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# Pathogenic Fitness of *Pyrenophora tritici-repentis* Isolates Causing Tan Spot of Winter Wheat in Oklahoma

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## ABSTRACT

Tan spot of wheat (*Triticum aestivum L.*) caused by the fungus *Pyrenophora tritici-repentis* can be a significant disease, and thus developing resistant varieties using fit and virulent isolates is critical to wheat breeding programs. Hence, variability in fitness characters and virulence was determined for *P. tritici-repentis* isolates collected in three different decades (1980s, 1990s, and 2000s) from Oklahoma wheat fields. Growth (hyphal extension on media), conidia production on media, and conidia production on wheat leaves of a susceptible (TAM 105), an intermediate (Deliver) and a resistant (Red Chief) variety were determined for 17 isolates. Isolates also were evaluated for pseudothecia production and ascospore maturity on wheat straw, and isolate virulence was determined on all three wheat varieties. Isolates differed significantly ($p<0.01$) for growth on media, and for conidia production on agar and on wheat leaves. Isolates varied significantly in pseudothecia production and ascospore maturity on wheat straw. Isolates differed significantly ($p<0.01$) in virulence (percent leaf area infection) on the three wheat cultivars. Isolates collected from the 2000s were more virulent than those collected in the 1980s and 1990s, and also were deemed to be more fit than isolates collected in the 1980s and 1990s in terms of traits such as growth, conidia production, pseudothecia formation, mature ascospore production, and virulence. This fitness could affect the competitiveness of these isolates in the field, as well as facilitate selection of isolates used to test wheat germplasm lines for reaction to tan spot.

**Key words:** *Pyrenophora tritici-repentis*; Isolate fitness; Wheat; Tan spot; Germplasm

## INTRODUCTION

Tan spot, caused by the ascomycete necrotrophic fungus *Pyrenophora tritici-repentis* (Died.) Drech. (anamorph: *Drechslera tritici-repentis* (Died.) Shoemaker), is a major disease of wheat worldwide [1]. Yield losses in wheat due to tan spot may range from 3 to 50% in the central plains of the United States [2]. Nearly 40% grain yield loss due to tan spot was reported in an experimental study in Kansas [3]. Lower thousand-kernel weight, reduced number of grain per head, shriveling and discoloration of seeds, and reduced milling quality have all been attributed to tan spot in wheat [4].

In Oklahoma in the southern Great Plains, winter wheat is typically planted on 3-4 million acres annually. Although tan spot disease has been observed in Oklahoma since the 1970s, this disease has become more of a concern due to shifting of cultivation practices from clean to minimum or no-till that leaves increased residue on the soil surface [5]. The tan spot fungus survives on wheat residue from the previous year and infects wheat the following spring. Ascospores produced in pseudothecia are the primary inoculum that initiates tan spot [6]. A positive correlation between the severity of tan spot and the amount of infected wheat residue left in the field has been reported [7]. This fact along with planting susceptible cultivars can contribute greatly to an increased occurrence of tan spot [8,9].

Variation in characters such as growth, sporulation, fruiting body (pseudothecia) formation, and virulence are important in disease onset and epidemics because these characters help define the fitness of the pathogen. Hunger and Brown [5] found significant variation in growth and sporulation on media among isolates of *P. tritici-repentis* collected from winter wheat in Oklahoma during the 1980s. Variation in the number of pseudothecia and ascospore development can also affect wheat tan spot epidemics. Previous studies showed that isolates of *P. tritici-repentis* differ significantly in virulence on wheat cultivars [10-14].

It is reported in other fungi that more recently collected isolates are more fit than older isolates [15,16]. For example, in the wheat-stripe rust pathosystem. Milus et al. [17] found that older populations of *Puccinia striiformis* f. sp. *tritici* have been replaced

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by a newer population that has a two day shorter latent period, and a spore germination rate that is double that of older isolates. Little is known about *P. tritici-repentis* isolates collected from wheat in different time periods. It might be that less fit isolates of *P. tritici-repentis* are being replaced by more virulent isolates over time. Thus, an investigation of fitness characters of isolates of *P. tritici-repentis* collected in Oklahoma was conducted. The objectives of this study were to compare the morphological and pathogenic fitness characters of *P. tritici-repentis* isolates collected over three decades (1980s, 1990s, and 2000s) in Oklahoma.

## MATERIALS AND METHODS

### Fungal isolates

Isolates of *P. tritici-repentis* originally collected from single ascospores or conidia in the 1980s, 1990s and 2000s were included in this study (Table 1). Wheat straw containing fungal pseudothecia was cut into 5-cm pieces and washed in 10% bleach for one min, rinsed thoroughly in sterile water, dried and then placed on 15% water agar (WA) in 90 mm petri plates. After 3 days incubation at 21°C, plates were examined using a dissecting microscope, and single ascospores ejected from pseudothecia were transferred to potato dextrose agar (PDA) using a sterile transfer needle. Conidial isolates were obtained from infected leaves. Leaves having typical tan spot symptoms were collected and surface sterilized as described above, and incubated in 12 h light at 23°C and 12 h dark at 16°C for 3 days. After identifying conidia using a microscope, single conidia were taken aseptically and placed onto PDA. Isolates were maintained on PDA while being used in experiments, but were kept in liquid nitrogen for long term storage.

### Fungal growth

Growth of fungal isolates was determined using the procedure as described by Hunger and Brown [5]. A 5-mm diameter mycelial plug, excised with a sterilized cork borer from the edge of an actively growing isolate, was placed on commercial PDA (CPDA) and PDA made from fresh potatoes (RPDA) (200 g potato, dextrose 20 g, agar 15 g in l L) and clarified V8 (CV8) juice agar (150 ml V8 juice, 3 g $CaCO_3$, 15 g agar, 850 ml water) in 90 mm petri plates. Plates were maintained in an incubator (Percival model I-36L, Boone, IA) at 23°C in dark for 5 days. Radial growth was measured by averaging the length of two opposite diameters and subtracting 5 mm from each reading. A two-factor (media and isolates) split-plot randomized complete block design was followed with four replications. Isolates were considered as main plots, where sub-plots (media) were arranged randomly in main plots within a block. Thus, each plate was a replication for a combination of isolate and medium.

### Fungal sporulation

*In vitro*: Conidial production of each isolate was determined on CV8 media following the procedure described by Hunger and Brown [5]. In brief, about 10 drops of sterile water was added onto 3-day old growing mycelia on CV8 media, which was then matted down using a sterile bent glass rod. Plates were then kept in the incubator for 12 h at 23°C in light (40 W, 30 $\mu Es^{-1}m^{-1}$) and 12 h dark at 16°C to produce conidiophores and conidia, respectively. Conidia were washed from the plate into a beaker using a stream of sterile water. One ml of conidial suspension was pipetted onto a segmented petri plate (40 mm), and the number of conidia per ml was used to determine the number of conidia produced per unit area of the fungal colony. A randomized complete block design was followed with four replications.

*In vivo*: The procedure of Rodriguez and Bockus [18] was followed to determine sporulation of each isolate on leaves of the susceptible wheat cultivar TAM-105, moderately susceptible cultivar Deliver, and resistant cultivar Red Chief. In brief, wheat seedlings were raised in commercial 'Ready-Earth' soil (SunGro Co., Bellevue, WA) in 6-in × 1.5 in. diameter plastic containers. Seedlings with three leaves fully expanded were inoculated by conidial suspension (2000 conidia $ml^{-1}$ of water) of each isolate using an atomizer (DeVilbiss Co., Somerset, PA). Inoculated plants were dried for 30 min and then were placed in a mist chamber that provided 100% relative humidity for 48h. Then inoculated plants were placed in a

**Table 1:** Isolates of *Pyrenophora tritici-repentis* collected from winter wheat in Oklahoma used in this study.

| Isolates | Collection year | County | Isolation propagule |
|---|---|---|---|
| OKA1 | 1983 | Garfield | Ascospore |
| OKA2 | 1983 | Garfield | Ascospore |
| OKD1 | 1983 | Blaine | Ascospore |
| OKD2 | 1983 | Blaine | Ascospore |
| OKD3 | 1983 | Blaine | Ascospore |
| OKD4 | 1983 | Blaine | Ascospore |
| OKD5 | 1983 | Blaine | Ascospore |
| RBB6 | 1996 | Kay | Ascospore |
| GYA3 | 1996 | Texas | Ascospore |
| El Reno | 2005 | Canadian | Conidia |
| Guymon | 2006 | Texas | Conidia |
| Cherokee | 2006 | Cherokee | Conidia |
| OK-06-1 | 2006 | Payne | Ascospore |
| OK-06-2 | 2006 | Payne | Ascospore |
| OK-06-3 | 2006 | Payne | Ascospore |
| Atoka | 2007 | Atoka | Conidia |
| Kiowa | 2007 | Pittsburg | Conidia |

greenhouse at 21°C following a cycle of 14 h light (510 $\mu Es^{-1}m^{-1}$) and 10h dark. After 5 days, the 2nd and 3rd leaves from the bottom were cut into 5-7 cm segments and placed on moist 90-mm petri plate. Petri plates, sealed by parafilm to retain moisture, were incubated in the light (30 $\mu Es^{-1}m^{-1}$) for 12 h at 23°C followed by 12 h dark at 16°C for 5 days. Leaf segments were cut again into smaller pieces (about 1 cm) and placed in a sterile test tube containing 25 ml sterile water. The tubes were vortexed vigorously for 30 sec. One ml of conidial suspension was transferred into a segmented petri plate and conidia were counted using a stereomicroscope. The average number of conidia produced per leaf was calculated. This experiment was conducted in a randomized complete design with four replications (five plants per replication).

### Pseudothecia production

The procedure of James et al. [19] was followed to determine pseudothecia production by each isolate. Wheat straw was cut into pieces (5 cm long), boiled in sterile water for 10 min at 80°C and autoclaved. About 2.5 ml of sterile water was added to filter paper in a petri plate, and four pieces of straw were placed parallel to each other on the moistened filter paper. A 5-mm diameter mycelial plug was placed in between each piece of straw. Petri plates were sealed with parafilm and were kept in dark at 21°C for 14 days during which time, pseudothecia developed. Plates were then incubated at 15°C for 23 days in a 12 h light (30 $\mu Es^{-1}m^{-1}$) and 12 h dark cycle for pseudothecia maturation. The total number of pseudothecia and mature pseudothecia per straw were counted. A pseudothecium was considered mature if at least one pigmented ascospore with clear septation was detected using a compound microscope [20]. The experiment was conducted in a randomized complete block design with four replications. Each petri plate with four pieces of straw was a replication for each isolate.

### Virulence testing

One isolate (OKD4) did not produce conidia on media, thus, virulence of the remaining 16 isolates was determined on wheat cultivars TAM 105 (susceptible), Deliver (moderately susceptible), and Red Chief (resistant) in the greenhouse. Isolates (main plot) and cultivars (sub plot) were arranged in a spit-plot randomized complete block design with four replications. Raising of wheat seedlings, inoculum preparation and inoculation by each isolate are described above. Inoculated seedlings were then placed in a greenhouse at temperatures of 25°C and 20°C for day and night, respectively. As leaf position may vary in resistance [21], only the 2nd and 3rd lower leaves of each seedling were collected after one week of inoculation, and the image of leaves was captured by scanning (EPSON 1650). Percentage leaf area infection from each leaf image was determined by ASSESS software [22]. Thus, twenty leaves from ten plants were rated and averaged for each replication.

### Statistical analysis

All statistical analyses were done using SAS 9.4 software (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was generated by PROC GLM module for sporulation on CV8, total and matured pseudothecia production of isolates, and means were separated by Fisher's protected least significance difference test ($\alpha$=0.05). ANOVA were generated using the PROC Mixed module for two factorial experiments (growth on media, sporulation and virulence on leaf of wheat cultivars). When significant, interactions of the factors in question were further assessed with an analysis of simple effects. Treatment means were separated by protected *t*-test using pair-wise difference (PDIFF) option at 5% level of significance.

## RESULTS

### Radial growth

Both isolates and media had significant influence on mycelial radial growth, however, a significant ($p<0.01$) isolate × media interaction was also detected (Table 2), thus, the simple effects of isolates and media are presented (Table 3). The greatest mean radial growth averaged over all three media was observed for isolate RBB6 and OK-06-3. GYA3 and Cherokee showed the lowest radial growth when average over all three media. The average radial growth of the recent isolates from the 2000s (41.8 mm) was greater than isolates collected during the 1980s and 1990s (38.4 mm). The mean radial growth was greater on RPDA and CV8 while it was least on CPDA.

### Sporulation

*In vitro:* Isolates differed significantly ($p<0.01$) for conidia production on clarified V8 (CV8) media (Table 4). OKD1, Guymon, Cherokee and Atoka produced the greatest number of conidia on CV8 while the fewest were produced by OKD3, GYA3, OKD2, OKD5, OK-06-2 and El-Reno. OKD4 did not produce conidia on CV8 (Table 5).

*In vivo:* Isolates also varied significantly in sporulation on the leaves of wheat cultivars, however, an interaction was detected between isolates and cultivars (Table 2). Thus, the simple effects of sporulation of isolates on the three cultivars are presented (Table 5). Kiowa produced the greatest number of conidia on wheat leaves, and the fewest were produced by GYA3, OK-06-2 and OKD3. The mean sporulation was 1023 conidia for the isolates collected in the 2000s while it was 450 and 336 conidia for the older isolates collected in 1980s and 1990s, respectively, on three wheat cultivars. As expected, isolates produced a greater number of conidia on susceptible cultivar TAM-105 while isolates produced fewer conidia on the resistant cultivar Red Chief (Table 5).

**Table 2:** Analysis of variance of radial growth of seventeen isolates on three growth media; and sporulation and percent leaf area infection (virulence) of sixteen isolates of *Pyrenophora tritici-repentis* on three wheat cultivars by PROC Mixed method.

| Source | d.f. | Radial growth (mm) | | Source | d.f. | Sporulation on leaf | | Leaf area infection (%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | *F* | *p* | | | *F* | *p* | *F* | *p* |
| Isolate | 16 | 56.94 | <.01 | Isolate | 15 | 56.94 | <0.01 | 34.8 | <0.01 |
| Media | 2 | 167.8 | <.01 | Cultivar | 2 | 167.8 | <0.01 | 342 | <0.01 |
| Isolate × Media | 32 | 9.17 | <.01 | Isolate ×Cultivar | 30 | 9.17 | <0.01 | 6.56 | <0.01 |

**Table 3:** Radial growth (mean ± standard error) of seventeen isolates of *Pyrenophora tritici-repentis* on commercial potato dextrose agar (CPDA), PDA made from fresh potatoes (RPDA) and clarified V8 (CV8) juice agar.

| Isolates | Radial growth (mm) on media[a] | | | Mean |
|---|---|---|---|---|
| | CPDA | RPDA | CV8 | |
| **1980s** | | | | |
| OKA1 | 37.8 ± 2.8 $^{a,B}$ | 45.3 ± 1.0 $^{c,A}$ | 49.9 ± 1.1 $^{cd,A}$ | 44.3 |
| OKA2 | 29.8 ± 2.3 $^{bc,B}$ | 48.5 ± 0.9 $^{bc,A}$ | 52.3 ± 0.6 $^{cd,A}$ | 43.5 |
| OKD1 | 17.8 ± 0.8 $^{efg,B}$ | 49.5 ± 0.6 $^{bc,A}$ | 48.9 ± 1.4 $^{cd,A}$ | 38.7 |
| OKD2 | 20.5 ± 1.5 $^{def,B}$ | 48.3 ± 0.8 $^{bc,A}$ | 48.1 ± 0.8 $^{cd,A}$ | 38.9 |
| OKD3 | 22.0 ± 1.4 $^{de,C}$ | 46.0 ± 0.7 $^{c,A}$ | 32.8 ± 4.3 $^{e,B}$ | 33.6 |
| OKD4 | 24.3 ± 1.1 $^{cd,C}$ | 53.8 ± 0.6 $^{ab,A}$ | 38.0 ± 2.3 $^{e,B}$ | 38.7 |
| OKD5 | 23.3 ± 1.8 $^{de,B}$ | 45.8 ± 0.7 $^{c,A}$ | 48.8 ± 1.9 $^{cd,A}$ | 39.3 |
| **1990s** | | | | |
| RBB6 | 37.0 ± 5.6 $^{a,B}$ | 58.4 ± 0.4 $^{a,A}$ | 53.5 ± 0.6 $^{bc,A}$ | 49.6 |
| GYA3 | 12.5 ± 1.5 $^{g,C}$ | 38.8 ± 0.5 $^{d,A}$ | 24.0 ± 1.3 $^{f,B}$ | 25.1 |
| **2000s** | | | | |
| El Reno | 23.8 ± 1.9 $^{d,B}$ | 49.3 ± 0.6 $^{bc,A}$ | 51.3 ± 0.5 $^{cd,A}$ | 41.4 |
| Guymon | 25.3 ± 4.1 $^{c,C}$ | 47.5 ± 0.9 $^{c,B}$ | 52.5 ± 0.9 $^{cd,A}$ | 41.8 |
| Cherokee | 15.3 ± 1.3 $^{fg,B}$ | 31.3 ± 0.6 $^{e,A}$ | 34.4 ± 3.2 $^{e,A}$ | 27 |
| OK-06-1 | 19.5 ± 2.1 $^{def,B}$ | 57.6 ± 0.3 $^{a,A}$ | 58.8 ± 1.3 $^{ab,A}$ | 45.3 |
| OK-06-2 | 25.3 ± 4.5 $^{cd,C}$ | 49.3 ± 2.2 $^{bc,B}$ | 60.5 ± 0.6 $^{a,A}$ | 45 |
| OK-06-3 | 23.3 ± 7.2 $^{de,B}$ | 59.5 ± 0.5 $^{a,A}$ | 63.3 ± 0.8 $^{a,A}$ | 48.7 |
| Atoka | 24.0 ± 1.9 $^{cd,B}$ | 49.9 ± 0.3 $^{bc,A}$ | 47.0 ± 1.4 $^{d,A}$ | 40.3 |
| Kiowa | 33.3 ± 1.3 $^{ab,C}$ | 47.5 ± 0.6 $^{c,B}$ | 53.5 ± 0.6 $^{bc,A}$ | 44.8 |
| Mean | 24.4 | 48.1 | 48.6 | |

[a]Means in a column (lower case) and row (upper case) having same letter did not differ significantly by pair-wise difference option (PDIFF) in PROC Mixed (α = 0.05); each mean is the average of four replications. Isolate collection decade is in bold.

**Table 4:** Analysis of variance by PROC GLM for conidia production (conidia number) on clarified V8-juice agar and pseudothecia production (total and percent matured) on wheat straw by seventeen isolates of *Pyrenophora tritici-repentis*

| Source | d.f. | Conidia number | | Total pseudothecia | | Matured pseudothecia | |
|---|---|---|---|---|---|---|---|
| | | *F* | *p* | *F* | *p* | *F* | *p* |
| Replication | 3 | 0.77 | 0.55 | 1.57 | 0.21 | 1.51 | 0.22 |
| Isolates | 16 | 13.78 | <0.01 | 16.6 | <0.01 | 18.5 | <0.01 |

**Table 5:** Sporulation (mean ± standard error) of seventeen isolates of *Pyrenophora tritici-repentis* on clarified V8 (CV8) juice agar and on leaves of wheat cultivars TAM-105 (susceptible), Deliver (moderately susceptible) and Red Chief (resistant)[a]

| Isolates[b] | Sporulation on CV8[c] | Sporulation on wheat leaf | | | Mean |
|---|---|---|---|---|---|
| | | TAM 105 | Deliver | Red Chief | |
| OKA1 (1983) | 30 ± 3.0 $^{de}$ | 765 ±36 $^{d,A}$ | 502 ±6 $^{efg,AB}$ | 198 ±44 $^{d-g,B}$ | 488 |
| OKA2 (1983) | 48 ± 2.1 $^{bc}$ | 780 ±12 $^{d,A}$ | 272 ±21 $^{gh,B}$ | 89 ±9 $^{fg,B}$ | 380 |
| OKD1 (1983) | 62 ± 8.7 $^{ab}$ | 860 ± 267 $^{d,A}$ | 779 ±21 $^{cde,A}$ | 178 ±14 $^{efg,B}$ | 605 |
| OKD2 (1983) | 24 ±.2 $^{def}$ | 850 ± 175 $^{d,A}$ | 621 ±16 $^{def,A}$ | 79 ±8 $^{g,B}$ | 516 |
| OKD3 (1983) | 11 ±1.3 $^{f}$ | 320 ±30 $^{e,A}$ | 153 ±24 $^{h,A}$ | 101 ±25 $^{fg,A}$ | 191 |
| OKD4 (1983) | - | - | - | - | - |
| OKD5 (1983) | 16 ± 2.1 $^{ef}$ | 932 ± 72 $^{cd,A}$ | 370 ± 32 $^{fgh,B}$ | 248 ± 19 $^{d-g,B}$ | 516 |
| RBB6 (1996) | 34 ± 5.6 $^{cd}$ | 764 ± 33 $^{d,A}$ | 528 ± 28 $^{efg,A}$ | 399 ± 45 $^{c-f,B}$ | 564 |
| GYA3 (1996) | 15 ± 1.6 $^{ef}$ | 157 ± 30 $^{e,A}$ | 80 ± 7 $^{h,A}$ | 87 ± 9 $^{fg,A}$ | 108 |
| El Reno (2005) | 17 ± .5 $^{ef}$ | 109 ± 21 $^{e,B}$ | 665 ±17 $^{def,A}$ | 240 ± 32 $^{d-g,B}$ | 338 |
| Guymon (2006) | 61 ± 9.2 $^{ab}$ | 2923 ±90 $^{a,A}$ | 1693 ±75 $^{ab,B}$ | 687 ± 51 $^{bc,C}$ | 1768 |
| Cherokee (2006) | 68 ± 8.6 $^{a}$ | 848 ± 53 $^{d,A}$ | 855 ± 87 $^{cd,A}$ | 478 ± 31 $^{cde,B}$ | 727 |
| OK-06-1 (2006) | 50 ± 2.1 $^{b}$ | 1174 ±107 $^{c,A}$ | 876 ± 90 $^{cd,A}$ | 493 ± 31 $^{cd,B}$ | 848 |
| OK-06-2 (2006) | 20 ± 2.2 $^{def}$ | 205 ± 38 $^{e,A}$ | 268 ± 37 $^{gh,A}$ | 83 ± 25 $^{g,A}$ | 185 |
| OK-06-3 (2006) | 29 ± 2.3 $^{de}$ | 1575 ± 250 $^{b,A}$ | 1020 ± 57 $^{c,B}$ | 815 ± 115 $^{b,B}$ | 1136 |

| | | | | | |
|---|---|---|---|---|---|
| Atoka (2007) | 62 ± 8.3 [ab] | 1740 ± 202 [b,A] | 1575 ± 221 [b,A] | 440 ± 72 [cde,B] | 1252 |
| Kiowa (2007) | 50 ± 7.1 [b] | 2629 ± 285 [a,A] | 1910 ± 315[a,B] | 1271 ± 224 [a,C] | 1936 |
| Mean | 37 | 1039 | 760 | 368 | |

[a]Sporulation means following the same letter in a column (lower case) and row (upper case for sporulation on wheat leaf) did not differ significantly by pair-wise difference (PDIFF) option in PROC Mixed using a protected *t*-test (α = 0.05); each mean is the average of four replications

[b]Year of isolate collection in parenthesis

[c]Conidia calculated per $cm^2$ mycelial colony area on CV8 media; means within this column having same letter did not differ significantly by Fisher's least significance test (α = 0.05)

[d]Did not produce conidia on CV8, and was excluded from analysis.

### Pseudothecia production

Isolates differed significantly ($p<0.01$) in production of pseudothecia and formation of mature ascospores within pseudothecia (Table 4). OKD2, OK-06-1, Guymon and OK-06-3 produced the highest number of pseudothecia while the least pseudothecia were produced by OKD4, GYA3 and El-Reno (Table 6). The percent of pseudothecia containing mature ascospores was highest for Guymon and OKA1 while OKD3, OKD4, El-Reno and OK-06-2 did not produce mature pseudothecia by the time the experiment was terminated. On an average the isolates from the 2000s produced 47 pseudothecia on wheat straw of which 20% were mature while the older isolates (from 1980s and 1990s combined) produced 42 pseudothecia of which 11% were mature (Table 6).

### Virulence of isolates

Isolates differed significantly ($p<0.01$) in virulence as determined by percent leaf area infection on three wheat cultivars, however, a significant isolate × cultivar interaction was also detected (Table 2), thus, the simple effects of isolates and cultivars on virulence are presented (Table 7). Overall, Cherokee was the most virulent isolate across all three cultivars followed by Kiowa, OK-06-1, OK-06-3 and OKA1. El-Reno was the least virulent followed by OKD3, OKD5 and OKA2. On average, tan spot severity was 33% in terms of leaf area infection (LAI) by the isolates collected in the 2000s while isolates of the 1980s and 1990s produced 21.4% and 19.3% LAI, respectively. Cultivars also differed significantly ($p<0.01$) in producing disease across all isolates. On an average, LAI of 43.4%, 29.5%, and 7.8% were observed on cultivars TAM-105 (susceptible), Deliver (moderately susceptible) and Red Chief (resistant), respectively.

## DISCUSSION

Growth is one important fitness character that enables a fungal pathogen to utilize nutrients from infected tissue. Loughman and Deverall [23] reported significantly greater intercellular mycelial growth of *P. tritici-repentis* on susceptible wheat cultivars after four days of inoculation as compared to mycelial growth in resistant cultivars. Mycelia growing on dead wheat plant tissue produce conidia that are the secondary inoculum for spreading tan spot in the field. However, in this experiment fungal growth was studied on artificial media which lacks host influence. Isolates grew well on real potato dextrose agar and clarified V8 juice which provided more vitamins and minerals. Mycelial growth was less on commercial potato dextrose agar, which may not be as nutrient and vitamin rich as agar made from fresh potatoes.

Sporulation or conidia production by a pathogen on host tissue is another important fitness parameter in disease epidemics, and Riaz et al. [24] reported that isolates of *P. tritici-repentis* produced significantly more conidia on susceptible compared to resistant wheat cultivars. Isolates used in this study varied significantly in conidia production both on an artificial medium and on wheat plants, with cultivar resistance influencing conidia production by different isolates.

Tan spot is a polycyclic disease because conidia produced on wheat leaves facilitate secondary infection that can lead to an epidemic in the field [18,25]. Comparing sporulation of *P. tritici-repentis* isolates on artificial media to sporulation on wheat leaves helps to identify isolates that may be most fit to create tan spot epidemics, and thereby be best to use in screening wheat breeder lines for reaction to tan spot in greenhouse studies. For example, isolates such as Guymon and Kiowa sporulated on artificial media at a high level as well as producing high numbers of conidia on wheat plants. Isolates such as Cherokee and OKD1 produced higher number of conidia on CV8 media while their sporulation was much less on all three wheat cultivars (Table 5). On the contrary, isolate OK-06-3 produced fewer conidia on artificial media but produced more conidia on wheat cultivars. Isolates OKD3 and GYA3 produced only few conidia on both artificial media and on wheat leaves. It is evident from our study that careful selection of fungal isolates that are not only virulent but also have the ability to produce higher numbers of conidia on wheat leaves would be crucial for screening wheat germplasm for reaction to tan spot.

Tan spot of wheat is favored by conservation tillage because *P. tritici-repentis* completes its life cycle on wheat residue. The production of pseudothecia, number of asci per pseudothecia, and number of mature ascospores per ascus are important to tan spot epidemics but not as important to isolate selection for screening wheat lines for reaction to tan spot. However, results from this experiment show that a higher number of pseudothecia does not ensure mature ascospores. For example, OKD2, OKD3, OKD5, OK-06-2, OK-06-3 produced a greater number of pseuthothecia but few or no mature ascospores were observed when the experiment was terminated after 23 days of incubation. Ascospore maturation is a continuous process; however, early maturation of ascospores would be an important competitive character of an isolate to initiate and establish tan spot in the late winter in hard winter wheat fields. Although previous laboratory studies showed nutrient amended media influenced pseudothecia production and ascospore maturity in this fungus [19,26], no supplemental nutrients were provided in this study because *P. tritici-repentis* colonize saprophytically in nature on wheat residue left in the field [27].

The isolates of *P. tritici-repentis* used in this study varied in virulence, which confirms similar variation reported by Schilder and Bergstrom [28]. This variation in virulence indicates the importance of selecting isolates for germplasm screening because highly virulent isolates would be most useful in identifying wheat lines resistant (percent leaf area infection) to tan spot as compared to using isolates with lesser virulence [10]. In this study, inclusion

**Table 6:** Total and matured pseudothecia (mean ± standard error) produced by the seventeen isolates of *Pyrenophora tritici-repentis* collected from winter wheat in Oklahoma[a]

| Isolates[b] | Total pseudothecia[c] | Matured pseudothecia (%)[d] |
|---|---|---|
| OKA1 (1983) | 51.0 ± 12.8 bcd | 41.6 ±11.0 ab |
| OKA2 (1983) | 49.7 ± 5.4 bcd | 21.6 ± 4.2 def |
| OKD1(1983) | 41.5 ± 3.4 cd | 7.4 ± 1.8 gh |
| OKD2(1983) | 84.5 ± 9.1 a | 0.0 ± 0.0 i |
| OKD3(1983) | 49.2 ± 3.9 bcd | 5.0 ± 2.1 h |
| OKD4(1983) | 12.3 ± 3.1 e | 0.0 ± 0.0 i |
| OKD5(1983) | 36.1 ± 1.4 d | 3.4 ± 2.3 hi |
| RBB6 (1996) | 44.7 ± 6.7 bcd | 12.9 ±1.6 efg |
| GYA3 (1996) | 12.0 ± 1.7 e | 0.8 ± 0.7 i |
| El Reno (2005) | 1.5 ± 0.8 f | 0.0 ± 0.0 i |
| Guymon (2006) | 59.6 ± 4.4 abc | 54.2 ± 8.1 a |
| Cherokee (2006) | 49.7 ± 1.2 bcd | 34.9 ±3.3 bc |
| OK-06-1 (2006) | 69.4 ± 5.4 ab | 22.0 ± 2.5 def |
| OK-06-2 (2006) | 47.1 ± 3.9 bcd | 0.0 ±0.0 i |
| OK-06-3 (2006) | 61.4 ± 5.7 abc | 11.7 ± 2.9 fg |
| Atoka (2007) | 35.3 ± 4.2 d | 23.8 ± 1.4 cd |
| Kiowa (2007) | 47.7 ± 3.4 bcd | 22.3 ± 1.2 cde |

[a]Means following the same letter within a column did not differ significantly by Fisher's least significance difference test (α = 0.05). A mean is the average of four replications

[b]Year of collection in parenthesis

[c]5-cm long wheat straw of cv. Deliver was infested by mycelial plug and pseudothecia were counted on it after 14 days of incubation

[d]Data were analyzed after arcsine root transformation.

**Table 7:** Percent leaf area infection (mean ± standard error) of sixteen isolates of *Pyrenophora tritici-repentis* on wheat cultivars TAM-105 (susceptible), Deliver (moderately susceptible) and Red Chief (resistant) under greenhouse condition

| | Percent leaf area infection on wheat cultivars[a] | | | | |
|---|---|---|---|---|---|
| Isolates | TAM-105 | Deliver | Red Chief | Mean | Mean[c] |
| OKA1 (1983)[b] | 54.6 ± 8.5 bcd,A | 46.8 ± 5.3 b,A | 4.0 ± 0.3 cde,B | 35.1 | |
| OKA2 (1983) | 27.1 ± 4.6 f,A | 16.9 ±2.2 hi,A | 6.3 ± 0.3 b-e,B | 16.8 | |
| OKD1 (1983) | 44.2 ± 3.7 de,A | 27.9 ±2.3 efg,B | 4.0 ± 0.9 cde,C | 25.4 | |
| OKD2 (1983) | 47.6 ± 4.4 cde,A | 17.9 ±1.5 ghi,B | 12.2 ± 2.3 a-d,B | 25.9 | |
| OKD3 (1983) | 14.8 ± 1.9 g,A | 11.3 ±1.6 i,AB | 1.6 ± 0.2 e,B | 9.2 | |
| OKD5 (1983) | 24.9 ± 4.9 fg,A | 19.8 ±1.5 ghi,A | 2.9 ± 0.4 de,B | 15.9 | 21.4 |
| RBB6 (1996) | 53.5 ± 2.4 bcd,A | 23.8 ± 3.7 e-h,B | 6.2 ± 0.8 b-e,C | 27.8 | |
| GYA3 (1996) | 18.6 ±1.6 fg,A | 11.8 ±1.2 i,AB | 1.6 ± 0.2 e,B | 10.7 | 19.3 |
| El Reno (2005) | 0.7± 0.1 h,A | 0.4 ± 0.1 j,A | 0.1 ± 0.0 e,A | 0.4 | |
| Guymon (2006) | 58.5±12.2 b,A | 34.1 ±3.5 de,B | 5.2±1.2 b-e,C | 32.6 | |
| Cherokee (2006) | 74.7± 9.5 a,A | 70.0 ± 2.0 a,A | 21.4 ± 1.9 a,B | 55.4 | |
| OK-06-1 (2006) | 57.6 ± 2.1 bc,A | 51.3 ± 3.8 b,A | 6.0 ± 0.9 b-e,B | 38.3 | |
| OK-06-2 (2006) | 57.3 ± 3.4 bc,A | 23.0 ± .1 fgh,B | 1.8 ± 0.3 de,C | 27.4 | |
| OK-06-3 (2006) | 53.4 ± 6.5 bcd,A | 39.0 ± 4.2 cd,B | 14.4 ± 2.1 abc,C | 35.6 | |
| Atoka (2007) | 37.8±4.2 e,A | 30.5 ±2.3 def,A | 15.0±1.4 ab,B | 27.8 | |
| Kiowa (2007) | 69.5±5.8 a,A | 47.7 ±1.6 bc,B | 21.4±1.5 a,C | 46.2 | 33 |
| Mean | 43.4 | 29.5 | 7.8 | | |

[a]Means following the same letter in a column (lower case) and row (upper case) did not differ significantly by pairwise difference (PDIFF) in PROC Mixed option (α = 0.05); each mean is the average of four replications

[b]Year of collection in parenthesis

[c]Mean percent leaf area infection of isolates from 1980s, 1990s and 2000s, respectively.

of additional isolates from the 1990s would have added more accuracy, but even using only two isolates from the 1990s, a wide variation in virulence was noted. The isolates collected in the 2000s were more virulent than isolates collected in the 1990s or 1980s on average, which suggests it would be prudent to consistently renewing isolates used in testing on a regular basis.

## CONCLUSION

Isolates collected in the 2000s in this study appeared to be more fit than isolates collected in the 1990s and 1980s as indicated by radial growth, sporulation on artificial media and live hosts, pseudothecia formation, ascospore maturation, and virulence. The results of this study indicated that selection of isolates for use in epidemiological studies or for testing wheat lines for reaction to tan spot are crucial, and should be considered when conducting studies on tan spot of wheat. This study demonstrated that fitness of *P. tritici-repentis* isolates varies, with the most recently collected isolates demonstrating the highest virulence. Hence, using recently collected isolates of *P. tritici-repentis* in studies involving tan spot and to screen for resistance to this disease is recommended so that isolates are more representative of current *P. tritici-repentis* populations in the field.

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