with cross walls and produces sexual and asexual spores. The sexual spores or the perfect stage are called ascospores are being formed within asci. Asci are formed directly in cavities within a stroma or matrix of mycelium called pseudothecia or an ascostroma (Pfender et al., 1991; Schoch et al., 2006). Pseudothecia are black with double walls, and 0.2 to 0.35 mm in diameter with dark spines surrounding the short beaks (Zillinsky, 1983). Ascospores are brown with three transverse septa and are oval to globose (Ciuffetti and Tuori, 1999). The asexual spores, (anamorph, or the imperfect stage) are called conidia and are born on septate conidiophores with a swollen base. The conidia are subhyaline, cylindrical, with four to seven septa (Ciuffetti and Tuori, 1999). On potato dextrose agar (PDA), pathogen growth is a dense, fluffy, greenish-grey mycelium without sporulation and white to light grey when grown on clarified V8 juice agar (CV8) (Schilder and Bergstrom, 1993).

The fungus survives through summer, fall and winter primarily as pseudothecia on wheat straw residues on the soil between seasons. Tan spot has become prevalent in most of the wheat growing regions of the world, including the Central Plains of the United States (Sarova et al., 2002; Carigano et al., 2008; Morris et al., 2010). This is in part due increasing employment of conservation-tillage farming, in which crop residue is retained on the soil surface between seasons to reduce soil erosion (Carigano et al., 2008). Ascospores released from pseudothecia are the primary source of inoculum. They discharge from pseudothecia under humid conditions at night in late winter and early in the spring and infect the lower leaves (Rees and Platz, 1983). Secondary infection on upper leaves is caused by conidia, and this secondary infection is

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primarily related to yield losses (Rees and Platz, 1983). Fungal spores usually have a lower respiration and metabolic activity but the presence of substrates such as cereal residues or other nutrients results in a transformation of spores to an active phase characterized by adsorption of water, increase in respiration and biosynthesis of cell components (Magan, 1988). Morphological changes including germ tube formation and elongation occur and ultimately an active vegetative mycelium is formed. This process is influenced by stress imposed by water availability. Spores of fungal species able to overcome such stress would have maximum ecological advantage, resulting in preferential colonization and exploitation of substrata (Magan, 1988).

Water potential is a measure of how much energy is required to extract water from crop residues. Water potential is a fundamental concept is widely used in the biological and soil sciences for quantifying the energy of water in plants, microorganisms, soils, and other related systems (Papendick and Campbell, 1981; Abdelmagid et al., 2015). Water potential is an abbreviated expression for the "potential energy of water" (Papendick and Mulla, 1986). Water potential is measured in units of pressure and is commonly represented by the Greek letter Ψ (Psi). Pure water at standard temperature and pressure (or other suitable reference condition) is defined as having a water potential of zero. The addition of solutes to water lowers its potential (makes it more negative). Water moves from an area of higher water potential to an area of lower water potential (Papendick and Mulla, 1986).

Fungi as a part of any thermodynamic system tend to achieve water potential equilibrium with the surrounding environment. Water flows spontaneously from high to low potentials (from low negative to more negative) and the availability of water for physiological processes decreases as the potential is lowered (Papendick and Mulla, 1986; Abdelmagid et al., 2015).

Total soil water potential is the sum of many components including matric, osmotic, pressure and gravitational potentials (Cook and Al-Hamadani, 1986). In agricultural soils, matric and osmotic potentials are the most important components of water potential which govern water flow and availability for physiological process (Cook and Al-Hamadani, 1986). Osmotic potential is due to the presence of solutes in soil water and it is important in saline soils or soils amended with fertilizers and organic waste (Palacios et al., 2014). Matric potential includes both adsorption and capillary effects and it is the most important factor affecting fungal growth in soil, crop residues or root surfaces (Cook and Al-Hamadani, 1986).

The effect of water potential on the maturation of pseudothecia on wheat straws has not been investigated before. In soil and cereal crop residues matric potential is the major component of the total water potential (Griffin, 1981). The matric potential affects growth of soil fungi and maturation of fungal spores on crop residues more than osmotic potential (Griffin, 1981). No work has been done before to compare the effect of osmotic and matric potentials on the

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maturation and viability of the pseudothecia of PTR. Also, the effect of water potential on mycelial growth, conidia formation, and germination on artificial media *in vitro*, has seldom been considered.

Better understanding of the interaction between abiotic factors and pseudothecia maturation, conidia sporulation and germination is important to develop improved control programs. Hence, the objectives of this research were to (1) determine the role of water potential on mycelial growth, conidia formation and germination of PTR; (2) determine the effect of water potential on initiation and maturation of pseudothecia of PTR on wheat straws; and (3) investigate the impact of water stress on the infection of wheat by PTR.

MATERIALS AND METHODS

Effect of water potential on the mycelial growth, conidia formation and germination of *Pyrenophora tritici-repentis* (PTR)

Three PTR isolates, OKD2, RBB6 and OK-06-3 were used in this study. The three isolates were collected from Oklahoma in 1983, 1996 and 2006, respectively. The isolates were previously confirmed as wheat pathogens following koch's postulate (Rober M. Hunger, Oklahoma State University, personal communication). Isolates were maintained on PDA (200 g potato, dextrose 20 g, agar 15 g in 1 L water) acidified to pH 5.5 containing 100 ppm of streptomycin sulfate to suppress bacterial contamination. Mycelial plugs (5-mm diameter) were excised from the advancing margin of each PTR isolate grown for 6 days on PDA. The plugs were placed in the center of 9 cm Petri dishes filled with 15 ml of clarified V8 juice agar medium (CV8) (200 ml of V8 juice®, 3g CaCO3, 20 g agar and 800 ml distilled water) containing 100 ppm of streptomycin sulfate to prevent bacterial contamination. CV8 was osmotically modified over the range of -0.5 to -4 MPa with KCl (Ritchie et al., 2006).

Total osmotic potential was the sum of the water potential of the CV8 -0.24 MPa and the osmotic potential of the added osmoticum. Osmotic potential was calculated according to (Liddell, 1993). CV8 also was adjusted matrically over the range -0.29 to -2.0 MPa using polyethylene glycol 8000 (PEG 8000) (Union Carbide Chemicals and Plastics, Danbury, CT) (Michel and Kaufmann, 1973; Magan, 1988; Palacios et al., 2014). It previously has been shown that the water potential generated by PEG 8000 is predominantly (99%) due to matric forces (Steuter et al., 1981).

The actual osmotic and matric potentials of all media were checked using a Vapor Pressure Osmometer (VAPRO 5520, Wescor, Utah, USA). All media were sterilized for 20 minutes. Inoculated plates of the same water potential were sealed with parafilm, placed inside plastic bags and incubated for 7 days at 21±2 ºC.

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Radial growth was measured by averaging the length of two opposite diameters and substracting 5 mm from each reading. Five replicates (plates) were used for each treatment. The experiment was repeated twice. These same CV8 plates were used to determine conidial production. Ten drops of sterile water was added to each plate and mycelia were mated down using a sterile, bent glass rod. Plates were then kept in the incubator for 12 hr at 23 ºC with cool-white fluroscent tubes (40 W, 30 µEs-1 m-1) to produce conidiophores. This was followed by 12 hr dark at 16 ºC to induce conidia production (Raymond and Bockus, 1982). Conidia were harvested by flooding each plate with 15 ml of distilled water and dislodging the conidia with a bent glass rod. The resulting suspension was filtered through cheesecloth (Moreno et al., 2008). One ml of conidial suspension was pipetted into a segmented petri plate (40 mm) and examined using a stereomicroscope to determine the number of conidia produced.

To determine germination of conidia, 1 ml of conidial suspension was added to 9 ml sterile water amended to the corresponding osmotic or matric potential using KCl and PEG 8000, respectively. The final concentration of spores was in the range of 1-5 x 10^5 per mL. Solutions were left at room temperature. After 4-6 hours, 1 ml of each solution was pipetted into a segmented petri plate (40 mm), and a compound microscope was used to determine germination. Spores were considered germinated when the germ-tube length was equal to or longer than the diameter of the spore (Ramirez et al., 2004).

Determine the effect of water stress on initiation and maturation of pseudothecia of *Pyrenophora tritici-repentis* on wheat straw

To determine the effect of water stress on pseudothecia production by each PTR isolate on wheat straws, the procedure of James *et al.* (1991) as modified by Kazi Kader (Oklahoma State University, personal communication) was followed. Wheat straw collected from the field was cut into pieces (80 mm long) and autoclaved. Three pieces of (9 cm) sterilized Whatman filter paper were placed in Petri dishes (9 cm) and 20 ml of each PEG 8000 solution was added to the sterilized filter papers in concentrations as listed in (Table 1) to create different water potentials. Five pieces of wheat straws were placed parallel to each other on the filter papers. Then, three (5 mm) dia. mycelia plugs of each PTR isolate were placed between the wheat straws. Petri plates were sealed with parafilm to prevent water evaporation. Plates were placed in dark at 21 ºC for two weeks and then transferred to an incubator with 12 h light (30 µEs-1 m-1) and 12 h dark periods at 15 ºC for 24 days. The total number of pseudothecia and mature pseudothecia per wheat straw were counted. A pseudothecium was considered mature only if at least one mature ascospore was found as indicated by the presence of pigmentation and clear septation using a compound microscope (Friesen et al., 2003). The experiment was repeated twice in a randomized complete block design with four replicates.

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Table 1. Required concentrations of polyethylene glycol (PEG 8000) to attain 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

The effect of water stress on the infection of wheat by *Pyrenophora tritici-repentis*

The wheat cultivar TAM 105, which is a tan spot susceptible cultivar, was used in this study. Seedlings were raised in commercially prepared 'Ready-Earth' soil (Sun Gro Co., Bellevue, WA) in 6-in. X 1.5 in. dia plastic cylinders till four weeks old. To apply the water stress, PEG 8000 solutions of various water potentials were prepared according to (Michel and Kaufmann, 1973) (Table 1). Four pots (replicates) were used per water potential (treatment) and each pot had 2 seedlings. Plants were divided into nine groups (eight different water potentials plus the water control). Each group of plants was placed in a plastic tray (38.1 cm x 29.2 cm x 15.2 cm) (Sterilite, Townsend, MA, USA). Water stress was applied to plants in the second week by pouring each PEG 8000 solution into the bottom of its assigned plastic tray. In the water control group, water was used to keep seedlings well irrigated. Conidia were produced on CV8 as described above. A conidial suspension was adjusted to 2 x 10^3 conidia ml^{-1} and 0.05% Tween ® 20 was added as a surfactant. Seedlings with three leaves fully expanded were inoculated with the conidial suspension of each isolate using an atomizer (DeVilbiss Co., Somerest, PA) following the procedure of Rodriguez and Bockus (1996). Inoculated plants were allowed to dry for 30 min so conidia adhered to leaves and then were placed in a mist chamber at 21 ºC and 14 light: 10 dark cycle (510 µEs-1m-1) was applied. After 48 hr, plants were placed in a greenhouse at 21ºC. The disease reaction was recorded six days post-inoculation using the rating system of Lamari and Bernier (1989). The experiment has been repeated twice.

The rating system used was: 1 = small dark brown to black spots without any surrounding chlorosis or tan necrosis, 2 = small dark brown to black spots with very little chlorosis or tan necrosis (moderately resistant), 3 = small dark brown to black spots completely surrounded by a

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distinct chlorotic or tan necrotic ring (lesions generally not coalescing), 4 = small dark brown or black spots completely surrounded with chlorotic or tan necrotic zones (some of the lesions coalescing), 5 = the dark brown or black centers may or may not be distinguishable (most lesions consist of coalescing chlorotic tissues or tan necrotic zones).

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 twice. 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, conidia formation and germination of *Pyrenophora tritici-repentis* on CV8 agar amended to various water potentials.

Osmotic (ψs) and matric (ψm) potentials significantly (P=0.05) affected the vegetative growth of PTR (Tables 2 and 3). Mycelial growth responses of the three PTR isolates to decreasing osmotic and matric potentials were similar. Area under mycelial growth curve (AUMGC) decreased when osmotic and matric potentials decreased (i.e. the stress increased). In osmotic potential studies (Table 2), there were no significant (P=0.05) differences among AUMGC values of isolate RBB6 for the control -0.24, -0.5 and -1.0 MPA. For OK-06-3 and OKD2, there were no significant (P=0.05) differences among AUMGC values for the control and -0.5 MPa. In general, there was a trend toward lower AUMGC values with increasing osmotic stress (Table 2) but OK-06-3 had greater growth than RBB6 and OKD2 over the different osmotic potentials (Table 2).

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Table 2. Area under mycelial growth curve (AUMGC) values for *Pyrenophora tritici-repentis* (PTR) grown on clarified V-8 juice (CV8) agar amended to different osmotic potentials (ψ_s) using KCl.

PTR isolates	Osmotic potentials in MPa	AUMGC[1]
RBB6	-0.24	33.26 a[1]
RBB6	-0.50	32.99 a
RBB6	-1.00	32.01 a
RBB6	-1.50	30.11 b
RBB6	-2.00	24.59 b
RBB6	-2.50	24.23 c
RBB6	-3.00	19.74 d
RBB6	-3.50	18.25 de
RBB6	-4.00	16.79 e
OK-06-3	-0.24	57.14 a
OK-06-3	-0.50	59.57 a
OK-06-3	-1.00	52.83 b
OK-06-3	-1.50	43.56 c
OK-06-3	-2.00	36.94 d
OK-06-3	-2.50	34.87 d
OK-06-3	-3.00	34.11 d
OK-06-3	-3.50	30.28 e
OK-06-3	-4.00	27.09 e
OKD2	-0.24	35.21 a
OKD2	-0.50	34.43 ab
OKD2	-1.00	32.29 b
OKD2	-1.50	29.80 c
OKD2	-2.00	29.57 c
OKD2	-2.50	25.97 d
OKD2	-3.00	25.85 d
OKD2	-3.50	25.83 d
OKD2	-4.00	23.24 e

[1] Two means in the same column and within the same level of isolate with the same letters are not significantly different at a 0.05 level of significance.

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In matric potential studies (Table 3), greatest AUMGC values were observed at the control -0.24 MPa for isolates OK-06-3 and OKD2. For isolate RBB6, greatest AUMGC values were observed at the control -0.24 MPa and at -0.29 MPa. Smallest AUMGC values were recorded at -2.00 MPa for RBB6 and OK-06-3 and at -1.61 and -2.00 MPa for isolate OKD2.

Table 3. Area under mycelial growth curve (AUMGC) values for three isolates of *Pyrenophora tritici- repentis* (PTR) grown on clarified V-8 juice (CV8) agar amended to different matric potentials (ψ_m) using polyethylene glycol 8000

Isolates	Matric potentials in MPa	AUMGC[1]
RBB6	-0.24	31.28 a[1]
RBB6	-0.29	30.67 ab
RBB6	-0.39	29.30 b
RBB6	-0.54	26.76 c
RBB6	-0.73	24.56 d
RBB6	-0.97	23.16 d
RBB6	-1.27	20.51 e
RBB6	-1.61	19.33 e
RBB6	-2.00	16.46 f
OK-06-3	-0.24	54.49 a
OK-06-3	-0.29	52.15 b
OK-06-3	-0.39	49.85 c
OK-06-3	-0.54	41.49 d
OK-06-3	-0.73	37.67 e
OK-06-3	-0.97	36.74 e
OK-06-3	-1.27	33.52 f
OK-06-3	-1.61	25.19 g
OK-06-3	-2.00	16.12 h
OKD2	-0.24	38.50 a
OKD2	-0.29	32.96 b
OKD2	-0.39	31.78 bc
OKD2	-0.54	30.76 c
OKD2	-0.73	30.25 c
OKD2	-0.97	25.75 d
OKD2	-1.27	24.69 d
OKD2	-1.61	21.62 e

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OKD2	-2.00	20.45 e

[1]Two means in the same column and within the same level of each isolate with the same letters are not significantly different at a 0.05 level of significance.

Osmotic potential (P=0.05) affected conidia production of the three PTR isolates on CV8 (Table 4). RBB6, OK-06-3, and OKD2 produced the greatest number of conidia at the control -0.24 MPa and at -0.5 MPa. The fewest conidia were produced by the three isolates at -4.0 MPa. However, there was no significant difference between -3.0, -3.5 and -4.0 MPa for RBB6, or between -3.5 and -4.0 MPa for OK-06-3 (Table 4). Osmotic potential did not significantly (P=0.05) affect conidial germination of RBB6 and OK-06-3, but significantly (P=0.05) affected conidia germination of OKD2 (Table 4). Obviously there was a numerical trend toward lower conidia number and germination with increasing osmotic stress (Table 4).

Table 4. Conidia production and germination percentage of condia produced by three isolates of *Pyrenophora tritici-repentis* (PTR) grown on clarified V-8 juice agar (CV8) amended to different osmotic potentials (ψ_s) using KCl

PTR isolates	Osmotic potentials in MPa	Conidia produced[1]	Conidia germination (%)
RBB6	-0.24	133.6 a[2]	22.47 a[2]
RBB6	-0.50	116.6 a	23.26 a
RBB6	-1.00	83.4 b	22.33 a
RBB6	-1.50	76.8 bc	17.95 a
RBB6	-2.00	63.4 bcd	21.84 a
RBB6	-2.50	56.0 cd	17.05 a
RBB6	-3.00	49.8 de	17.46 a
RBB6	-3.50	44.2 de	19.71 a
RBB6	-4.00	30.4 e	17.41 a
OK-06-3	-0.24	125.0 a	24.26 a
OK-06-3	-0.50	117.0 a	26.49 a
OK-06-3	-1.00	95.0 b	21.80 a
OK-06-3	-1.50	86.2 c	24.62 a
OK-06-3	-2.00	72.8 d	19.70 a
OK-06-3	-2.50	66.8 d	21.23 a
OK-06-3	-3.00	62.6 d	23.08 a
OK-06-3	-3.50	45.8 e	25.74 a

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OK-06-3	-4.00	32.2 e	18.19 a
OKD2	-0.24	35.21 a	22.06 a
OKD2	-0.50	34.43 ab	20.06 ab
OKD2	-1.00	32.29 b	20.04 ab
OKD2	-1.50	29.80 c	19.57 abc
OKD2	-2.00	29.57 c	18.32 abcd
OKD2	-2.50	25.97 d	16.16 bcd
OKD2	-3.00	25.85 d	15.01 bcd
OKD2	-3.50	25.83 d	14.52 cd
OKD2	-4.00	23.24 e	13.76 d

[1]Conidia produced (number/1 ml) by PTR isolates RBB6, OK-06-3, OKD2 on CV8 amended to different osmotic potentials (ψs) using KCl.

[2]Two means in the same column and within the same level of each isolate with the same letters are not significantly different at a 0.05 level of significance.

All three isolates exhibited a similar response in conidia production and germination to decreasing matric potential (Table 5). The greatest number of conidia was produced by the three isolates at control -0.24 MPa and -0.29 MPa, while the fewest were produced on -2.0 MPa for RBB6, and at -1.61 and -2.0 MPa for OK-06-3 and OKD2 (Table 5). Matric potential also significantly (P=0.05) affected % conidia germination of RBB6 and OKD2, but did not affect conidia germination of OK-06-3 (Table 5). As with osmotic potential (Table 4), a similar numeric lowering of conidia number and germination was observed in response to matric stress increase.

Table 5. Conidia production and germination percentage of condia produced by three isolates of *Pyrenophora tritici-repentis* (PTR) grown on clarified clarified V-8 juice agar (CV8) amended to different matric potentials (ψ_m) using polyethylene glycol (8000)

Isolates	Matric potentials in MPa	Mean Conidia[1]	Conidia germination (%)
RBB6	-0.24	126.2 a[2]	17.94 a[2]
RBB6	-0.29	113.2 a	17.16 a
RBB6	-0.39	90.0 b	17.13 a
RBB6	-0.54	78.6 bc	15.53 ab
RBB6	-0.73	64.6 cd	14.84 ab
RBB6	-0.97	53.4 d	14.50 ab

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RBB6	-1.27	48.6 de	13.85 ab
RBB6	-1.61	35.8 e	12.80 ab
RBB6	-2.00	14.8 f	9.22 b
OK-06-3	-0.24	124.8 a	19.39 a
OK-06-3	-0.29	122.8 a	15.32 a
OK-06-3	-0.39	85.6 b	16.90 a
OK-06-3	-0.54	86.2 b	15.96 a
OK-06-3	-0.73	70.0 bc	18.56 a
OK-06-3	-0.97	67.6 c	18.38 a
OK-06-3	-1.27	40.6 d	20.61 a
OK-06-3	-1.61	32.4 de	16.31 a
OK-06-3	-2.00	22.2 e	17.42 a
OKD2	-0.24	116.4 a	16.22 a
OKD2	-0.29	106.0 a	13.18 ab
OKD2	-0.39	84.8 b	13.09 ab
OKD2	-0.54	79.2 bc	12.27 ab
OKD2	-0.73	67.0 cd	11.12 b
OKD2	-0.97	59.6 d	10.52 b
OKD2	-1.27	42.4 e	10.01b
OKD2	-1.61	29.0 ef	9.96 b
OKD2	-2.00	19.2 f	8.94 b

[1]Conidia produced (number/1 ml) by PTR isolates RBB6, OK-06-3, OKD2 on CV8 amended to different matric potentials (ψm) using PEG 8000.

[2]Two means in the same column and within the same level of each isolate with the same letters are not significantly different at a 0.05 level of significance.

The effect of water stress on formation and maturation of pseudothecia by *Pyrenophora tritici- repentis* on wheat straw.

Pseudothecia production by RBB6, OK-06-3, OKD2 decreased with increasing matric stress (Table 6). However, matric potential significantly (P=0.05) affected pseudothecia production on wheat straw by OK-06-3 and OKD2 but not by RBB6 (Table 6). Different matric potentials significantly (P=0.05) affected maturation of pseudothecia for isolates RBB6 and OK-06-3, while no significant differences (P<0.05) occurred with OKD2. A similar downward trend of

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pseudothecia maturation by the three isolates of PTR was observed in response to matric stress increase (Table 6).

Table 6. Pseudothecia production and maturation by three isolates of *Pyrenophora tritici-repentis* (PTR) produced on wheat straw treated with polyethylene glycol 8000 to create different matric potentials (ψ_m)

PTR isolate	Matric potentials in MPa	Mean Pseudothecia produced[1]	Mature pseudothecia (%)
RBB6	-0.24	55.40 a[2]	9.18 a[2]
RBB6	-0.29	58.00 a	9.06 ab
RBB6	-0.39	42.80 a	6.22 ab
RBB6	-0.54	48.00 a	5.79 ab
RBB6	-0.73	53.00 a	5.57 ab
RBB6	-0.97	40.60 a	5.56 ab
RBB6	-1.27	48.60 a	4.57 ab
RBB6	-1.61	44.80 a	4.17 ab
RBB6	-2.00	40.20 a	4.06 b
OK-06-3	-0.24	82.60 a	13.51 a
OK-06-3	-0.29	72.00 ab	7.26 b
OK-06-3	-0.39	68.00 ab	6.22 b
OK-06-3	-0.54	59.60 bc	4.61 b
OK-06-3	-0.73	37.20 d	4.58 b
OK-06-3	-0.97	41.40 dc	4.41 b
OK-06-3	-1.27	38.20 d	4.03 b
OK-06-3	-1.61	14.60 e	3.33 b
OK-06-3	-2.00	10.40 e	2.91 b
OKD2	-0.24	81.60 a	2.95 a
OKD2	-0.29	73.80 a	1.75 a
OKD2	-0.39	66.60 ab	1.70 a
OKD2	-0.54	56.00 bc	1.54 a
OKD2	-0.73	43.80 cd	1.36 a
OKD2	-0.97	30.60 d	1.19 a

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OKD2	-1.27	28.40 de	1.09 a
OKD2	-1.61	11.60 ef	0.96 a
OKD2	-2.00	7.00 f	0.76 a

[1]Mean pseudothecia number produced by RBB6, OK-06-3, OKD2 of PTR on 5 pieces of wheat straw (80 mm each) treated with PEG 8000 to create different matric potentials (ψ_m)

[2]Two means in the same column and within the same level of each isolate with the same letters are not significantly different at a 0.05 level of significance

The effect of water stress on the infection of wheat by *Pyrenophora tritici-repentis*

Increasing water stress imposed on TAM 105 was associated with a greater susceptibility to tan spot as indicated by greater disease reactions associated with the highest three water stresses ranging from -1.03 to -1.76 MPa (Table 7). This was consistently observed with all three isolates.

Table7. Rating of hard red winter wheat (TAM 105) to infection by three isolates of *Pyrenophora tritici-repentis* (PTR) when water stressed using PEG 8000

PTR isolate	Water stress in MPa	Disease rating[1]
RBB6	-0.00	3.85d[2]
RBB6	-0.05	4.05 d
RBB6	-0.15	4.35 c
RBB6	-0.30	4.40 bc
RBB6	-0.49	4.30 c
RBB6	-0.74	4.60 ab
RBB6	-1.03	4.70 a
RBB6	-1.37	4.75 a
RBB6	-1.76	4.70 a
OK-06-3	-0.00	3.80 de
OK-06-3	-0.05	3.65 e
OK-06-3	-0.15	3.95 cd
OK-06-3	-0.30	4.05 bc
OK-06-3	-0.49	3.95 cd
OK-06-3	-0.74	4.05 bc

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OK-06-3	-1.03	4.15 abc
OK-06-3	-1.37	4.25 ab
OK-06-3	-1.76	4.30 a
OKD2	-0.00	3.25 e
OKD2	-0.05	3.25 e
OKD2	-0.15	3.45 cde
OKD2	-0.30	3.55 bcd
OKD2	-0.49	3.40 de
OKD2	-0.74	3.55 bcd
OKD2	-1.03	3.65 abc
OKD2	-1.37	3.75 ab
OKD2	-1.76	3.80 a

[1]Disease reaction is the average value of rating 4 replicates on a scale of 1-5, where 1= small dark brown to black spots without any surrounding chlorosis or tan necrosis, 2= small dark brown to black spots with very little chlorosis or tan necrosis (moderately resistant), 3= small dark brown to black spots completely surrounded with a distinct chlorotic or tan necrotic ring (lesions generally not coalescing), 4= small dark brown or black spots completely surrounded with chlorotic or tan necrotic zones (some of the lesions coalescing), 5 = most lesions consist of coalescing chlorotic tissues or tan necrotic zones.

[2]Two means in the same column and within the same level of each isolate with the same letters are not significantly different at a 0.05 level of significance

DISCUSSION

Decreased tillage combined with limited use of rotation crops in the southern Great Plains has led to an increase in the incidence and severity of tan spot of winter wheat (*Triticum aestivum* L.) (Morris et al., 2010)

Most research investigating the effect of water potential on the biology of plant pathogenic fungi has focused on soil-borne pathogenic fungi. Few studies have been done in the past investigating the effect of water potential on the biology of air-borne fungi such as *P. tritici repentis*. The likely rationale for this is that air-borne fungi are not in direct contact with soil, and it is in the soil where water potential effects are most commonly considered. However, a foliar pathogen such as PTR survives on plant residue between agricultural seasons, and the involvement of water potential and its components on such a system may significantly affect survival and infection by the pathogen. In this regard, some research has been conducted studying the

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presence of microbial antagonists on PTR and the interaction between PTR and potential antagonists on wheat straw under different environmental factors (Pfender et al., 1991; Pfender et al., 1988; Pfender, 1988; Summerell and Burgess, 1989). However, there is no available data in the literature about the effect of osmotic and matric potentials directly on mycelial growth, conidia production and conidia germination of PTR.

In our study, area under mycelia growth curve (AUMGC) values of the three isolates decreased when osmotic (ψs) and matric (ψm) potentials decreased (i.e, the stress increased). However, mycelial growth of the three isolates was not totally inhibited at any of the osmotic and matric potentials used in this study. The response of mycelial growth of the three isolates was similar to decreasing osmotic and matric potentials. We do believe that the observed responses were caused by changes in osmotic and matric stresses rather than by toxicity of KCl or PEG 8000 (Ferrin and Stanghellini, 2006). Toxic effects of KCl and PEG 8000 would cause inconsistency in the mycelial growth of the three isolates among different treatments which was not observed (Abdelmagid et al., 2015). Ionic solutes such as KCl and NaCl have been used in several water potential studies involving various plant pathogenic fungi such as *Fusarium moniliforme* (Woods and Duniway, 1986); *Verticillium dahlia* (Ioannou et al., 1977) and *Sclerotinia minor* and *S. sclerotiorum* (Abdelmagid et al., 2015). PEG 8000 also has been used in many studies investigating the effect of matric potential on several soil plant pathogenic fungi such as *Rhizoctonia solani* (Ritchie et al., 2006); *Fusarium graminearum* (Ramirez et al., 2004) and *Sclerotinia minor* and *S. sclerotiorum* (Abdelmagid et al., 2015)

The water potential generated by PEG 8000 is predominantly (99%) due to matric forces (Steuter et al., 1981). The ability of a fungus to grow under osmotic stress and the exact optimal water potential depends on the fungus species and in some cases on the osmoticum, temperature, or other factors in the environment (Cook and Al-Hamadani, 1986; Abdelmagid et al., 2015). 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). In this study, the mycelial growth of the three isolates of *P. tritici repentis* was not inhibited at osmotic and matric potentials below -1.5 MPa, which is about the same or lower than the permanent wilting point of mesophytic higher plants (Slayter, 1967). Conidia production and germination by the three isolates were reduced significantly in response to increasing osmotic and matric stress. The reduction of conidia produced may be correlated to reduced mycelial growth.

Tan spot is a disease favored in wheat produced under conservation tillage because PTR completes its life cycle on wheat residue. Conservation tillage, in which crop residue is left on

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the soil surface between cropping seasons to reduce soil erosion and increase water retention, is becoming increasingly common (Pfender, 1988). The production of pseudothecia, and number of mature ascospores per ascus are important to tan spot epidemics. Survival of PTR on and in infested straw differs with its position (i.e. buried or on soil surface), or microenvironment, in the field (Pfender et al., 1991). In a study of fungal communities associated with conservation-tillage wheat straw in Kansas, Pfender and Wootke (1988) found that the fungus persisted in straw retained on a mulch layer above the soil surface. PTR was rarely recovered from buried straw or straw retained for several months directly on the soil surface beneath the mulch layer. In our study, pseudothecia number and maturation of pseudothecia produced by PTR in artificially infested wheat straw stored without soil contact decreased significantly (P=0.05) when the matric potential decreased.

In soil and cereal crop residue, matric potential is the major component of the total water potential (Magan and Lynch, 1986). Griffin (1981) suggested that matric potential would affect growth of soil fungi more than osmotic potential. High water potential is not in itself detrimental to growth or pseudothecia production by PTR. Summerell and Burgess (1988) reported that PTR requires water potentials above approximately -1.5 MPa for pseudothecia production on osmotically adjusted agar or on adjusted wheat residue. Although maximal growth of this pathogen occurs at high water potential (i.e., less water stress); it can grow in wheat residue at water potentials as low as -8.5 MPa (Pfender et al., 1988).

Growth at such low water potentials could enable PTR to avoid competition from micro-organisms more limited in their moisture stress tolerance. PTR on wheat straw buried in soil has been displaced by actinomycetes and soil borne fungi than on straws on soil surface (Pfender, 1988). Nevertheless, because of the relatively high water potential requirement for pseudothecia production, PTR must at least occasionally interact with micro-organisms under wet conditions if it is to produce its primary inoculum (Pfender et al., 1991).

Whether a disease develops depends upon the influence of environmental factors on the genetically controlled response of the host plant to the presence of the pathogen or its metabolites. The tendency of non-genetic factors, acting prior to infection, to affect the susceptibility of plants to disease is called predisposition (Schoeneweiss, 1975). In the course of their development, plants may frequently be exposed to temporary water deficiency which is intrinsic to most abiotic forms of stress, not only during drought, but also at low temperature and when the soil contains high concentrations of ions. This can occur not only in arid and semi-arid regions, but also under continental climatic conditions (Hoffmann and Burucs, 2005).

Various plant varieties differ in their ability to survive long periods of water deficiency and in the strategies they employ to counteract the adverse effects of water stress. This depends

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primarily on the water use efficiency of the variety and on its genetically determined drought tolerance (Chaves *et al*., 2003). In the present study, water stress predisposed TAM 105 seedlings to infection by PTR. Disease severity on the wheat variety TAM 105 increased when water stress increased. Major changes in climate over a period of years have been implicated as stress factors affecting the incidence and severity of many diseases (Schoeneweiss, 1975). In the United States, ash dieback, maple decline, sweetgum blight, birch dieback, oak decline, dry face of slash pine, and pitch streak of slash pines have been associated with an extended period of below normal precipitation in the 1930s (Schoeneweiss, 1975). Short term droughts of days or weeks during the growing season may also predispose plants to diseases (Cook, 1973).

In another study, Beddis and Burgess (1992) found that *Fusarium graminearum* was able to colonize water stressed wheat seedlings to a greater height than seedlings grown under non-stress conditions. Water stress like other abiotic stresses may increase the concentration of reactive oxygen species (ROS), which may cause damage to macromolecules, leading to the death of the cells (Beddis and Burgess, 1992). The stress response can also vary depending on the developmental stage during which wheat is subject to stress (Pereyra and Torroba, 2003).

In conclusion, low water potential (high stress) decreased vegetative growth, conidia production and germination, pseudothecia production and maturation on wheat straw without soil contact, and increased water stress was associated with increased tan spot severity on wheat seedlings (TAM 105). In no till systems in dry years, the pathogenicity parameters that allow PTR to survive on wheat straw may be negatively affected. Future work should be extended to the field and include more wheat varieties to develop residue management and biological control procedures for reducing primary inoculum (i.e., pseudothecia) of PTR in conservation-tillage wheat residue.

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