Gradients of Tan Spot of Winter Wheat from a Small-Area Source of Pyrenophora tritici-repentis

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ABSTRACT

Wheat plots (15.2 X 15.2 m) were established near Manhattan and Hesston, Kansas, to measure changes in area under the disease progress curve (AUDPC) with distance from a small-area (0.6 X 0.6 m) source of Pyrenophora tritici-repentis. At both locations during 2 yr, relatively steep gradients of tan spot occurred away from the primary inoculum. Exponential decay equations significantly (P < 0.0001) fit data and estimated that a 90% reduction in AUDPC occurred at 3.6-5.4 m from the source. These results indicate that disease spread by primary and secondary inoculum is limited in Kansas, and that fields where P. tritici-repentis does not occur will not be affected greatly by neighboring diseased fields. According to data obtained in vitro and in the greenhouse, high temperatures (32.5 and 40 C) that can occur in the spring in Kansas do not have a large effect on conidial germinability; however, temperatures of 40 C for 8 hr per day for 2 days immediately after inoculation significantly reduced disease severity. Thus, high temperature may be partially responsible for the relatively limited disease spread in Kansas but is probably not a major factor.

Tan spot, caused by Pyrenophora tritici-repentis (Died.) Drechs. (ana- morph = Drechslera tritici-repentis (Died.) Shoemaker), is an economically important disease of winter wheat (Triticum aestivum L.) in many regions of the world (22). Synonyms for tan spot are yellow leaf spot, yellow leaf blotch, leaf blotch, wheat leaf blight, and eye spot (6). Tan spot was first reported in Kansas in 1947, and its severity has increased until it is one of the most important wheat diseases in that state. Grain yield losses of up to 40% from tan spot have been reported (3). One of the main reasons for the increase of tan spot in Kansas is the adoption of minimum- and no-till crop management practices, which leave more wheat residue on the soil surface (1,4,9).

The fungus survives between crops of wheat in infested wheat residue, saprophytically colonizing the tissue. Ascomycetes are initiated on wheat straw on or above the soil surface in the summer or fall, and they mature after a cold period early in the following spring (1,23). When moisture is present, usually from spring rains, the primary inoculum (ascospores) is forcibly ejected and then deposited onto wheat leaves. Ascospores do not move very far; Rees (10) reported an 80% reduction in primary infections 10 m from infested wheat stubble. Similarly, Schilder and Bergstrom (13) found a 90% reduction in ascospores trapped 0.2 m from a source of primary inoculum compared with the number trapped 0 m from the source. In Kansas, the first symptoms of the disease are usually visible in the middle of March or early April (erection of the pseudostem, Feekes scale growth stages [GS] 4-5). Depending on the host genotype, lesions may be chlorotic, necrotic, or both (7). Conidiophores and conidia are then produced from lesions or from dead, infected leaves and serve as secondary inoculum. Conidia are reported to be windblown for longer distances (100 m) (13) than ascospores, and infections by conidia cause most of the loss (14). With prolonged rains or dew, the disease spreads from lower to upper leaves, ultimately causing death of infected leaves and limiting yields by reducing seed number and size (5,14).

Crop rotation and burning or incorporation of crop residue into soil have been shown to control tan spot (4,17-19). When the wheat straw is destroyed or buried, pseudotheca of the fungus are not produced (8). These management practices reduce primary inoculum within a given field; however, they would have no effect on secondary inoculum produced in adjacent fields of continuously cropped, reduced-tillage wheat. Therefore, the potential exists for the pathogen to spread from neighboring fields and negate the benefit obtained from rotation, residue burial, or residue destruction from burning. The experiments reported here were conducted to quantify tan spot spread from a small-area source of P. tritici-repentis. The ultimate objective was to determine if neighboring diseased fields might affect fields that are free of tan spot. This information would contribute to understanding pathogen ecology, dispersal of new strains, timing of damage to wheat plants, and proper timing of disease controls.

MATERIALS AND METHODS
Field experiments. The winter wheat cultivar AGSECO 7837 was seeded (67 kg/ha) the first week in October at two locations during the 1991-92 and 1992-93 winter wheats seasons. Standard fertilizer regimes for the areas were used. The cultivar was chosen because it is resistant to prevalent races of the leaf rust pathogen (Puccinia recondita Roberge ex Desmaz. f. sp. tritici (Eriks & E. Henn.) D.M. Henderson) in Kansas but susceptible to tan spot. One location was at the Rocky Ford Experimental Farm near Manhattan, Kansas, on a Chase silty clay loam; and the second location was at the Harvey County Experimental Field near Hesston, Kansas, on a Ladysmith silty clay loam. The sites at the Manhattan location had been fallowed the previous year, and the previous crop at the

Fig. 1. Plot design of field experiments to measure the spread of tan spot of winter wheat from a small-area source of Pyrenophora tritici-repentis. Dotted lines delimit individual plots (15.2 m square), solid lines are 0.61-m mowed alleys dividing plots into four quadrants (1, 2, 3, and 4), and x = 12.5 g of oat-kernel inoculum of P. tritici-repentis.
Heston location was spring oats both years. In Kansas, pseudotheica in the field do not produce ascospores for more than one year (W. W. Bockus, unpublished); therefore, the sites were presumed free of *P. tritici-repentis*. All sites were at least 50 m from other wheat fields, and none of the other fields were under reduced tillage. To eliminate any possible seedborne inoculum (12), seed was treated with captan (Captain 400D, 1.3 ml/kg) and triadimenol (Baytan 30F, 0.82 ml/kg).

Plots were arranged in a north–south orientation using a randomized complete-block design with four replications and two treatments (inoculated and non-inoculated). Wind direction can oscillate but is primarily from the southwest during early fall, late spring, and summer, and from the northwest during late fall, winter, and early spring. Plot size was 15.2 × 15.2 m, and two 0.61-m alleys intersected at right angles at the center of each plot (Fig. 1). Alleys were maintained by periodic mowing to produce four quadrants in each plot and allowed for rating of plants without excessive disturbance of the wheat canopy.

Inoculum for the field experiments consisted of autoclaved oat kernels (50 g per inoculated plot = replication) infested with *P. tritici-repentis* and spread on the soil surface. Inoculum (12.5 g) was applied in a 0.3 × 0.3 m area in early November in the extreme central corner of each quadrant (Fig. 1); noninoculated plots received autoclaved oat kernels not infested by *P. tritici-repentis*. The fungus produced pseudotheica and mature ascii on infested kernels during the fall and winter, which initiated the tan spot epidemic in the following spring (9).

**Disease assessment.** Tan spot was evaluated seven times at intervals of about 2 wk at the Manhattan site starting 20 March 1992 and 30 March 1993, and at the Heston site starting 13 March 1992 and 23 March 1993. Disease severity was rated along the alleys in four directions (north, south, east, and west) at 0, 1.52, 3.05, 4.57, and 6.10 m from the center (inoculum) of each plot. The leaves were scored for percent leaf area affected by chlorosis and necrosis, and placed into one of the following categories: 0, 1, 5, 10, 25, 50, 75, or 100% of the leaf area affected (4). At each evaluation time, the top three fully expanded leaves of 10 plants per direction per distance were rated. The rating times corresponded to the following growth stages (Feeskes scale): leaf sheath strongly erected (GS 5), first node of stem visible (GS 6), second node visible (GS 7), flag leaf emerging (GS 8), boot (GS 10), flowering–watering ripe (GS 10.5–10.5.4), and milk–soft dough (GS 11.1–11.2).

**Statistical analysis of field data.** Average disease severities were calculated from the ratings of all 30 leaves. The area under the disease progress curve (AUDPC) for each replication for each direction and each distance from the inoculum was calculated by Shaner and Finney's method (15). Adjusted AUDPCs also were calculated by subtracting values for the noninoculated plot from those of the corresponding inoculated plot in that replication. After attempting to fit other models (linear, quadratic) to the data, the exponential decay equation \[ Y = A \times \exp(-B \times X) \] was used to model the change in AUDPC with distance from the inoculum, where \( Y = \) AUDPC, \( X = \) distance from primary inoculum in meters, \( A = \) intercept, and \( B = \) decay parameter. Nonlinear regression involving iterations (GraphPAD, ISI Software, Philadelphia, PA) was used to generate exponential equations that best described the data. Using the equations for each location–year, distances were estimated that would produce 50, 90, or 99% reduction in AUDPC compared with the 0 distance.

**Conidial germinability after exposure to high temperatures.** Field data collected in the first year indicated that the disease did not spread very far from an inoculum source. One possible explanation could be rapid inactivation of conidia (secondary inoculum) by relatively high

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**Fig. 2.** Area under the disease progress curve (AUDPC) regressed against distance from a small-area source of *Pyrenophora tritici-repentis* at two locations in Kansas during 1992. The exponential equations for the regression lines are (A) \( Y = 1.516 \times \exp(-0.4863 \times X) \) (standard error for A parameter \( \text{SE}_A = 52.5 \), standard error for B parameter \( \text{SE}_B = 0.034 \), \( R^2 = 0.859, P < 0.0001 \) for the Manhattan location and (B) \( Y = 1.254 \times \exp(-0.6381 \times X) \) (SE\(_A\) = 52.5, SE\(_B\) = 0.059, \( R^2 = 0.804, P < 0.0001 \)) for Heston.
air temperatures that occur in Kansas during the spring. The months of May and June average 7 and 20 days, respectively, with maximum temperatures >30°C. Therefore, the following experiments were conducted to determine the effect of high temperatures on conidial germination and infectivity.

Three isolates of *P. triticici-repentis* (MCR-6, FH-86, and AUB) collected from infected wheat leaves in Kansas were selected for experiments in vitro and in the greenhouse. Conidial inoculum was produced by transferring a mycelial plug of the fungus to plates containing V8 agar (11). The plates were incubated for 4–5 days in a plastic box (21 ± 2°C) lined with aluminum foil to exclude light. Aerial mycelium then was knocked down with a sterile, bent-glass rod, and plates were placed 20 cm below four fluorescent lights (30 W, cool white) for 12–24 hr (21 ± 2°C) to produce conidiospores. These conidiospores were transferred to the dark at 15°C for 12–24 hr to obtain conidia. After conidia formed, the agar plates were air dried for 12 hr in a sterile, laminar-flow hood and stored at 4°C until used.

To prepare spore suspensions, dried agar disks containing conidia were cut with sterile scissors into pieces about 0.5–1 cm square. The dried agar and conidia from two plates were transferred into a 25 × 200 mm test tube with 25 ml of sterile distilled water, and suspensions were made by vortexing. Then, 0.2 ml of the suspension was spread onto each of 12 plates containing water agar with two antibiotics (ampicillin and oxytetracycline hydrochloride) each at about 50 ppm. To simulate different diurnal temperatures that can occur in the spring in Kansas, four plates were inoculated at each of three temperature regimes: 25/25°C, 32.5/25°C, and 40/25°C (12/12 hr, light/dark). Germination rates were determined under a dissecting microscope by observing 25 conidia per plate after 1, 2, 4, and 8 days incubation. This experiment was conducted three times.

**Disease severity after exposure to high temperatures.** Inoculum suspensions were made as described above. Six seeds of the wheat cultivar AGSEO 7837 were planted in each of 30 pots (7 × 7 × 6 cm), and plants were grown to the four-leaf stage in the greenhouse (20 ± 5°C). The experiment was arranged in a split-plot design with six treatments and five replications (pots). Temperature treatments (25/25°C, 32.5/25°C, and 40/25°C for 8–16 hr, respectively, and 12/12 light/dark) were used as main plots, and presence and absence of inoculum were subplots. Plants in each inoculated pot were sprayed with 2 ml of spore suspension (about 5 × 10⁷/ml) and allowed to dry for 20–30 min so conidia would adhere to the leaves. The pots and leaves then were wrapped with paper towels moistened with distilled water and placed into plastic bags. Distilled water was sprayed liberally onto the inside surface of the plastic bags. The bags were closed and placed in incubators for 2 days at the temperatures described above. After 2 days, plants were taken out of the plastic bags and placed in a randomized complete-block design in the greenhouse.

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**Table 1.** Predicted distances (m) from a small-area source of *Pyrenophora triticici-repentis* resulting in 50, 90, or 99% reductions in area under the disease progress curve (AUDPC) for tan spot of wheat compared with the 0 distance during four location-years.

<table>
<thead>
<tr>
<th>Location-year</th>
<th>AUDPC (0-m)</th>
<th>Reduction %</th>
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<tbody>
<tr>
<td>Manhattan–1992</td>
<td>1.516</td>
<td>1.43a  4.73  9.47</td>
</tr>
<tr>
<td>Hesston–1992</td>
<td>1.254</td>
<td>1.09  3.61  7.22</td>
</tr>
<tr>
<td>Manhattan–1993</td>
<td>1.834</td>
<td>1.62  5.37  10.74</td>
</tr>
<tr>
<td>Hesston–1993</td>
<td>1.035</td>
<td>1.20  3.98  7.96</td>
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*Values (in meters) were calculated from exponential decay equations of AUDPC regressed against distance for the four location-years.*
(21 ± 6°C, natural light). Tan spot severity was rated at 2-day intervals from 2-14 days after inoculation, according to the scale described above. This experiment was conducted twice.

Statistical analyses of in vitro and greenhouse data. Mean percent germination and severity scores were calculated for each replicate. An arcsine square-root transformation of germination percentages was used in data analysis. Mean values of transformed data were separated by analysis of variance (ANOVA) followed by LSD (P = 0.05). Infectivity data were analyzed by regressing disease severity against time and fitting linear models to the data. Slopes and estimates of the intercepts were compared (P = 0.05) for lines generated by the different temperatures.

RESULTS
Spread of tan spot in the field. The environment was conducive to development of tan spot at both sites during both years (3; W. W. Bockus, unpublished). Powdery mildew (caused by Blumeria graminis (DC.) E.O. Speer) occurred early in the season at the Heston site during 1992 but did not progress after flag leaf emergence (GS 8) and was confined to the lower leaves. Speckled leaf blight (caused by Septoria tritici Roberge in Desmaz.) was prevalent at Heston throughout 1993. Besides tan spot, no significant foliar diseases were noted in either year at the Manhattan site.

Heavy fall rains at Heston during the 1992-93 crop year washed some oat kernel inoculum along the drill rows to the north and south. For this location and year, only the east and west directions were used in the analysis. Significant interactions for direction (north, south, east, and west) occurred only during 1992 at the Heston site. These were due to relatively minor increases in AUDPCs (decay parameter) in the north compared with the other directions; therefore, data from all directions for each location and year were combined for analysis.

When the exponential association was used to model changes in AUDPC with distance from primary inoculum for the inoculated plots by themselves, asymptotes occurred at relatively high values (867-2,171, not shown). These values were due to leaf chlorosis and necrosis from causes besides tan spot, including senescence, powdery mildew at Heston in 1992, and speckled leaf blight at Heston in 1993. However, when corresponding direction-distance values for the noninoculated plots first were subtracted from the inoculated, asymptotes approaching 0 were achieved (Figs. 2 and 3). This allowed modeling of tan spot effects by themselves and made it easier to estimate distances where 50, 90, and

<table>
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<tr>
<th>Table 2. Germination (%) of Pyrenophora tritici-repentis conidia in vitro at 40/25 C (12/12 hr) after 1, 2, 4, or 8 days</th>
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<tr>
<td>Days</td>
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<tr>
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</tr>
<tr>
<td>1</td>
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<td>2</td>
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<td>4</td>
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<td>8</td>
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*AVERAGE percent germination of 25 conidia on four replicates of water agar containing two antibiotics. Data were arcsine square root transformed for analysis and back transformed for presentation. Values in a column followed by a common letter are not significantly different according to analysis of variance followed by LSD (P = 0.05). 

Fig. 4. Tan spot severity on leaves of winter wheat in the greenhouse after exposure to 25/25 C, 32.5/25 C, or 40/25 C (8/16 hr) during the first 48 hr of the infection process. Linear regression lines are (A) Y = 8.43X - 12.49 (R² = 0.7671, P = 0.0001) for 25/25 C, Y = 7.60X - 13.77 (R² = 0.9168, P = 0.0001) for 32.5/25 C, and Y = 5.89X - 20.76 (R² = 0.9663, P = 0.0001) for 40/25 C and (B) Y = 3.49X - 19.71 (R² = 0.8269, P = 0.0001) for 25/25 C, Y = 2.07X - 13.49 (R² = 0.6411, P = 0.0001) for 32.5/25 C, and Y = 0.46X + 0.29 (R² = 0.3201, P = 0.0032) for 40/25 C.
99% reductions in AUDPC occurred. Subtraction resulted in some negative AUDPC values for individual data points, but the means for a given distance and the curves remained positive.

For all four experiments, maximum AUDPC values occurred 0 m from the inoculum source and declined sharply as distance from the source increased. Simple exponential decay equations significantly ($P < 0.0001$) fit data from all locations and years. Distances from primary inoculum that would result in 50, 90, and 99% less AUDPC were calculated using these equations (Table 1).

**Effect of temperature on conidial germination.** Virtually all conidia germinated on agar after 1 day of incubation at 25/25 or 32.5/25 C. At 40/25 C, there was a significant delay in germination with relatively few germinated conidia on days 1 or 2 (Table 2). However, after 4 or 8 days incubation at 40/25 C, germination was 100%.

**Effect of temperature on disease severity.** In the greenhouse experiments, tan spot developed only on plants in inoculated pots. However, at 40/25 C, and to a lesser extent at 32.5/25 C, there was some chlorosis and necrosis on leaves in noninoculated pots due to high-temperature injury, although the affected area did not exceed 15%. Therefore, to analyze only the effect on tan spot, ratings for noninoculated pots were subtracted from those for inoculated pots before analysis.

Linear models significantly ($P < 0.05$) fit increases in disease severity with time for the three temperature regimes (Fig. 4). For the first experiment, the slope of the line for the 40/25 C treatment was lower ($P = 0.0129$) than that for the 25/25 C treatment (Fig. 4A). The slopes of lines for the 25/25 and 32.5/25 C treatments were similar; however, estimates of intercepts for the 25/25 C regime were significantly ($P = 0.0031$) higher than those for 32.5/25 C. Similarly, the intercept estimates for 32.5/25 were higher ($P < 0.0001$) than those for 40/25 C. Similar results were obtained from the repeat experiment, except that the slopes for all lines were significantly different (Fig. 4B).

**DISCUSSION**

Our results indicate that tan spot in Kansas has a relatively steep gradient away from a small-area source of primary inoculum. Direction did not have an effect on the gradient. Exponential decay curves for AUDPC regressed against distance were similar in shape to curves reported for dispersal of ascospores and conidia (13). Schilder and Bergstrom (13) reported that 60–100% of conidia were deposited on sticky slides within 25 m of the inoculum source. Results here showed a somewhat steeper disease gradient, with 90% reduction in AUDPC at 3.6–5.4 m from the source.

Conidia of P. tritici-repentis have been reported to move for relatively long distances (100 m [13], 80 km [16]). Because tan spot is a multiple-cycle disease, such dispersal would be expected to result in a flatter disease gradient than we measured. Possible explanations for the steep disease gradient are rapid dilution of inoculum (13) coupled with poor leaf deposition, infection efficiency, and/or relatively few secondary cycles. The deposition or infection efficiency of conidia onto leaves is not known. Under optimum conditions, P. tritici-repentis can complete a cycle in as few as 8 days (11); however, it is unlikely that cycles are completed that rapidly in the field. Another contributing factor may be that relatively few conidia of P. tritici-repentis are produced and dispersed from an infected leaf (11) compared with other pathogens causing multiple-cycle diseases. For example, long-distance spore dispersal of the cereal rust fungi is known to be important for epidemics (2,21). Also, urediniospores of rust fungi can increase up to 10,000-fold (20,22), which is many orders of magnitude greater than the increase for P. tritici-repentis (11).

Germinability of conidia on agar was not affected by exposure to 32.5 C for 12 hr per day. However, exposure to 40 C delayed germination for 3–7 days compared with 25 C. Additionally, during the first 48 hr after inoculation, significantly less tan spot developed in the greenhouse on plants exposed to 32.5 or 40 C for 8 hr per day compared with plants at 25 C. Therefore, the relatively high temperatures that can occur in Kansas in the field may adversely affect the spread of tan spot and contribute to the observed steep disease gradients. Further research is necessary to quantify conidial germinability and disease severity with high temperatures under field conditions. However, we believe that the negative effects of high temperature on disease are relatively minor and should not be major factors limiting disease spread in the field.

In our experiments, tan spot did not spread very far from an area source of P. tritici-repentis inoculum. If long-distance dispersal of conidia occurs under Kansas conditions, it is of little consequence epidemiologically. However, only a small-area source was used in these experiments, and the strength of the inoculum source is an important component to conidial spread (13). A large-area source might result in flatter disease gradients than we observed. Nevertheless, during a 4-yr experiment in Kansas, no interplot interference was observed between 9.1 × 15.2 m no-till and rotated plots separated by only 10.7 m (4). Therefore, we believe that fields in Kansas that are free of P. tritici-repentis as a result of crop rotation or residue destruction will not be significantly affected by neighboring fields that have inoculum of the fungus.

**LITERATURE CITED**

and host effects on latent and infectious periods and on urediniospore production of *Puccinia recondita* f. sp. *tritici*. Phytopathology 73:414-419.

