Effects of Wheat Genotype, Time After Inoculation, and Leaf Age on Conidia Production by **Drechslera tritici-repentis**

M. Riaz, W. W. Bockus, and M. A. Davis

Graduate student, professor, and research assistant, respectively, Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan 66506-5502.

Present address of first author: Agricultural Research Institute, Dera Ismail Khan NWFP, Pakistan.

Research supported in part by the TIPAN project of USAID. Contribution 91-386-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan.

Accepted for publication 18 June 1991 (submitted for electronic processing).

**ABSTRACT**


Regression analysis of the number of spores per leaf versus days after inoculation indicated a significantly higher slope (292.7) for a susceptible wheat cultivar (TAM 105) than for an intermediate cultivar (Triumph [93.5]) and two resistant cultivars (Karl [95.6] and Auburn [72.8]). When leaf age, expressed as position, was introduced as a variable, younger leaves produced spores less rapidly than older leaves for the resistant cultivar Auburn. Based on these results, cultivars that are resistant to **Drechslera tritici-repentis** will produce fewer conidia than susceptible cultivars, which should slow the secondary spread of the pathogen. Additionally, a procedure is described to quantify sporulation of **D. tritici-repentis** on wheat leaves. Leaves were harvested at various times after inoculation, placed on moist filter paper in petri dishes and exposed to alternating periods of 12 h light (35 μmol m⁻² s⁻¹, 25±1 C) and 12 h dark (16±1 C) for 5 or 6 days. The number of spores produced per leaf then was determined.

*Additional keywords: Pyrenophora tritici-repentis, yellow leaf spot.*

**Pyrenophora tritici-repentis** (Died.) Drechs., and its anamorph **Drechslera tritici-repentis** (Died.) Shoemaker, is the causal agent of tan spot, a foliar disease of wheat (**Triticum aestivum** L.) and many other members of the family Gramineae (5,10,12,28,29). This fungus has been described as having the widest host range within the genus **Drechslera** (28). It has been reported in many countries throughout the world (6,7) and is considered a problem on durum, winter, and spring wheat (2,4,16,27).

Tan spot on wheat can cause significant losses under severe epidemic conditions. Losses are manifested in total yield, kernel weight (25,26), and number of grains per head (25). Four factors are important for development of disease and, consequently, reduction in yield: presence and amount of primary inoculum (1,23), duration of leaf wetness after inoculation (8,9), stage of crop development at the time of infection (21,25), and host genotype (8,20).

Tan spot resistance has been reported to be similar in both seedling and adult plants for a given genotype (5,8,19). Resistance to the pathogen in wheat cultivars can profoundly influence the amount of yield loss from the disease. Hosford and Busch (8) reported 8, 12, 23, and 28% reduced yield for the cultivars Hercules, Wells, Chris, and Waldron, respectively. Raymond et al (19) reported 17.2% yield loss by the resistant cultivar Red Chief but 27.7% loss by susceptible TAM 105 under moderate to severe epidemic conditions. As well as yield loss, significant differences in disease severity among cultivars have been reported (13). Resistance can be manifested as reduced necrosis or chlorosis with susceptibility to the necrotic symptom inherited dominantly (14).

Leaf position or age has a significant effect on appearance of symptoms and disease severity (3,9,19,30). Watkins et al (30) noted coalescence of lesions on lower (older) leaves, but lesions on upper (younger) leaves appeared restricted. Raymond et al (19) observed the same phenomenon and reported that older leaves had higher disease ratings than younger leaves, in spite of being inoculated at the same time. Cox and Hosford (3) reported tan spot ratings (0-5 scale) of 2.1, 2.9, and 4.1 on the top, second, and third leaves of adult plants, respectively.

Other studies have described the effects of substrate, temperature, and photoperiod on sporulation of the fungus (11,18). Light and dark periods in the range of 12/12 are required for optimum formation of conidiophores and conidia, respectively (11,18). Ranges of temperature for conidiation in vitro are 10-31 C for conidiophores and 10-25 C for conidia (18,19). However, all of these sporulation studies were conducted in vitro and did not measure any possible interactions between the fungus and host tissues. Nelson and Tung (17) studied sporulation of a related fungus, **Bipolaris maydis** (Nisikado & Miyake) Shoemaker, on corn (**Zea mays** L.) and showed that sporulation on infected host tissues is important and has a direct relationship with disease development. Therefore, considering the importance of sporulation, its effect on disease development, and significant differences in disease severity among cultivars and among leaves of different positions, a study was undertaken to quantify sporulation by **D. tritici-repentis**. Sporulation was quantified on different cultivars, at different times after inoculation, and on leaves of different positions.

**MATERIALS AND METHODS**

**Preparation of inoculum and inoculation.** Four isolates of **P. tritici-repentis** (AUB II, MCR II, MCR VI, and PT-1C), collected from different fields of infected winter wheat across Kansas, were used in the studies. Fresh single-spore cultures of each isolate were established on slants of potato-dextrose agar and stored at 4 C. To produce spores for inoculation, isolates were transferred to plates with V8 juice agar and maintained at room temperature in the dark for 3-5 days. A 25-mm² mycelial plug then was transferred to a fresh plate of V8 juice agar and kept in the dark for 3-5 days. Aerial mycelium was knocked down with a sterile, bent glass rod (19), and plates were incubated for 24 h at 20 ± 1 C in the light (about 35 μmol m⁻² s⁻¹) for conidiophore formation and then for 12-24 h in the dark at 16 ± 1 C for conidiation (19). When conidia were formed, 10 ml of sterile distilled water (600 ml of water + one drop of Triton B-1956,
a surfactant) was added to each plate, and conidia were dislodged with a rubber policeman.

Plants were inoculated at the four-leaf stage. Equal quantities of suspensions (3 × 10^7 propogules per milliliter) of each isolate were mixed, and 2 ml per plant was applied with an atomizer at 60 kPa pressure. Inoculated plants were allowed to dry and placed in a plastic-covered mist chamber for 48 h. Mist was provided by centrifugal humidifiers operating for 1.5 min during every 10-min interval. After 48 h, plants were removed from the chamber and placed on greenhouse benches until leaves were harvested. During this time, pots were watered from the bottom to avoid additional leaf wetness beyond that provided in the mist chamber. Temperature in the greenhouse ranged from 15 ± 2 to 25 ± 2°C during all experiments.

**Induction of sporulation.** Preliminary experiments were conducted to determine temperature, moisture, and light conditions required for sporulation from host tissue. From the results of these experiments (M. Riaz, unpublished, 24), the following procedure was adopted. Four leaves, representing a replicate, were harvested, cut into 5-cm pieces, and placed in a glass petri dish lined with Whatman No. 1 filter paper. Initially, 2.5 ml of distilled water was poured onto the filter paper, with an additional 1.0 ml added after 24 h in some experiments. Leaves in petri dishes were incubated with alternating periods of 12 h light at 25 ± 1°C and 12 h dark at 16 ± 1°C for maximum sporulation. Light for each shelf in the incubator was provided by three 20-W cool-white fluorescent lamps suspended approximately 7.5 cm above the petri dish lid. Illumination was about 35 μmol·m⁻²·s⁻¹ when measured at leaf level.

**Recovery and enumeration of conidia.** After incubation, leaves from each replicate plate were cut into small pieces (about 1 cm) with scissors. Leaf pieces then were comminuted in a small blender (Waring model 91-264, Dynamic Corporation of America, New Hartford, CT) for 12–15 s in 15 ml of distilled water with one drop of Triton B-1956 per 500 ml of water to prevent clumping of conidia. After blending, two samples were taken using a Pasteur pipette and placed on a counting grid. Conidia were examined under a dissecting microscope at ×25 in six 0.01-ml volumes for each sample. There were no significant differences between analyses if spore production data were expressed on a total leaf area basis (length × width of blade) or per leaf basis; therefore, data were presented on a per leaf basis.

**Incubation time.** Optimum time to incubate leaves before recovering and counting conidia was evaluated by planting two seeds of the wheat cultivar TAM 105 (susceptible to *P. triticicoris*) in a steamed mixture of silky clay loam soil, peat, and vermiculite (1:1:1 by volume) in each of 30 plastic pots (225 ml). Plants were later thinned to one plant per pot, fertilized (20:20:20, N:P:K) at the three-leaf stage, inoculated at the four-leaf stage, and placed in the greenhouse for 10 days. The four inoculated leaves from each pot (representing a replication) were harvested and incubated under the conditions described above to induce sporulation. Experimental design in the incubator was a randomized complete block with six inoculation times (3, 4, 5, 6, 7, and 8 days) and five replications. After incubation, the number of conidia per leaf was determined as described above.

**Effect of host genotype.** Four winter wheat cultivars, TAM 105 (susceptible), Triumph (intermediate), Karl (resistant), and Auburn (resistant) were selected based on their known response to *D. tritici-repentis*. The four cultivars were compared at five harvesting times (3.75, 6.75, 9.75, 12.75, and 15.75 days after inoculation) for a total of 20 treatments. Experimental design in the greenhouse and incubator was a split plot with five replications. Cultivars were main plots and times of harvesting were subplots. Four leaves (one leaf was closest to the soil line) per replication per treatment were harvested on each date and incubated for 5 days under conditions described above. Conidia per leaf was the dependent variable.

**Leaf age.** An experiment was established in a split-split plot design with four replications. Main plots were two cultivars (TAM 105 susceptible and Auburn resistant), split plots were six harvesting times (2, 5, 8, 11, 14, and 17 days after inoculation), and split-split plots were three leaf positions (leaves two, three, and four). Six to seven seeds per pot (2.3 L) were sown in the soil mixture described previously and later thinned to four plants per pot. Plants were inoculated at the four-leaf stage, and fertilization and handling of pots were similar to the above experiments. Harvesting of leaves was started immediately after removal of pots from the mist chamber (2 days after inoculation). A replication consisted of four leaves (one from each of the four plants) from one of the leaf positions (leaf two, three, or four) from each pot on a particular harvest date. Immediately after harvest, leaves were incubated for 6 days to induce sporulation.

**Statistical analysis.** All experiments mentioned here were repeated at least once with similar results. Regression analyses were used to compare response curves. For linear models, statistics were calculated by the REG procedure of SAS (SAS Institute, Cary, NC). Estimates of slope parameters obtained for linear models were compared using the general linear models (GLM) procedure of SAS. Estimated Y intercepts of linear regression models with equal slope parameters were compared using the common slope model of GLM. Polynomial models and associated statistics were calculated for data that showed a nonlinear response with Graph PAD (ISI Software, Philadelphia, PA). Log transformations of the conidial counts did not consistently improve

![Fig. 1. Effect of days of incubation of detached leaves infected by Drechlera tritici-repentis in a conducive environment on number of spores produced per leaf. For experiment 1: \( Y = -2.214 + 1.385X - 120X^2 \) \((r^2 = 0.793, P < 0.005)\); for experiment 2: \( Y = -1.854 + 1.258X - 113X^2 \) \((r^2 = 0.915, P < 0.002)\).](image)

![Fig. 2. Effect of time after inoculation on number of spores per leaf produced by Drechlera tritici-repentis on four cultivars of winter wheat. Linear regression equations: TAM 105, \( Y = -2.222 + 292.7X \) \((r^2 = 0.710, P < 0.0001)\); Triumph, \( Y = -253.8 + 93.5X \) \((r^2 = 0.470, P = 0.0003)\); Karl, \( Y = -644.0 + 95.6X \) \((r^2 = 0.590, P < 0.0001)\); Auburn, \( Y = -410.9 + 72.8X \) \((r^2 = 0.583, P < 0.0001)\).](image)
RESULTS

After inoculation, the number and size of lesions on leaves were similar to what is seen in the field under moderate to severe epidemic conditions. Resistant cultivars, particularly their younger leaves, had restricted lesions compared with susceptible TAM 105. Three to 4 days after inoculation, small developing lesions first became visible. These lesions continued to develop for another 5–6 days whether leaves remained attached to the plant (incubation time experiments) or were placed in the incubator (host genotype and leaf age experiments). After this time, most leaves of TAM 105 were completely necrotic. No conidia were produced on infected leaves kept in greenhouse conditions. Sporulation from incubated leaves was primarily observed from the central one-half to three-fourths of the lesion area. When the fungus completely killed a leaf, sporation usually occurred where discrete lesions had been and not across the entire leaf surface.

Incubation time. Number of spores produced per leaf followed a significant \( (P<0.05) \) quadratic response with time of incubation (Fig. 1). No conidia were observed on infected leaves in petri dishes after 1 day of incubation and very few or no conidia were observed on leaves after 2 days. Significant numbers of spores were produced after leaves were incubated for 3 days in an environment conducive to sporation. Maximum spore production occurred between 5 and 6 days of incubation. Numbers of spores produced per leaf declined after 7 or 8 days.

Effect of host genotype. Significant \( (P < 0.0003) \) linear relationships occurred for all four cultivars when the number of spores produced per leaf was determined for leaves between 8.75 and 20.75 days after inoculation (Fig. 2). A few conidia were detected on all four cultivars on leaves 8.75 days after inoculation. The slope of the line for the susceptible cultivar (TAM 105 = 292.7 spores per leaf per day) was significantly higher than the slopes for all other cultivars. Estimates for the linear slope parameter for the intermediate (Triumph = 93.5) and two resistant cultivars (Karl = 95.6 and Auburn = 72.8) were not significantly different. Using the common slope model, the estimated \( Y \) intercept for Triumph was significantly higher \( (P < 0.0008) \) than those for Karl or Auburn, which were not different from each other \( (P = 0.2986) \).

Leaf age. In the leaf age experiments, a few spores were detected on leaves 8 days after inoculation (Fig. 3). This was true for all three of the leaf positions on Auburn as well as TAM 105. Few differences were detected in slopes or intercepts when comparing leaf positions for the susceptible cultivar TAM 105 (Fig. 3A, Table 1). Those that did occur were not consistent between runs of the experiment (data not shown). In contrast, leaf position had a significant effect on the slope of the regression line for the resistant cultivar Auburn (Fig. 3B, Table 1). On Auburn, the youngest leaf (leaf four) produced significantly fewer spores per unit of time than the other two older leaves of Auburn and all three leaves of TAM 105.

DISCUSSION

A procedure is described to quantify conidial production by D. tritici-repentis on recently detached leaves. Although measurement of sporulation on attached leaves may be more realistic, detached leaves allow for a high degree of environmental control, are easy to manipulate, and provide relatively greater precision in measurement of conidial production. Additionally, spore production from incubated leaves was similar in location (central

![Graph](image)

**Fig. 3.** Effect of time after inoculation on number of spores per leaf produces by *Drechslera tritici-repentis* on two cultivars and three leaf positions. Leaves counted from the soil line up on seedlings with three fully expanded leaves. Linear equations for A, cultivar TAM 105: leaf 2, \( Y = -1.004 + 185.2X \) \( (r^2 = 0.679, P < 0.0001) \), leaf 3, \( Y = -2.800 + 346.6X \) \( (r^2 = 0.619, P = 0.0003) \), leaf 4, \( Y = -2.653 + 292.2X \) \( (r^2 = 0.791, P < 0.0001) \); B, cultivar Auburn: leaf 2, \( Y = -2.800 + 246.1X \) \( (r^2 = 0.850, P < 0.0001) \), leaf 3, \( Y = -1.694 + 186.5X \) \( (r^2 = 0.713, P < 0.0001) \), leaf 4, \( Y = -262.6 + 29.2X \) \( (r^2 = 0.527, P = 0.0015) \).

<table>
<thead>
<tr>
<th>Cultivar, leaf number, slope</th>
<th>TAM 105</th>
<th>Auburn</th>
</tr>
</thead>
<tbody>
<tr>
<td>(leaf number)</td>
<td>2</td>
<td>3, 4</td>
</tr>
<tr>
<td></td>
<td>185</td>
<td>346, 292</td>
</tr>
<tr>
<td>Table 1. Probability values for comparing slopes of linear regression models for two cultivars of winter wheat and three leaf positions when regressing number of spores of <em>Drechslera tritici-repentis</em> per leaf on time after inoculating leaves.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*1 Leaf two, three, or four from the soil line.*

*2 Eight, 14, and 17 days after inoculation.*

*3 Slopes are given in number of spores per leaf per day and analysis was by the general linear models procedure of SAS.*
area of lesions) to sporulation on leaves in the field (W. W. Bockus, unpublished). The procedure simulated a conducive environment for sporulation and was useful to detect differences between host genotypes and leaf positions in the ability of the fungus to produce spores.

Once a leaf is thoroughly colonized, spores can be produced in as few as 3 days under proper conditions (Fig. 1). After placing infected leaves in a conducive environment, maximum spore production was obtained after 5 or 6 days. Unexpectedly, the numbers of conidia enumerated declined after this time. This may have been due to conidia that were produced during the early phase of the incubation period becoming fragile and being destroyed by the blending process. More likely, however, conidia that were produced several days earlier germinated, became distorted, and were not distinguishable from mycelial or conidiophore fragments during the counting process. Due to these results, we chose 5 or 6 days of incubation for all of the subsequent experiments.

In our experiments, D. tritici-repentis began to produce conidia 8 days (Fig. 3A) or 8.75 days (Fig. 2) after inoculation. Thus, under optimum conditions, the fungus can produce a secondary disease cycle about every 8 days. Furthermore, infected leaves of susceptible cultivars can produce up to about 4,000 conidia per seedling leaf. Larger leaves of adult plants may allow for even more conidial production per leaf in the field. However, the resistance response of wheat to D. tritici-repentis is similar in seedlings and adult plants (3). Consequently, we believe that differences observed in our experiments with seedling leaves should reflect the influence of host genotype on epidemics in the field.

Conidia were quantified at a single point in time for a given leaf. It is not known how many spores per leaf are produced if multiple harvests of conidia per leaf are taken into account. Similarly, no attempt was made in our experiments to correlate sporulation to the amount of necrotic or chlorotic leaf area or, perhaps more importantly, to the amount of tissue colonized.

Very few conidia were produced when leaves were harvested early (3.75 or 2 days) after inoculation (Figs. 2 and 3). At this time, fungal colonization of leaves is not sufficient to allow the physiological or spatial requirements to initiate reproduction. Other results presented here support this hypothesis since there was a positive linear relationship between days after inoculation and number of spores produced per leaf. The longer the fungus was allowed to colonize the leaf in the greenhouse, the more conidia were produced in the incubator. This relationship would only hold up to a point as evidenced by the fact that, for the leaf age experiments, harvest days 14 and 17 were outside the linear part of the curve. Before these later dates, the fungus had probably thoroughly colonized the leaves; in fact, most infected leaves were completely necrotic. In the older leaves of Auburn produced significantly more conidia than younger leaves. Because younger leaves are more resistant to the fungus (3.9, 19, 30), less colonization had occurred (15). Older leaves are nearer to their natural senescence, and the fungus is probably better able to infect these leaves, cause their premature death, colonize them, and produce spores. It is not clear whether enhanced sporulation on older leaves is due to increased physiological reproduction capability of the fungus or to increased infection efficiency and tissue colonization.

Results presented here showed substantial differences between wheat cultivars in the ability of D. tritici-repentis to produce spores. On the susceptible cultivar TAM 105, the fungus sporulated more rapidly, with a greater slope, as compared with the resistant cultivars Karl or Auburn. The youngest leaves of Auburn contributed the most to these differences. There was about 6-0.12X greater slope for leaves of TAM 105 or old leaves of Auburn compared with young leaves of Auburn. Based on results presented here, cultivars selected for resistance under experimental conditions using small plots (3,9,13,15,16,19,22,26) may perform better than expected when planted on larger areas. In plots, spores produced on susceptible cultivars would result in more inoculum being dispersed onto neighboring resistant cultivars than would occur in a commercial situation (31). Differences in sporulation of the magnitude reported here should result in greatly reduced secondary spread of the pathogen in fields planted to resistant cultivars when compared with those planted with susceptible ones.

LITERATURE CITED
