

Differences Among Isolates of *Pyrenophora tritici-repentis* in Production of Conidia on Wheat Leaves

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ABSTRACT

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Isolates of *Pyrenophora tritici-repentis* were compared for their ability to produce conidia on host tissue. In the first set of experiments, four isolates were compared on the tan spot-susceptible cultivar TAM 105. In the second set of experiments, three isolates were compared on four wheat cultivars differing in their necrotic and chlorotic reactions to tan spot. Number of conidia and percentage of disease severity were regressed against time after inoculation to compare slopes and estimates of the intercepts of linear response curves. Comparison of slopes for conidia production on the susceptible cultivar TAM 105 indicated that isolate MCR-6 had a significantly higher slope (up to 12-fold) than the other isolates. Additionally, the estimates of the intercepts for the other isolates were all different from each other (up to 5.7-fold). Comparisons of slopes and estimates of the intercepts for conidia production on cultivars differing in resistance showed that MCR-6 had a significantly higher slope than isolates MCS-I and PTF on all cultivars, and that the estimates of the intercepts of MCS-I were higher than those of PTF on all cultivars except on the highly resistant Karl 92. Thus, large differences in sporulation ability occurred within the population of *P. tritici-repentis*, and the ranking of an isolate remained the same on all cultivars. Differences in conidial production among isolates were not explained by differences in aggressiveness (disease severity). The large differences in sporulation capacity of strains of *P. tritici-repentis*, if shown to occur in the field, have implications for their rate of spread and would be determining factors in the time required for a new strain to attain a significant frequency in the fungal population.

Tan spot of wheat (*Triticum aestivum* L.) is caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph = *Drechslera tritici-repentis* (Died.) Shoemaker). The disease can be very destructive on durum, winter, and spring wheat (3,7,8,15,19). *Pyrenophora tritici-repentis* is also pathogenic to 26 other species of Poaceae (13) including several prairie grasses (20).

Tan spot reduces total yield, kernel weight (29,30), number of grains per head (29), total biomass (12), and grain quality because of red-smudge symptoms (5). In recent years, tan spot has increased to damaging levels in the Great Plains of the

U.S. Additionally, the disease has been reported in areas where it was not detected before and under a wide range of environmental conditions (4). The increase in disease incidence and severity has been attributed to changes in cultivars, tillage practices, and improved diagnosis (11,15).

Control of tan spot is achieved through crop rotation, burning or incorporating crop residue, spraying wheat with foliar fungicides, or planting resistant cultivars. Crop rotation and residue management may reduce surface debris and, thus, the amount of primary inoculum. However, under a favorable environment for disease development, even small amounts of residue can result in significant disease (26). Additionally, in locations where wheat is grown continuously, large amounts of residue on the soil surface are desirable to avoid erosion, conserve soil moisture, and reduce expenditures on fuel (1). Therefore, host resistance is preferable for control of this disease.

Host plant resistance reduces the amount of yield loss from tan spot (24). Resistance has been reported to be under polygenic (21) or monogenic control (17, 18) and of intermediate to high heritability (21). Many workers have reported that tan spot resistance is similar in both seedling

and adult plants for a given genotype (2,8, 9,15,24); however, other reports indicate that the reactions of adult plants and seedlings are not always the same (2,5,9). Although resistance can be incorporated into wheat cultivars to reduce losses, this resistance allows asexual reproduction of the pathogen (27).

According to Rotem et al. (28), studies of sporulation in vivo have been neglected because of the tedious experiments required, the difficulty in counting spores, high experimental error, and a misconception that sporulation in petri dishes necessarily reflects the situation in nature. *Pyrenophora tritici-repentis* has been reported as a difficult organism to study because of variation in magnitude of disease symptoms between studies, and significant interactions among isolate, genotype, and environment (14). Furthermore, some wheat cultivars that are resistant in one part of the world appear to be susceptible in another (3). A possible explanation for such variation is that isolates can vary between inoculations in the reaction they cause, particularly on susceptible hosts (14,31). Therefore, variation between isolates of *P. tritici-repentis*, including variation in conidial production on leaves, needs to be quantified.

Studies on conidiation of *P. tritici-repentis* revealed the effects of substrate, temperature, isolate, and photoperiod on sporulation of the fungus (10,13,23). However, these studies were carried out in vitro and did not compare sporulation in vitro with sporulation on living host tissues. Riaz et al. (27) quantified conidiation of *P. tritici-repentis* in vivo and studied the effect of wheat genotype, time after inoculation, and leaf age on conidial production. Resistant cultivars produced fewer conidia than susceptible cultivars and younger leaves produced fewer conidia than older leaves. However, those researchers used a mixture of four isolates of *P. tritici-repentis* collected from infected winter wheat across Kansas.

Our study expanded on those findings by determining the sporulation ability of four individual isolates of *P. tritici-repentis*. A secondary objective was to compare sporulation of different isolates on susceptible, intermediate, and resistant winter wheat cultivars. Disease severity

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resulting from inoculation by individual isolates was determined to see if this was related to differences among isolates in sporulation ability. Such information is important, because the number of conidia produced provides a means for the quantitative evaluation of host resistance (27) and fitness among the isolates, which are important parameters in breeding for resistance to tan spot. Additionally, knowledge of the sporulation ability of different isolates is useful in the study of epidemics and in forecasting of disease.

MATERIALS AND METHODS

Preliminary tests were conducted on the susceptible winter wheat cultivar TAM 105 using several isolates of *P. tritici-repentis*. During these tests, the linear part of the conidial-production curve was determined, and leaf harvest intervals were set. Based upon a possible range in sporulation ability from leaves, four isolates (MCR-6, MCS-I, AUB-II, and PTF) were chosen for further study. The single-conidial isolates were collected originally from infected wheat fields in Kansas (MCR-6 and MCS-I from McPherson County and AUB-II and PTF from Riley County) and stored on potato dextrose agar (PDA) slants at 4°C. All four isolates were nec+ and chl+ (16). Isolates were compared for ability to sporulate in vitro using the method of Hunger and Brown (10).

In the first set of experiments, the isolates' responses on TAM 105 were compared. In a second set of experiments, three isolates (MCR-6, MCS-I, PTF) were compared for sporulation on each of four winter wheat cultivars differing in their resistance to tan spot. Based upon the necrosis/chlorosis model of tan spot symptoms (17), the reaction of the cultivars is as follows: TAM 105 is susceptible and develops necrosis and chlorosis; Arkan develops chlorosis but not necrosis; Victory develops necrosis but not chlorosis; and Karl 92 develops neither necrosis nor chlorosis. In both experiments, percentage of disease severity caused by individual isolates also was compared. Both experiments were repeated.

Preparation of plant material. Plants for all experiments were grown in pots containing 270 cm³ of a 2:1 mixture of Kennebeck silty-clay loam and sand that had been steamed (60°C) for 3 h. All seedlings were fertilized twice with N-P-K (20-20-20), when the plants had one and three leaves. They were grown in the greenhouse (21 ± 5°C) to the four-leaf stage before being inoculated with the conidial suspensions. For the first set of experiments, the four fungal isolates were compared for sporulation on leaves of TAM 105, which were harvested at either 5, 10, 15, or 20 days after inoculation. Five replicate, nonvernalized plants were used for all treatments (one isolate at one harvest time) in a randomized complete block design, with the four oldest leaves

(leaves 1 to 4) of each plant harvested. For the second set of experiments, three isolates (MCR-6, MCS-I, and PTF) and seedlings of TAM 105, Arkan, Victory, and Karl 92 were used. Four replicate plants were used per treatment (one isolate, one cultivar, one harvest time), with the oldest four leaves per plant harvested either 5, 10, 15, or 20 days after inoculation.

Preparation of inoculum. Mycelial plugs from single-spore cultures of the isolates were transferred to one-fourth-strength PDA in petri plates. Then, each isolate was transferred and stored on agar slants at 4°C as a source of mycelium to produce inoculum for all experiments. Conidia used for inoculations were obtained by transferring mycelial plugs from slants to V8 agar plates, which were kept in the dark (20 ± 2°C) for about 6 days. The aerial mycelium was then flattened with a sterile, bent, glass rod, and plates were placed 40 cm below four cool-white fluorescent tubes (40 W, about 30 μE s⁻¹ m⁻²) for 24 h at 20°C for conidiophore formation and then incubated in the dark for 24 h at 16°C for conidiation (27).

Conidia were harvested from petri plates by adding distilled water to the surface of the plates and dislodging conidia with a sterile, bent, glass rod (24). This previously published method was slightly modified to obtain a homogeneous conidial suspension. Because Tween 20 sometimes results in clumping of conidia, it was omitted, and conidial suspensions instead were agitated with a vortex mixer. Inoculum density was determined by using a spore-counting grid and concentrations adjusted by adding distilled water to obtain 4,000 conidia per ml for the first set of experiments and 3,000 conidia per ml for the second set of experiments. Because conidiophores and mycelial fragments are much less infective (less than 1/25) than conidia (6), they were not included in the counts. Conidial germination, determined on water agar, was always more than 97% for all isolates.

Inoculation. Plants that had a fully expanded fourth leaf were inoculated with conidial suspensions using an atomizer (model 152, DeVilbiss Co., Somerset, PA) operated at 69 kPa pressure. Plants were sprayed at a rate of 1.5 ml of conidial sus-

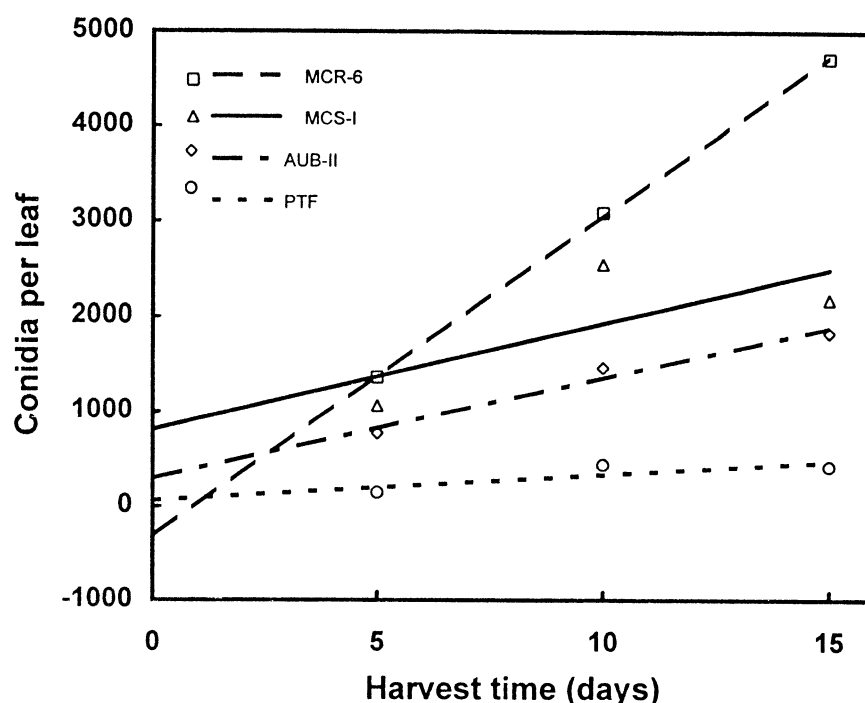


Fig. 1. Number of conidia per leaf regressed against time after inoculation for four isolates of *Pyrenophora tritici-repentis* on the susceptible wheat cultivar TAM 105. Linear equations are as follows: MCR-6, $Y = -303.8 + 335.9X$ ($P = 0.0001$); MCS-I, $Y = 816.7 + 111.9X$ ($P = 0.0078$); AUB-II, $Y = 219.9 + 105.9X$ ($P = 0.0077$); and PTF, $Y = 55.2 + 27.8X$ ($P = 0.0349$).

Table 1. Probability values for comparing slopes and estimates of the intercepts of linear regression models for conidial production per leaf regressed against time after inoculation for four isolates of *Pyrenophora tritici-repentis* on susceptible wheat cultivar TAM 105

Isolate	Isolate					
	MCS-I		AUB-II		PTF	
	Slope	Intercept	Slope	Intercept	Slope	Intercept
MCR-6	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001
MCS-I	—	—	0.9176	0.0287	0.1453	0.0001
AUB-II	—	—	—	—	0.1756	0.0002

pension per plant and dried for 30 min to allow spores to adhere to the leaves. Plants then were placed in a plastic-covered mist chamber for 48 h, and mist was provided by two centrifugal atomizing humidifiers electrically controlled to operate for 1 min every 9 min, which produced continual leaf wetness. After that, plants were removed from the chamber and placed on greenhouse benches until harvest. Pots were placed in shallow pans and watered from the bottom to prevent free water from touching the leaves during incubation. Temperature in the greenhouse ranged from 16 to 26°C during the studies.

Assessment of sporulation on leaves. The four oldest leaves of the plants were harvested either 5, 10, 15, or 20 days after inoculation. Harvested leaves were rated for disease severity based on the percentage of leaf area affected by necrosis and/or chlorosis and assigned to one of the following classes: 0, 1, 5, 10, 25, 50, 75, or 100% of the leaf blade affected. Entire

harvested leaves from each plant were cut into 5- to 7-cm pieces and placed on filter paper moistened with 2.5 ml of distilled water in a glass petri dish, which was then sealed with Parafilm. The petri dishes were placed 30 cm under cool-white fluorescent lights (20 W, about $65 \mu\text{E s}^{-1} \text{m}^{-2}$ and incubated with 12 h light at 25°C and 12 h dark at 16°C for 5 days.

After this 5-day period, all segments of each leaf were cut into 1-cm pieces with scissors and blended in 15 ml of distilled water in a small blender (model 91-264, Dynamic Corporation of America, New Hartford, CN) for 15 s. After blending, two subsamples were taken using a pipette and put onto counting grids. For each subsample, conidia were counted under a dissecting microscope on grid areas having a capacity of 0.06 ml (six units of 0.01 ml each). Similar results were obtained whether expressing conidial numbers on a unit-area or per-leaf basis; therefore, data were presented only on a per-leaf basis.

Statistical analysis. Both experiments were repeated with similar results. Disease severity data were arcsine square root transformed for analysis and back transformed for presentation. Harvest day 20 was omitted from the analyses, because it was usually outside the linear part of the curve. The number of conidia produced per leaf, or transformed severity, was regressed against the time after inoculation when leaves were harvested. Data were analyzed using the SAS (SAS Institute, Cary, NC) general linear model (GLM) procedure, and regression analyses were used to compare slopes of response lines of the different isolates. Additionally, the equal-slopes model of GLM was used to compare estimates of the intercepts (as opposed to actual intercepts) of response curves. This procedure sets the slope of lines to zero and then compares where they intercept the y axis.

RESULTS

Significant differences occurred among isolates in conidia produced in vitro. For each isolate, the number of conidia produced per petri plate, and mean separation letters, were as follows: MCS-I, 1.60×10^5 a; MCR-6, 1.27×10^5 b; AUB-II, 1.19×10^5 b; and PTF, 0.97×10^5 c. Similar results were obtained in a second experiment.

Disease severity and sporulation of isolates from a susceptible cultivar. Repetitions of the experiment were combined for analysis. Significant ($P < 0.05$) linear relationships occurred for all four isolates when the number of spores produced per leaf was regressed against harvest time (Fig. 1). MCR-6 had a significantly higher slope than all of the other isolates (Table 1; Fig. 1). Similarly, the estimates of the intercepts for the other three isolates were significantly different from each other. Over the entire course of these experiments, MCS-I produced 62% as many conidia as MCR-6, AUB-II produced 43%, and PTF produced only 11% as many conidia.

Significant ($P < 0.05$) linear relationships occurred for all four isolates when transformed disease severity data were regressed against harvest time. Comparisons of the slopes of the regression lines for severities indicated that all isolates had similar rates of increase (slopes) (Table 2; Fig. 2). However, when estimates of the intercepts were compared, all isolates were significantly different from each other except MCR-6 and AUB-II.

Disease severity and sporulation of isolates from cultivars differing in resistance. Repetitions of both experiments were combined for analysis. With two exceptions, significant ($P < 0.05$) linear relationships occurred for all three isolates on all four cultivars when the number of spores produced per leaf was regressed against harvest time. Comparisons of the slopes of the lines produced by each iso-

Table 2. Probability values for comparing slopes and estimates of the intercepts of linear regression models for percentage of disease severity regressed against time after inoculation for four isolates of *Pyrenophora tritici-repentis* on susceptible wheat cultivar TAM 105

Isolate	Isolate					
	MCS-I		AUB-II		PTF	
	Slope	Intercept	Slope	Intercept	Slope	Intercept
MCR-6	0.8149	0.0006	0.4298	0.2198	0.8935	0.0011
MCS-I	—	—	0.3002	0.0242	0.9191	0.0001
AUB-II	—	—	—	—	0.3497	0.0001

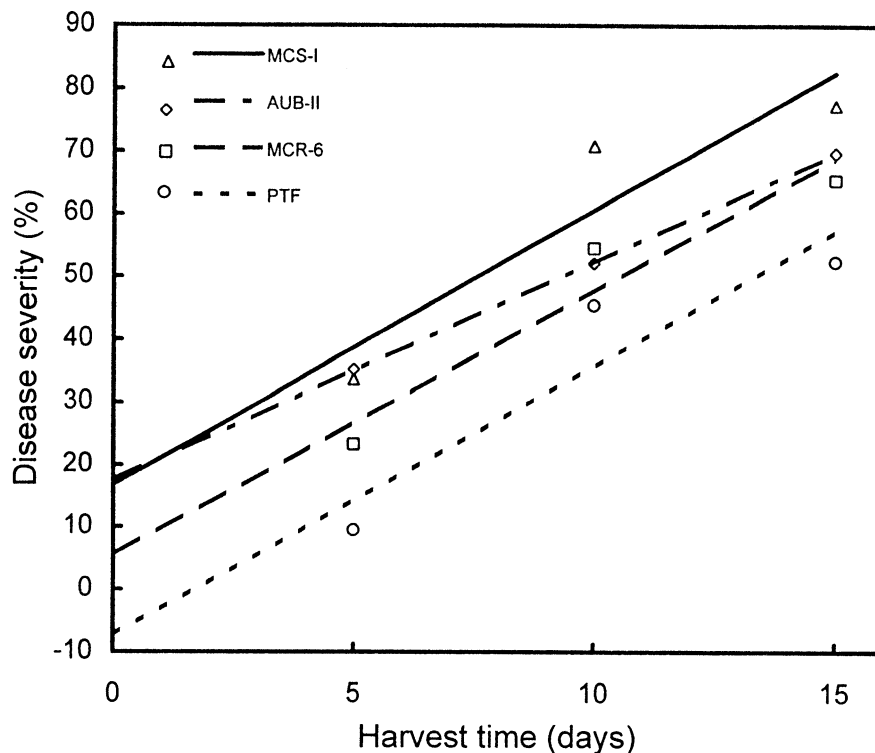


Fig. 2. Percentage of disease severity regressed against time after inoculation for four isolates of *Pyrenophora tritici-repentis* on susceptible wheat cultivar TAM 105. Linear equations are as follows: MCR-6, $Y = 6.2 + 4.2X$ ($P = 0.0001$); MCS-I, $Y = 16.8 + 4.4X$ ($P = 0.0001$); AUB-II, $Y = 17.8 + 3.5X$ ($P = 0.0001$); and PTF, $Y = -7.1 + 4.3X$ ($P = 0.0001$).

late indicated that MCR-6 had a significantly higher slope than MCS-I and PTF on all cultivars (Table 3; Fig. 3). Similarly, analysis of the estimates of the intercepts showed that MCS-I was significantly different from PTF on three of the four cultivars. The exception occurred on the highly resistant cultivar Karl 92, where the lines of MCS-I and PTF could not be separated statistically.

With two exceptions, significant ($P < 0.05$) linear relationships occurred for all three isolates on all four cultivars when transformed disease-severity data were regressed against harvest time. The exceptions were for the regressions of MCS-I and PTF on Karl 92.

Comparisons of the slopes of the lines for transformed disease severity produced by each isolate indicated that the slopes for all isolates were not significantly different on any cultivar (Table 4; Fig. 4). Similarly, all isolates produced similar estimates of the intercepts, except that PTF was significantly less than MCR-6 and MCS-I on Arkan.

DISCUSSION

In vitro sporulation studies have provided insights into physiological, biochemical, and genetic phenomena, which usually are more complicated to study on the living host. Nevertheless, studies in vitro may not mimic the sporulation patterns of the fungus on live tissue, because they do not account for the influence of the host on reproduction (28).

The number of spores produced by an isolate of a pathogen on its host is an important fitness character and can be influenced by components of the resistance mechanisms in the host (28). Furthermore, numerical values of sporulation are basic ingredients of simulation models of epidemics and also assist other research efforts such as control and breeding. As an example, Nelson and Tung (22), working with *Bipolaris maydis* on corn, found that sporulation on infected tissues had a direct relationship with disease development. Therefore, data presented here should prove useful in future attempts to predict tan spot and understand epidemic development. Although many factors affect sporulation, we concentrated on the magnitude of differences among isolates of *P. tritici-repentis*.

The experiments using a single, susceptible cultivar showed large differences among the four isolates of *P. tritici-repentis* in their ability to produce conidia on leaves. This was manifested by as much as a 12-fold difference in rates (slopes) when number of conidia was regressed against harvest time (Fig. 1). Even between isolates with statistically similar rates of increase, there was as much as a 5.7-fold difference in total number produced during the experimental period. We conclude that large variability exists in the

population of *P. tritici-repentis* in ability to produce conidia on host tissue.

In the second set of experiments, large differences in conidial production among isolates were also evident and occurred on all four of the host cultivars studied. Furthermore, isolates responded similarly to each other on all cultivars. In other words, MCR-6 produced the most conidia and PTF produced the least on all cultivars. Our experiments produced no evidence of qualitative cultivar-by-isolate interactions for sporulation ability on leaves. Therefore, the production of conidia by an isolate on one cultivar, relative to other isolates, should be a good indicator of its relative conidial production on other cultivars. However, it must be pointed out that our data were from conditions that were nearly optimum for sporulation; somewhat

different results may occur under less than optimum environments.

The four cultivars used in the second set of experiments represented the four possible combinations in symptom expression; necrotic and chlorotic reaction, chlorotic but not necrotic, necrotic but not chlorotic, and neither necrotic nor chlorotic (15,17). Except for the highly resistant Karl 92, isolates maintained their respective rankings on all cultivars. For Karl 92, MCS-I and PTF could not be separated, probably because of the high level of resistance, which reduced the number of conidia produced by all isolates. Aside from this, there was no evidence of physiological specialization among the isolates tested for sporulation capacity on host tissue. This finding parallels reports of small to intermediate, quantitative, isolate-by-

Table 3. Probability values for comparisons of slopes and estimates of the intercepts for conidia production per leaf regressed against time after inoculation for three isolates of *Pyrenophora tritici-repentis* on four wheat cultivars

Wheat cultivar	Isolates compared					
	MCR-6 and MCS-I		MCR-6 and PTF		MCS-I and PTF	
	Slope	Intercept	Slope	Intercept	Slope	Intercept
TAM 105	0.0153	0.0001	0.0063	0.0001	0.7390	0.0278
Arkan	0.0394	0.0001	0.0006	0.0001	0.1334	0.0001
Victory	0.0150	0.0240	0.0006	0.0001	0.2659	0.0228
Karl 92	0.0018	0.0457	0.0008	0.0003	0.8072	0.0789

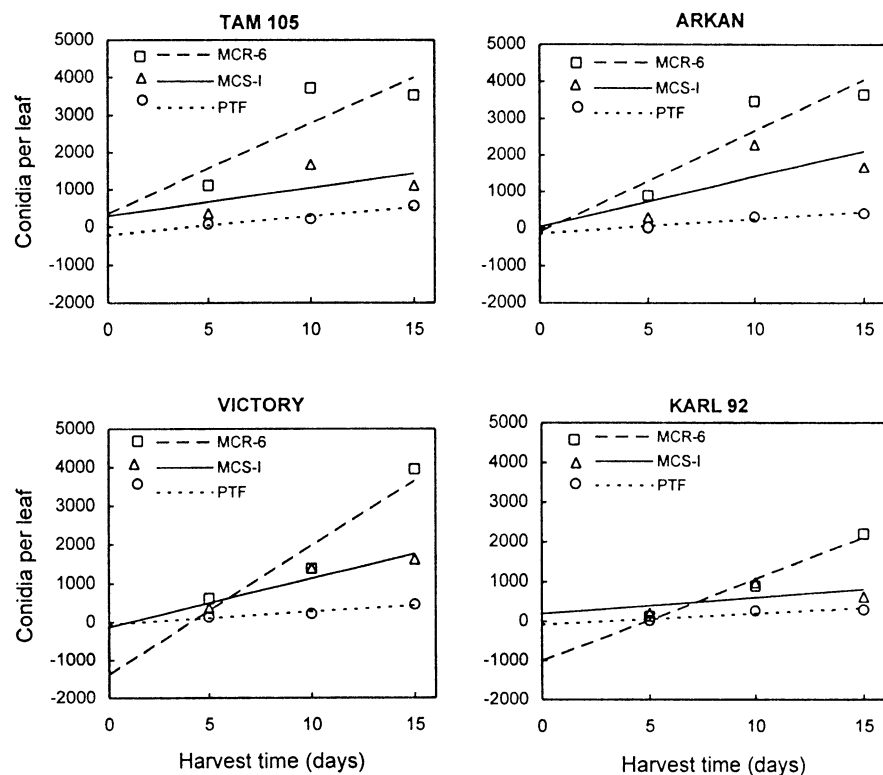


Fig. 3. Number of conidia per leaf produced by three isolates of *Pyrenophora tritici-repentis* regressed against time after inoculation on four wheat cultivars. Linear equations of the isolates are as follows: on TAM 105; MCR-6, $Y = 359.4 + 243.0X$ ($P = 0.0053$); MCS-I, $Y = 299.5 + 75.8X$ ($P = 0.0504$); PTF, $Y = -203.1 + 49.2X$ ($P = 0.0107$); on ARKAN; MCR-6, $Y = -93.7 + 276.6X$ ($P = 0.0001$); MCS-I, $Y = 39.1 + 138.3X$ ($P = 0.0188$); PTF, $Y = -135.4 + 38.3X$ ($P = 0.0012$); on VICTORY; MCR-6, $Y = -1362.0 + 334.4X$ ($P = 0.0024$); MCS-I, $Y = -127.6 + 125.8X$ ($P = 0.0003$); PTF, $Y = -57.3 + 32.8X$ ($P = 0.0038$); and on KARL 92; MCR-6, $Y = -994.8 + 206.3X$ ($P = 0.0009$); MCS-I, $Y = 205.7 + 39.8X$ ($P = 0.2059$); PTF, $Y = -75.5 + 27.3X$ ($P = 0.0065$).

cultivar interactions for virulence and aggressiveness in *P. tritici-repentis* (4,14,16,31).

Some of the isolates used in our experiments differed significantly in their abilities to produce disease (Tables 2 and 4; Figs. 2 and 4); others have reported similar differences (3,14). However, no consistent correlation occurred between an isolate's ability to cause disease and the amount of conidia produced. As one example in the first experiment, isolate MCS-1 produced significantly more disease than MCR-6, but the reverse was true for conidial production. On the other hand, isolate PTF produced the least amount of disease with a standard inoculum concen-

tration and also produced the fewest conidia. Therefore, although an isolate's aggressiveness may have an effect, differences in conidial production were not due solely to differences in aggressiveness among the isolates. Further research is necessary to determine the magnitude of the role that aggressiveness might play in conidial production.

Similarly, some of the isolates used in our experiments differed significantly in their ability to produce conidia in vitro; other researchers have reported similar differences (10). However, no significant correlation ($r = 0.4698$) occurred between an isolate's ability to produce conidia in vitro and in vivo. For example, isolate

MCS-1 produced significantly more conidia in vitro than MCR-6, but the reverse was true for conidial production on leaves. On the other hand, isolate PTF produced the least amount of conidia in vitro and the fewest conidia on leaves. Therefore, although an isolate's ability to sporulate in vitro may have an effect, differences in conidial production on leaves were not due solely to differences in sporulation ability in vitro.

The most important part of fungal dissemination in tan spot epidemics occurs from the secondary spread of airborne conidia; ascospores play a minor role (20, 25). This fact, coupled with our findings, has important implications for the rate of spread of new strains of *P. tritici-repentis*. The large differences (over 10-fold) in sporulation capacity of isolates shown in our experiments, if shown to occur in the field, would greatly affect the rate of spread of a strain. Therefore, the genotypic background, relative to sporulation, in which a new strain occurs could be a large determinant of the time required for the new strain to attain a significant frequency in the fungal population.

Table 4. Probability values for comparisons of slopes and estimates of the intercepts for disease severity regressed against time after inoculation for three isolates of *Pyrenophora tritici-repentis* on four wheat cultivars

Wheat cultivars	Isolates compared					
	MCR-6 and MCS-I		MCR-6 and PTF		MCS-I and PTF	
	Slope	Intercept	Slope	Intercept	Slope	Intercept
TAM 105	0.1294	0.5468	0.9742	0.1996	0.1154	0.4966
Arkan	0.1170	0.1061	0.6979	0.0008	0.2350	0.0001
Victory	0.6195	0.3163	0.5702	0.1116	0.9508	0.5532
Karl 92	0.1489	0.0869	0.0778	0.4394	0.7226	0.3502

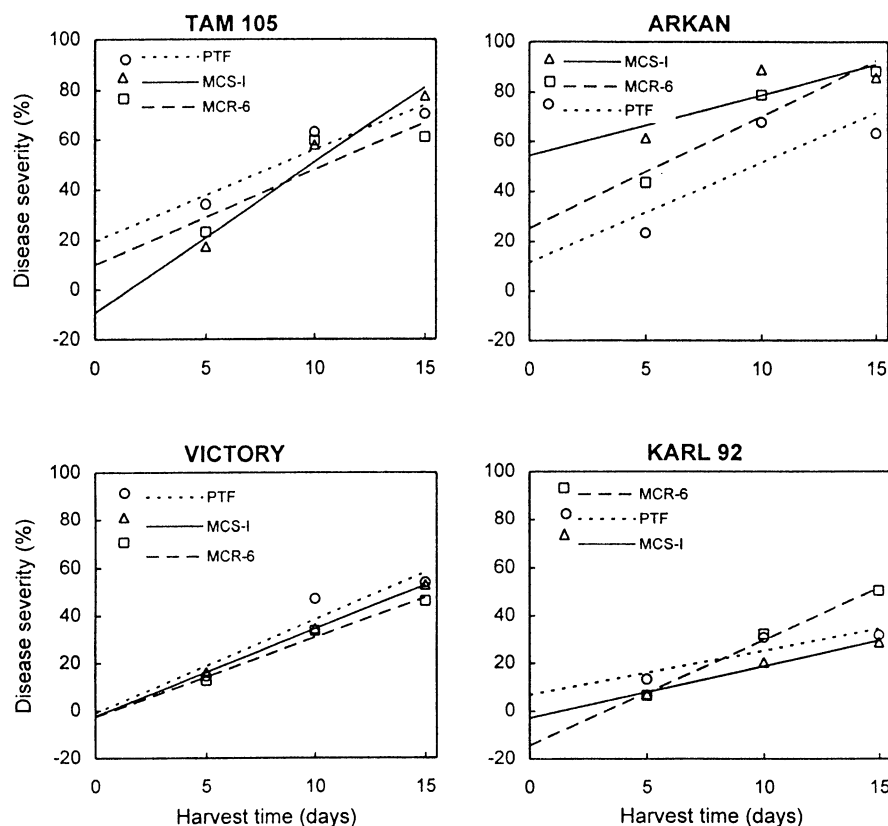


Fig. 4. Percentage of disease severity caused by three isolates of *Pyrenophora tritici-repentis* regressed against time after inoculation on four wheat cultivars. Linear equations of the isolates are as follows. On TAM 105: MCR-6, $Y = 10.9 + 3.7X$ ($P = 0.0059$); MCS-I, $Y = -8.7 + 6.0X$ ($P = 0.0001$); PTF, $Y = 19.7 + 3.6X$ ($P = 0.0004$). On ARKAN: MCR-6, $Y = 25.4 + 4.5X$ ($P = 0.0001$); MCS-I, $Y = 54.4 + 2.4X$ ($P = 0.0141$); PTF, $Y = 11.7 + 4.0X$ ($P = 0.0006$). On VICTORY: MCR-6, $Y = -2.6 + 3.6X$ ($P = 0.0008$); MCS-I, $Y = -3.6 + 3.9X$ ($P = 0.0001$); PTF, $Y = -1.7 + 3.9X$ ($P = 0.0001$). On KARL 92: MCR-6, $Y = -14.3 + 4.4X$ ($P = 0.0004$); MCS-I, $Y = -2.9 + 2.2X$ ($P = 0.0599$); PTF, $Y = 8.5 + 1.6X$ ($P = 0.1761$).

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