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Pathogenicity of Three Isolates of *Rhizoctonia* sp. From Wheat and Peanut on Hard Red Winter Wheat

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Abstract: *Rhizoctonia*-induced root diseases can significantly affect wheat and peanut production where these two field crops are grown in rotation. Hence, this study characterized two isolates of *Rhizoctonia* sp. from wheat [*R. cerealis* (RC) and *R. solani* (RSW)] and one from peanut [*R. solani* (RSP)] for cultural traits and pathogenicity on Hard Red Winter Wheat (HRWW). The RSP had a higher optimum temperature than RC and RSW for growth (hyphal extension) on media (25 to 27.5°C versus 20 to 25°C), seedling emergence, shoot and root weight and disease severity (≥25°C versus <25°C). Of six HRWW cultivars tested, TAM 101, Tonkawa and Custer demonstrated some resistance to RSP at 30°C. Field trials revealed that RC and RSW significantly reduced seedling emergence in late-planted wheat when soil temperature was cool (<27°C), but did not reduce seedling emergence in early-planted wheat when soil temperature was high (>35°C). In contrast, RSP significantly reduced seedling emergence in both early and late-planted wheat and also reduced forage production in early-planted wheat in fumigated soil. Results suggest that *Rhizoctonia* from peanut (a warm weather crop) are more virulent on wheat at higher soil temperatures associated with early planting, whereas *Rhizoctonia* obtained from wheat (a cool weather crop) are more pathogenic on wheat at lower soil temperatures associated with late planting. Hence, soil temperature as affected by planting date should be considered in areas where wheat is planted following peanut if root disease caused by *Rhizoctonia* sp. is a concern.

Key words: Wheat, peanut, crop rotation, *Rhizoctonia* sp., pathogenicity

INTRODUCTION

Winter wheat (*Triticum aestivum* L.) and peanut (*Arachis hypogaea* L.) are 2 important agricultural commodities in the United States. In 2008, winter wheat was planted on nearly 14 million hecter from which just over 1.5 billion bushels of grain was harvested; peanut was planted on nearly 610 thousand hecter from which just over 5.1 billion pounds of peanut were harvested. Wheat peanut rotations are also prevalent in parts of the United States and in various other parts of the world (Aslam *et al*., 1999; Filonow *et al*., 1988; Swaroop, 2009; Yao, 2004). In parts of the United States including Oklahoma, winter wheat is often used as a dual-purpose crop providing forage for cattle and grain for human consumption

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(Hossain *et al*., 2004). In such a system, wheat is planted in late August or September when soil temperatures are elevated (30-35°C) and then grazed by cattle during autumn and winter (Carver *et al*., 2001). Cattle are removed in late February or early March and grain is then harvested in May or June (Hossain *et al*., 2003). If winter wheat is grown as a grain-only crop, the optimum planting date is during October when soil temperatures are usually lower than 25°C. Peanut produced in Oklahoma, which is mostly used for human consumption, is generally planted in early May and harvested in early to late October.

Root disease caused by *Rhizoctonia* sp. can occur on both of these crops, with *R. cerealis* [Van der Hoeven (teleomorph = *Ceratobasidium cereale* D. Murray and L.L. Burpee)] causing sharp eyespot of wheat (Lipps and Herr, 1982) and *R. solani* [Kühn (teleomorph = *Thanatephorus cucumeris* (A.B. Frank) Donk] causing Rhizoctonia root rot on wheat (Burton *et al*., 1988; Mazzola *et al*., 1996; Rush *et al*., 1994). *R. solani* can also infect peanut, where it can attack any part of the plant at any stage of growth (Brenneman, 1996). *Rhizoctonia* alone, or in association with other pathogens like *Pythium, Rhizopus, Fusarium* and *Aspergillus* may cause seed decay or seedling damping-off (Brenneman, 1996). Hence, although both crops can be affected by *Rhizoctonia*, there is a greater potential for economic damage to wheat grown in Oklahoma due to the greater importance of this crop to the state's economy.

Rotation of wheat with other crops is practiced in North America to facilitate production and to reduce diseases (Cook and Veseth, 1991). One such rotation occasionally used in Oklahoma is to grow peanuts followed by a late-planted (late October or November) wheat crop harvested for grain, which is followed the next year with early-planted (September) wheat used to provide forage for cattle and grain. Peanuts are planted again following the wheat harvest. The effects of this rotation on the incidence and severity of root rot diseases caused by *Rhizoctonia* sp. on wheat has not been thoroughly investigated. Hence, the objectives of this study were to:

- Evaluate the growth of three isolates of *Rhizoctonia* sp. obtained from wheat and peanuts, on artificial media and evaluate in growth chamber studies the influence of temperature on their pathogenicity on hard red winter wheat
- Determine the pathogenicity of these three isolates on early and late-planted hard red winter wheat in field studies

MATERIALS AND METHODS

Rhizoctonia Isolates

The *R. cerealis* isolate, designated as RC, was isolated in 1995 from a sharp eye spot lesion found on a wheat stem (variety 2180) growing near Haskell, OK. The *R. solani* isolate from wheat, designated as RSW, was isolated in 1998 from roots of wheat plants showing root rot symptoms growing near Tipton, OK. The *R. solani* isolate from peanut, designated as RSP, was isolated in 1982 from peanut pods showing symptoms of pod rot collected from Hughes County, OK (Filonow *et al*., 1988). Isolates were maintained on potato dextrose agar slants in the dark, at 20-22°C and were transferred twice or thrice a year to fresh slants for long term storage. For use in this study, the isolates were maintained at 22-25°C on PDSA [Potato Dextrose Agar (1/5 strength PDA, Difco, Detroit, Michigan, USA) amended with streptomycin sulfate (0.3 g L^{-1})] and were transferred to fresh plates once every 3-4 days (Carling and Sumner, 1992).

Growth on PDSA

Growth, measured as Hyphal Extension (HE), was determined by centrally placing 5 mm diameter mycelial plugs of isolates onto PDSA in a 9 cm petri dish (O'Sulivan and Kavanagh, 1991). Three replicates (three plates) of each isolate were incubated in laboratory incubators at 7 different temperatures (10°, 15°, 20°, 22.5°, 25°, 27.5° and 30°C) and the colony diameter was measured 24, 48 and 72 h after inoculation. The experiment was repeated thrice and the data were analyzed using proc MIXED (PC SAS Version 9.1, SAS Institute Inc., Cary, NC).

Inoculum Preparation

In a 250 mL Erlenmeyer flask, 23 mL of water was added to 25 g of wheat seeds and autoclaved for 20 min (121°C, 1 kg cm^{-2}) and cooled overnight at room temperature (20-25°C) (Carling and Summer, 1992). Each flask was then inoculated with the respective isolate by adding three 5 mm plugs excised from the outer margin of a colony growing on a plate of PDSA. Flasks were then kept at room temperature (20-25°C) for 7-8 days by which time hyphal growth had covered the wheat seeds. To avoid clumping of seeds, flasks were shaken vigorously each day. Ten seeds from each flask were then plated on to PDSA to ensure that the culture used to inoculate the wheat seeds was not contaminated.

Effect of Inoculum Level on the Pathogenicity of *Rhizoctonia* Isolates

Ten certified wheat seeds of the hard red winter wheat 2137 were planted in a row in 8×8 cm plastic pots at a depth of ~1.5 cm. Seeds were planted into non-sterile mixture of vermiculite and Canadian sphagnum moss (Redi-Earth Peat-lite mix; Scotts-Sierra Horticultural Products Company, 14111 Scottslawn Rd, Marysville, OH 43041). Inoculum was prepared as described previously and the inoculated seeds were evenly distributed along the row, over the healthy seeds (Carling and Sumner, 1992). Three inoculum levels (10, 20 or 30 infected seeds) and a control (0 infected seeds) were tested in these experiments that were conducted at 30°C. The pots were then placed for 14 days, in an incubator (Percival Scientific, model I-36LL) with a 16:8 photoperiod (36.35 and 21.2 μ $ES^{-1}m^{-2}$). The combination of three isolates (RC, RSW and RSP), three inoculum levels (10, 20, 30 infected seeds) and the control resulted in 10 treatments. Each treatment had three replications and the experiment was repeated twice. Seedling emergence was recorded 14 Days After Planting (DAP). The seedlings were gently removed from the pots 14 DAP and the soil was washed from the roots under a stream of tap water. The stem and the leaf sheath were then examined and rated for damage from *Rhizoctonia* (disease severity rating) on a scale ranging from 1 to 6 (1 = healthy: no discoloration of the leaf sheath or stem; 2 = slight discoloration on the leaf sheath or stem; 3 = distinct eyespots on the leaf sheath or the stem; 4 = rotting at the base of the stem or of the whole plant; 5 = post-emergence damping-off or yellowing; 6 = no emergence). A small portion of the leaf sheath was then placed onto PDSA, incubated at room temperature (20-25°C) and examined for presence of *Rhizoctonia* after 3 days. Analysis of variance was performed on all data using Proc MIXED (PC SAS Version 9.1, SAS Institute Inc., Cary, NC).

Effect of Temperature on the Pathogenicity of *Rhizoctonia* Isolates

As described previously, ten certified wheat seeds (2137) were planted in the soil mix in plastic pots and inoculated with each *Rhizoctonia* isolate. The pots were incubated at 7 temperatures (10, 15, 20, 22.5, 25, 27.5 and 30°C) in the dark (O' Sulivan and Kavanagh, 1991). Pots were removed from the incubator four days after planting when the seedlings at

30°C began emerging. Pots were then kept at room temperature (20-25°C) under supplemental light, [17.2 μE S^{-1} m^{-2} at pot height (15.24 cm)] for ten more days. The combination of 3 isolates (RSW, RSP and RC) and the uninoculated control and seven temperatures resulted in 28 treatments. Each treatment had 3 replications and the experiment was repeated thrice. Seedling emergence and disease severity rating were measured as described previously. The roots and shoots were separated just above the seed and the fresh root and shoot weights were determined. An analysis of variance was performed on all data using PROC MIXED (PC SAS Version 9.1, SAS Institute Inc., Cary, NC).

Pathogenicity of *Rhizoctonia* Isolates on Hard Red Winter Wheat Cultivars Grown in Growth Chambers

Six hard red winter wheat cultivars (2137, 2174, Custer, Jagger, Tonkawa and TAM-101) were used in this study. 2137, 2174, Custer, Jagger and Tonkawa were selected as they represented commonly cultivated varieties in Oklahoma at the time of these studies. The TAM-101 was selected because of its extended use in root rot trials over the past fifteen years in Oklahoma. Ten certified wheat seeds of the 6 wheat cultivars were inoculated as described previously with each of the *Rhizoctonia* isolates at a ratio of 1:1. Pots were then incubated at either 15 or 30°C (Percival Scientific, model I-35 LL and I-36 LL) with a 16:8 photoperiod (36.35 and 21.2 μ E S^{-1} m^{-2}) for 14 days. Seedling emergence and root and shoot weights were measured and the seedlings were rated for disease severity as previously described. There were 24 treatments at each temperature, three replications for each treatment and the experiment was conducted thrice. Analysis of variance was performed on all data using PROC MIXED (PC SAS Version 9.1, SAS Institute Inc., Cary, NC).

Pathogenicity of *Rhizoctonia* Isolates on Hard Red Winter Wheat Cultivar Tam-101 Evaluated in the Field

Field trials were conducted during 1998-1999 at the Agricultural Experiment Station Plant Pathology Farm located near Stillwater, OK, U.S.A. Micro-plots (2.4×2.4 m) constructed with rail road ties and filled with sandy-loam top soil (72% sand, 12% silt and 16% clay, pH 6.6) (Filonow *et al*., 1988) were used to test the pathogenicity of *Rhizoctonia* isolates on the HRRW cultivar TAM-101. Before planting, soil tests were used to add nitrogen, phosphorus and potassium for a yield goal of 3135.5 kg ha^{-1}. The factors of the experiment included three isolates of *Rhizoctonia* (RC, RSW and RSP) plus an uninoculated control, 2 planting dates (early and late), fumigated (0.68 kg can methyl bromide/plot) and non-fumigated microplots and four replications. The experiment was performed as a split-plot arrangement in a randomized complete block design. Planting date was the main-plot factor. The factorial combination of fumigation and isolate served as the split-unit factor. The micro-plots were watered until damp and then tilled. Half of the micro-plots were fumigated with methyl bromide. Fumigation was conducted a week before the early planting date and the micro-plots were left covered overnight with plastic sheeting. Micro-plots used in late planting were left covered until about a week before planting to limit introduction of other microorganisms. Five, 1.2 m rows were planted in each micro-plot, with 100 healthy seeds planted in each row at a depth of ~1.9 cm. Healthy seeds planted in each row were inoculated with one of the three *Rhizoctonia* isolates, RC, RSW or RSP. Wheat seed inoculum, prepared as described previously, was used at a ratio of 1:1 (100 inoculated seeds with 100 healthy seeds in each row). The inoculated seeds were distributed randomly with the healthy seeds. In control micro-plots, autoclaved wheat seeds were used instead of inoculated seeds. The

early planting date was the 04-Sep and the late planting date was the 23-Oct. The plots were irrigated as needed with sprinkler irrigation to avoid extreme drought stress. Soil temperature was monitored twice daily, once in the morning and once in the afternoon at a depth of 5 cm. Weeds were controlled in the fall by applying Glean (9 mL/0.4 ha in 79 L of water) approximately two weeks after the early and late planting dates and by hoeing in the spring. Wheat was sprayed as needed with Cygon 2 E (0.24 L/0.4 ha in 79 L of water) to control insect pests and with Quadris 2.08 SC (0.27 l/0.4 ha in 75.7 L of water) to control foliar fungal diseases.

Field Data Measurements and Analysis

All data except yield were collected from 0.3 m in each of the inner three rows of each plot. Seedling emergence (number of seedlings/0.3 m/ row) was recorded 20 and 21 Days After Planting (DAP), for early and late-planted wheat, respectively. Forage was cut from plants about 10 m above the soil surface, dried in an oven at 50°C for 5 days and the dry weight in grams was measured. Forage taken from the early-planted wheat 61, 84, 103 and 168 DAP was totaled to calculate cumulative forage produced, whereas forage from the late-planted wheat was only cut once at 119 DAP. The number of fertile heads/0.3 m was counted before harvest. All plants were harvested by hand from each of the three rows and yield per row and test weight were determined. After harvest, fifteen tillers were collected at random from one of the rows in each plot. The lowest internode on each of these tillers was then rated for disease using a scale from 1 to 4 where, 1 = healthy internode; 2 = discoloration, but no clearly defined lesions; 3 = clearly defined lesions visible; 4 = stem girdling lesions present. Percentage of tillers in each plot having a rating of >1 was determined and data were evaluated in a chi square test.

Analysis of variance was performed on all data, except disease incidence data, using PROC MIXED (PC SAS Version 9.1, SAS Institute, Cary, N.C.). When treatment effects were determined significant at the 0.05 level, pair-wise t tests were conducted with a DIFF option in an LSMEANS statement and the differences presented with superscripted letters in the appropriate table. For the disease incidence data, loglinear models were fit using CATMOD procedure (PC SAS Version 9.1, SAS Institute, Cary, N.C.) to assess the relationship of disease incidence with planting date, fumigation and isolate and related interaction. If significant interactions were detected (e.g., isolate by fumigation by planting date), frequency tables demonstrating the relationship of each factor to disease were created for all levels of the other independent factors (e.g., tables of disease incidence by isolate for each combination of fumigation and planting date) using the FREQ procedure (PC SAS Version 9.1, SAS Institute, Cary, N.C.).

RESULTS

Hyphal Extension on PDSA

Hyphal Extension (HE) was determined by measuring radial hyphal extension of the three isolates on PDSA at 7 temperatures ranging from 10-30°C after 24, 48 and 72 h. Growth was then calculated as HE per day over the three day period. The HE for RC was greatest at 20°, 22.5° and 25°C and least at 30°C (Fig. 1, upper case letters). The HE of RSW was greatest at 20 and 22.5°C and least at 10 and 30°C. HE of RSP was greatest at 25 and 27.5°C and lowest at 10°C. Comparing isolates within temperatures (Fig. 1, lower case letters), HE of RSW was significantly greater than HE of RC at 10°, 20° and 22.5°C and HE of RSP was significantly greater than RC and RSW at all temperatures ≥20°C.

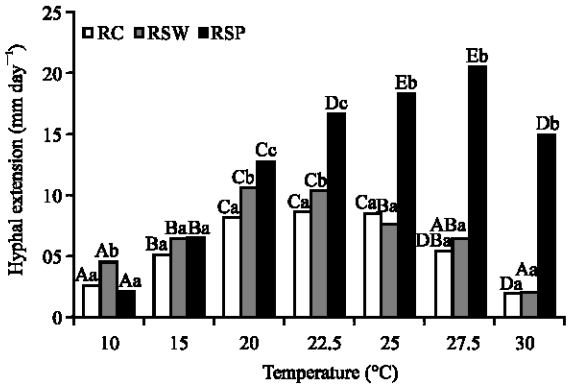

Fig. 1: Growth [Hyphal Extension (HE)] of *Rhizoctonia* isolates on potato dextrose agar at temperatures between 10° and 30°C. Uppercase letters compare HE within an isolate across temperatures and lower case letters compare isolates within a temperature. Bars with the same letter are not significantly different at $p \leq 0.05$

Effect of Inoculum Level on Pathogenicity

The effect of three different inoculum levels (10, 20 and 30 infected seeds) on the pathogenicity of the three *Rhizoctonia* isolates was evaluated in growth chamber studies. Results indicated that seedling emergence and disease severity was significantly affected by *Rhizoctonia* isolate but not by inoculum level. Inoculating with 10, 20 or 30 infected wheat grains did not significantly affect seedling emergence or disease severity for any of the 3 isolates.

Effect of Temperature on the Pathogenicity of *Rhizoctonia* Isolates

The effect of 7 temperatures on the pathogenicity of RC, RSW and RSP was evaluated in growth chamber studies. The parameters indicative of pathogenicity (shoot and root weights, seedling emergence and disease severity) were evaluated. Temperature and *Rhizoctonia* isolate significantly affected seedling emergence and disease severity of wheat and there was a significant interaction between these 2 factors. Fresh shoot and root weights were significantly affected by *Rhizoctonia* isolate but not by temperature and there was a significant interaction between the 2 factors.

In RC and RSW-inoculated seedlings, root weight, seedling emergence and disease severity were not significantly affected by temperature. The shoot weight of RC-inoculated seedlings was significantly lower at 15-22.5°C, than at 25-27.5°C and that of RSW-inoculated seedlings were significantly lower at 20°C than at temperatures >20°C (Fig. 2a). Comparing shoot and root weights and seedling emergence of RSP-inoculated seedlings across temperatures, the greatest reductions in all 3 parameters occurred at temperatures = 25°C (Fig. 2a-4, uppercase letters). Furthermore, RSP-inoculated seedlings had the highest disease severity at temperature > 25°C (Fig. 5, uppercase letters).

Pathogenicity of the three *Rhizoctonia* isolates (RC, RSW and RSP) as compared to an uninoculated control also was evaluated in this study. The RC and RSW significantly reduced shoot weight as compared to the uninoculated control at all temperatures ≥25°C, except at 22.5°C for RSW (Fig. 2b). However, RC and RSW did not significantly reduce root weight or seedling emergence at any temperature when compared to the uninoculated control

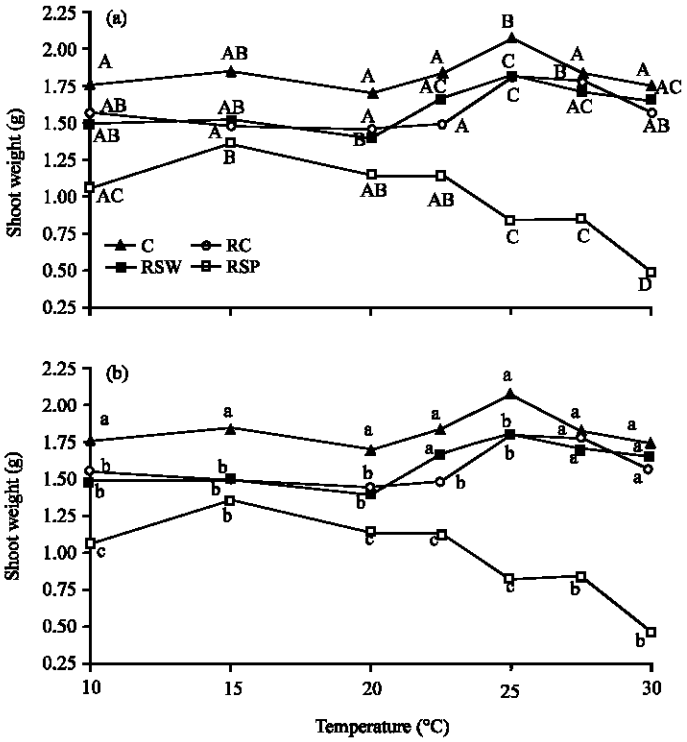

Fig. 2: Effect of temperature on shoot weight of wheat seedlings as affected by *Rhizoctonia cerealis* from wheat (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP). (a) Uppercase letters compare shoot weight as influenced by temperature within an isolate ($p \leq 0.05$). (b) Lowercase letters compare shoot weight as influenced by *Rhizoctonia* isolates ($p \leq 0.05$)

(Fig. 3, 4, lowercase letters). The RSP significantly reduced shoot weight and seedling emergence, as compared to the uninoculated control at all temperatures (Fig. 2b-4, lowercase letters,). The RSP also significantly reduced root weights as compared to the uninoculated control at temperatures >25°C (Fig. 3, lowercase letters). As expected, control seedlings had the lowest disease severity (≤1.4), at all temperatures (Fig. 5, lowercase letters). The RC-inoculated seedlings received average disease severity ratings between 1.4 and 1.9, which were significantly higher than the uninoculated control at only 15 and 30°C (Fig. 5, lowercase letters). Average disease severity ratings of RSW-inoculated seedlings ranged from 1.6 to 2.0 and were significantly higher than the uninoculated control at all temperatures (Fig. 5, lowercase letters). The average disease severity ratings for RSP-inoculated seedlings were between 2.8 and 4.8 and were significantly higher than the control at all temperatures and were also the highest among the three isolates (Fig. 5, lowercase letters).

Reactions (*In vitro*) of Hard Red Winter wheat Cultivars to *Rhizoctonia*

Pathogenicity of RC, RSW and RSP was evaluated on six wheat cultivars (2137, 2174, Custer, Jagger, Tonkawa and TAM-101), at two different temperatures (15 and 30°C). Parameters evaluated included shoot and root weights, seedling emergence and disease severity.

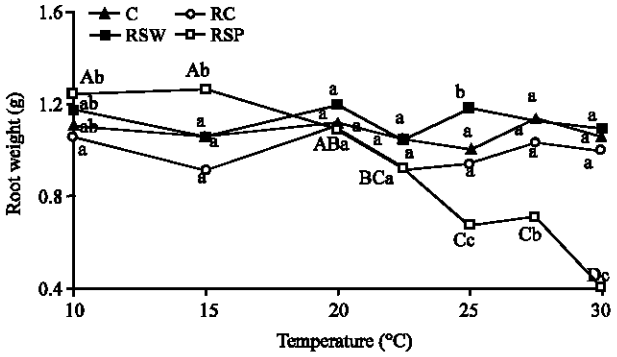

Fig. 3: Effect of temperature on root weight of wheat seedlings following inoculation with *Rhizoctonia cerealis* from wheat (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP). Lowercase letters compare root weight as influenced by *Rhizoctonia* isolates ($p<0.05$). Uppercase letters compare root weight as influenced by temperature within the isolate RSP ($p<0.05$)

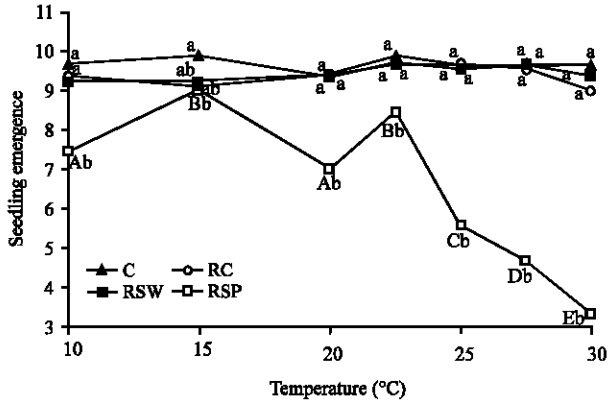

Fig. 4: Effect of temperature on wheat seedling emergence (number of emerged seedlings out of ten seeds planted) following inoculation with *Rhizoctonia cerealis* from wheat (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP). Lowercase letters compare seedling emergence as influenced by *Rhizoctonia* isolates ($p<0.05$). Uppercase letters compare seedling emergence as influenced by temperature within the isolate RSP ($p<0.05$)

Reactions at 15°C

There was no significant interaction between variety and isolate and *Rhizoctonia* isolate had no significant effect on seedling emergence. Shoot and root weights and disease severity were significantly affected by *Rhizoctonia* isolate and variety and there was a significant interaction between the two factors.

No consistent interaction was observed between the RC and RSW isolates and variety with respect to any of the parameters indicative of pathogenicity and hence, this data has not been presented. The RSP inoculated-seedlings did not show a significant reduction in seedling emergence. Comparing RSP-inoculated seedlings across all varieties, seedlings of 2174, Jagger and TAM 101 had significantly higher shoot weights

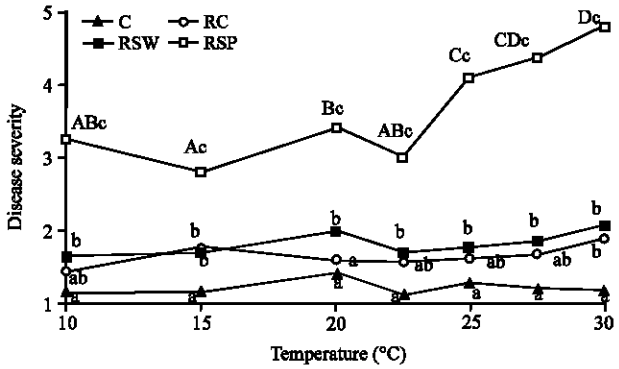

Fig. 5: Effect of temperature on disease severity on wheat seedlings following inoculation with *Rhizoctonia cerealis* from wheat (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP). Disease severity was determined by rating damage on the stem and leaf sheath on a scale ranging from 1 to 6, where 1 = healthy: no discoloration of the leaf sheath or stem; 2 = slight discoloration on the leaf sheath or stem; 3 = distinct eyespots on the leaf sheath or the stem; 4 = rotting at the base of the stem or of the whole plant; 5 = post-emergence damping-off or yellowing; 6 = no emergence. Lowercase letters compare disease severity as influenced by *Rhizoctonia* isolates ($p<0.05$). Uppercase letters compare disease severity as influenced by temperature within the isolate RSP ($p<0.05$)

Table 1: Effect of *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on fresh shoot weight of hard red Winter wheat varieties after 14 days at 15 and 30°C

	Temperature/isolate					
	15°C		30°C			
Wheat variety	C	RSP	C	RC	RSW	RSP
2137	1.6^{A}	1.5^{A}	1.9^{A}	1.8^{A}	1.7^{A}	0.5^{AB}
2174	2.0^{C}	1.8^{B}	2.1^{AB}	2.1^{ABC}	1.8^{A}	0.4^{A}
Custer	1.6^{A}	1.6^{A}	2.2^{AB}	2.2^{C}	2.2^{B}	0.8^{B}
Jagger	1.7^{AB}	1.8^{B}	2.1^{AB}	1.9^{AB}	1.7^{A}	0.6^{AB}
Tonkawa	1.9^{BC}	1.6^{A}	2.3^{B}	2.2^{BC}	2.2^{B}	0.8^{B}
TAM 101	1.9^{BC}	1.8^{B}	2.3^{B}	2.4^{C}	2.2^{B}	1.3^{C}

Letters compare shoot weight of varieties at a fixed temperature for each isolate. Values with the same letter within a column are not significantly different at $p \leq 0.05$

(Table 1). Comparing RSP-inoculated seedlings across varieties, seedlings of 2137 had the highest fresh root weight, which was significantly greater than those of Custer, Jagger and Tonkawa, which had the lowest root weights (Table 2). Among the RSP-inoculated seedlings, TAM 101 had a significantly lower disease severity, compared to all other varieties (Table 3).

Reactions at 30°C

Fresh shoot and root weights were significantly affected by *Rhizoctonia* isolate and wheat variety and there was no significant interaction between the 2 factors. Also, *Rhizoctonia* isolate and variety significantly affected seedling emergence and disease severity and there was a significant interaction between these 2 factors.

Table 2: Effect of *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on fresh root weight of hard red Winter wheat after 14 days at 15 and 30°C

	Temperature/isolate					
	15°C		30°C			
Wheat variety	C	RSP	C	RC	RSW	RSP
2137	1.0^{A}	1.2^{A}	1.0^{A}	0.9^{A}	0.9^{AB}	0.3^{AB}
2174	1.2^{AB}	1.1^{AB}	1.0^{A}	0.9^{A}	0.9^{AB}	0.2^{A}
Custer	1.3^{B}	1.0^{B}	1.0^{A}	1.0^{A}	1.0^{AB}	0.4^{BC}
Jagger	1.1^{A}	1.0^{B}	1.0^{A}	0.9^{A}	0.8^{A}	0.3^{AB}
Tonkawa	1.2^{B}	1.0^{B}	1.0^{A}	1.0^{A}	1.1^{B}	0.5^{BC}
TAM 101	1.1^{AB}	1.1^{AB}	0.9^{A}	1.0^{A}	0.9^{AB}	0.6^{C}

Letters compare root weights as influenced by isolates across varieties at each temperature. Values with the same letter are not significantly different at $p \leq 0.05$

Table 3: Disease severity on wheat seedlings at 15 and 30°C following inoculation with *Rhizoctonia solani* isolated from peanut (RSP) compare to uninoculated control (C) seedlings

	Temperature/Isolate			
	15°C		30°C	
Wheat variety	C	RSP	C	RSP
2137	1.3^{AB}	2.5^{B}	1.1^{A}	4.9^{C}
2174	1.1^{A}	2.7^{BC}	1.2^{A}	5.0^{C}
Custer	1.6^{B}	2.6^{B}	1.2^{A}	4.3^{B}
Jagger	1.3^{AB}	3.0^{C}	1.2^{A}	4.6^{C}
Tonkawa	1.2^{AB}	3.1^{C}	1.1^{A}	4.2^{B}
TAM 101	1.0^{A}	2.0^{A}	1.1^{A}	3.4^{A}

Letters compare disease severity as influenced by isolates across varieties at each temperature. Values with the same letter are not significantly different at $p \leq 0.05$. Disease rating was based on a scale from 1-6, where 1: Healthy: no discoloration of the leaf sheath or stem; 2: Slight discoloration on the leaf sheath or stem; 3: Distinct eyespots on the leaf sheath or the stem; 4: Rotting at the base of the stem or of the whole plant; 5: Post-emergence damping-off or yellowing; 6: No emergence

Table 4: Emergence (percentage) of uninoculated (C) wheat seedlings and seedlings inoculated with *Rhizoctonia solani* from peanut (RSP) after 14 days at 30°C

	Rhizoctonia isolates	
Wheat variety	C	RSP
2137	97.8^{A}	34.4^{A}
2174	98.9^{A}	43.3^{ABC}
Custer	98.9^{A}	48.9^{BC}
Jagger	96.7^{A}	42.2^{AB}
Tonkawa	97.8^{A}	55.6^{C}
TAM 101	96.7^{A}	71.1^{D}

Letters compare seedling emergence as influenced by isolate across varieties. Values with the same letter are not significantly different at $p \leq 0.05$

Seedling emergence and disease severity were not significantly different across varieties inoculated with RC or RSW (data not shown). The TAM 101, Custer and Tonkawa seedlings inoculated with RC and RSW had significantly higher shoot weights (Table 1). There were no significant differences in root weights of RC-inoculated seedlings across varieties (Table 2). A comparison of RSW-inoculated seedlings across varieties indicates that seedlings of Tonkawa had the highest and Jagger the lowest fresh root weights (Table 2). Comparing across the 6 varieties, RSP-inoculated TAM 101 seedlings had the highest shoot and root weights followed by Custer and Tonkawa (Table 1, 2). Furthermore, RSP-inoculated seedlings of TAM 101, followed by Custer and Tonkawa seedlings, had lower disease severity compared to 2137, 2174 and Jagger (Table 3). RSP-inoculated TAM 101, followed by Tonkawa, had the highest seedling emergence as compared to the other HRWW varieties (Table 4).

Table 5: Effect of *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on wheat seedling emergence (SE) per foot of row in fumigated and non-fumigated soil when planted early (04-Sep) and late (23-Oct)

		Rhizoctonia isolates			
Planting date	Fumigation	C	RC	RSW	RSP
Earlyplanted	Fumigated	23.7^{a}	22.4^{ab}	21.3^{ab}	16.4^{b}
	Non-fumigated	13.6^{a}	15.8^{a}	14.3^{a}	6.4^{b}
Lateplanted	Fumigated	28.1^{a}	16.7^{b}	15.9^{b}	10.0^{b}
	Non-fumigated	20.8^{a}	10.6^{b}	13.3^{b}	8.4^{b}

Emergence was determined 21 days (early-planted) and 22 days (late-planted) after planting. Letters compare SE values across isolates, within a planting date and fumigation treatment. Values with the same letters are not significantly different at $p \leq 0.05$

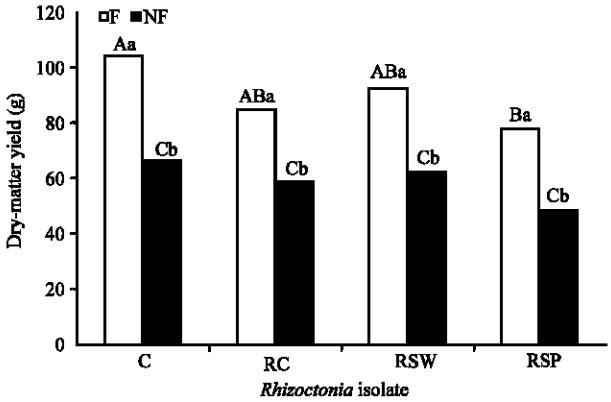

Fig. 6: Effect of *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on forage produced by wheat planted early in fumigated soil. Uppercase letters compare forage yield within fumigation treatment (fumigated or not fumigated) across isolates. Lower case letters compare forage yield as influenced by fumigation within each isolate. Bars with the same letters are not significantly different at $p < 0.05$

Field trials Using *Rhizoctonia* Isolates

Rhizoctonia isolate and fumigation significantly affected seedling emergence and there was a significant two-way interaction between planting date and isolate. When compared to the control, RC and RSW did not significantly reduce seedling emergence in wheat planted early in fumigated or non-fumigated soil (Table 5). The RSP, however, significantly reduced seedling emergence of wheat planted early in fumigated and non-fumigated soil when compared to the uninoculated control (Table 5). In comparison, RC, RSW and RSP all significantly reduced seedling emergence as compared to the uninoculated control in both fumigated and non-fumigated soil in late-planted wheat, with the greatest reductions occurring in the RSP-inoculated plots.

Planting date and fumigation significantly affected cumulative forage yield from early planted wheat with significant two-way interactions between planting date and fumigation. Yield of dry matter forage from wheat planted in fumigated soil was significantly higher than the forage yield from non-fumigated soil for all isolates and the control (Fig. 6, lower case letters). In early-planted wheat, RC and RSW did not significantly reduce forage yield as compared to an uninoculated control in fumigated or non-fumigated soil (Fig. 6, upper case letters). However, in early-planted wheat, RSP significantly reduced the forage yield as compared to the uninoculated control in fumigated soil (Fig. 6, upper case letters). No effect

Table 6: Percentage of tillers with a disease rating >1 on the first internode of wheat tillers of plants grown in fumigated and non-fumigated micro-plots planted early (04-Sep; "PD1") and late (23-Oct; "PD 2") and inoculated with *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) or *R. solani* from peanut (RSP)

		Rhizoctonia isolates			
Fumigation	Planting date	C	RC	RSW	RSP
Fumigated	Early planted	25.8^{Aa}	21.8^{Aa}	23.0^{Aa}	32.0^{Ab}
	Late planted	24.8^{Aa}	28.5^{Aa}	27.5^{Aa}	24.0^{Ba}
Non-fumigated	Early planted	25.8^{Aa}	27.5^{Aa}	29.0^{Aa}	32.3^{Aa}
	Late planted	22.5^{Aa}	27.5^{Aa}	28.0^{Aa}	25.8^{Aa}

The rating scale was from 1 to 4 [1 = healthy internode; 2 = discoloration, but no clearly defined lesion (s); 3 = clearly defined lesion(s) present; 4 = stem girdled with lesion(s)]. Uppercase letters compare the percentage of diseased tillers as influenced by planting dates within each isolate, within fumigation treatments. Lowercase letters compare the percentage of diseased tillers as influenced by isolates, within fumigation treatments and planting date. Values with the same letter are not significantly different at $p \leq 0.05$

was observed on forage produced by late-planted wheat. Additionally, for early- and late-planted wheat, there was no significant effect of isolate or fumigation on the number of fertile heads, yield, or test weight.

The percentage of diseased tillers (those with a rating >1) was not significantly different between the early and late planting dates in the fumigated or non-fumigated control, RC- and RSW-inoculated plots (Table 6, upper case letters). In RSP-inoculated plots, the percentage of diseased tillers was significantly higher in the early-planted fumigated plots, but there was no significant difference in the non-fumigated RSP inoculated plots (Table 6, upper case letters).

In early-planted wheat, the percentage of diseased tillers was significantly higher in RSP-inoculated wheat as compared to the control, RC- and RSW-inoculated wheat in fumigated plots (Table 6, lower case letters), but not significantly different in non-fumigated plots (Table 6, lower case letters). Additionally, no significant differences in percentage of diseased tillers as affected by isolate were observed in wheat planted late into either fumigated or non-fumigated soil (Table 6, lower case letters).

DISCUSSION

Effect of Temperature on Growth [Hyphal Extension (HE)] and Pathogenicity

The optimum temperature for HE on media was between 20-25°C for RC, 20-22.5°C for RSW and 25-27.5°C for RSP. Among isolates, RSP had a significantly higher HE rate compared to RC or RSW at all temperatures above 15°C. At warmer temperatures (≥25°C), the HE of RSP was significantly higher than both RC and RSW. Thus, results indicated that HE on media, which is a measure of the growth and possibly spread of the pathogen, depends on temperature. The rate at which a soil-borne fungal pathogen can grow and spread has practical implications in the field, since, this also determines the colonization efficiency of the pathogen on the roots of susceptible plants (Otten *et al.*, 2004). Thus, warmer soil temperatures may favor faster spread of RSP through the soil, which may result in damping-off of young seedlings.

Results from growth chamber studies also indicated that higher temperature favored the pathogenicity of RSP as compared to RC and RSW as measured by shoot and root weights, seedling emergence and disease severity. Neither RC nor RSW reduced seedling emergence or root weights from 10-30°C. RC and RSW, however, did reduce shoot weights when compared to the uninoculated control at temperatures ≤25°C. By contrast, RSP-inoculated

seedlings showed significantly reduced emergence, shoot and root weights and increased disease severity ratings when compared to the control at all temperatures. The greatest reductions in emergence root and shoot weights and the greatest increase in disease severity was at 30°C, followed by 27.5° and 25°C. Hence, results indicated that HE and pathogenicity of RSP on wheat is favored by temperatures ≥25°C and HE of RC and RSW is favored by temperatures ≤25°C. This is consistent with the crop of origin for these isolates, with peanut being a warm season crop that is cultivated in Oklahoma from May through October when temperature frequently exceeds 35°C. Such temperatures would provide the selection pressure to favor strains of *Rhizoctonia* sp. that have higher optimum temperatures for many traits. In contrast, RSW and RC were isolated from wheat, which is grown from September through May. Temperatures during these months usually are lower than 25°C, which would provide a selection pressure for isolates of *Rhizoctonia* sp. with optima at cooler temperature.

Effect of Inoculum Level on Pathogenicity

The level of inoculum (10, 20 or 30 infected wheat kernels/10 healthy wheat kernels) of *Rhizoctonia* used in *in vitro* tests indicated that inoculum level did not affect emergence of seedlings or disease severity of RC-, RSW- or RSP-inoculated seedlings. This agrees with Yitbarek *et al.* (1988), who tested the effect of various inoculum levels of *R. solani* isolates on canola and found that with increasing inoculum level, a plateau was reached after which there was no corresponding increase in disease incidence. In our study, the lowest level of inoculum used may have been sufficient to produce the maximum disease, after which the increase in inoculum had no effect on disease severity.

Reaction of Hard Red Winter Wheat to *Rhizoctonia* isolates (*in vitro* Tests)

No variety showed consistent resistance to RC or RSW at 15 or 30°C. The TAM 101 demonstrated some resistance to RSP as indicated by a significantly lower disease severity and a higher shoot weight at 15°C. At 30°C, besides TAM 101, Tonkawa and Custer showed significantly higher seedling emergence, shoot weights and lower disease severity ratings. These results indicate that TAM 101, Tonkawa and Custer may be more resistant to RSP, when compared to the other varieties used in this study. In the growth chamber studies, resistance of these three hard red Winter wheat varieties towards RSP was more evident at 30°C, than at 15°C. This is important since, the isolate is also more virulent at 30°C than at 15°C.

Results suggested that the temperature used to conduct *in vitro* tests for disease may directly impact the pathogenicity of RSP. RSP showed the highest rate of HE and pathogenicity at temperatures ≥25°C. The various plant parameters that were measured to evaluate the pathogenicity of *Rhizoctonia* isolates included, seedling emergence, shoot and root weight and disease severity. These parameters can also be employed for the fast and efficient screening of various breeder lines of wheat to identify the ones that have the greatest potential for resistance to *Rhizoctonia* isolates. The selected breeder lines could then be tested in field trials, which would save time and money. However, selecting the temperature for screening breeder lines for resistance to *Rhizoctonia* isolates is important because temperature optima vary for pathogen growth and pathogenicity, depending on the isolate used in the testing. The temperature at which there is a maximum rate of hyphal extension may be a direct indicator of the optimum temperature for pathogenicity of the isolate.

Field Trials

The RSP caused the greatest reduction in emergence and forage production in field trials. *Rhizoctonia* isolates from wheat (RC and RSW) also reduced emergence and forage production, but not to the extent of the reduction caused by RSP. RSP inoculation also resulted in significantly higher numbers of diseased tillers when compared to the control, RC and RSW-inoculated wheat in the fumigated early-planted plots. Thus, RSP was more virulent on early-planted wheat that was planted when soil temperature was higher (35-40°C) and remained above 27°C until the last week of September. These results correspond with results described previously in this study, where RSP had a greater hyphal extension, greater virulence at higher temperatures (≥25°C) and resulted in greater reductions in fresh root and shoot weights in experiments conducted in growth chambers. This also agrees with a study conducted by Mathieson (1991), who demonstrated that emergence of wheat from soil infested with *R. solani* decreased as temperature increased from 15 to 35°C. By comparison, soil temperature was much lower (<21°C) during and after the late planting date, where RC and RSW significantly reduced emergence but not forage production.

Although, the *Rhizoctonia* isolates used in these studies caused significant reduction in seedling emergence in the field, subsequent reductions in fertile heads, grain yield and/or test weight of grain were not observed. This may be related to several factors including the ability of wheat to compensate for differences in stand (emergence) by producing more tillers and heads. Such compensation would have been facilitated by the balanced fertility and irrigation supplied in the microplots in which these field experiments were conducted. These factors, alone or in combination, could have mitigated the effects from the root/stem infection by the *Rhizoctonia* isolates. Furthermore, the small plot field trials described in this paper in essence followed conventional tillage practices so there was no wheat straw or other plant residue left on the soil surface or incorporated in the top layer of soil at the time of planting or through the season. Such residue can serve as a nutrient source for root rot fungi and is consistent with the study conducted by Pumphrey *et al*. (1987), where he concluded that *Rhizoctonia* root rots are generally lower in wheat planted following conventional tillage. Furthermore, results from the growth chamber studies indicate that TAM 101, the wheat variety used in the field trials, exhibited some resistance to RSP. This may be one of the reasons why there was no significant reduction in yield from RSP, which was the most virulent isolate of *Rhizoctonia* used in these studies. According to Carling and Sumner (1992), it is important to record the yield along with root rot severity in the field, because the root-disease severity and root growth may not always correspond directly with the yield.

The fact that higher temperature favors RSP (which was the most virulent isolate in this study) is important to producers in the United States and other places where wheat and peanut are used in rotation. For instance, in Oklahoma, wheat is planted both early (late August to September) as a dual purpose (forage and grain) crop when the soil temperatures are high and late (early to late October) as a grain only crop when the soil temperatures are lower. Hence, if isolates such as RSP are present in fields following a peanut crop, producers may need to consider using that wheat crop as a graze-out crop if it was planted early, or if grain is desired, than planting should be delayed until late October when soil temperatures are lower. Such a delay in planting may help reduce the possibility of stand and forage losses from Rhizoctonia-caused root rot.

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