The Effect of Primary Inoculum Level of *Pyrenophora tritici-repentis* on Tan Spot Epidemic Development in Wheat

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ABSTRACT

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In field experiments in two crop years, three levels of primary inoculum of *Pyrenophora tritici-repentis*, covering approximately a 100-fold range in residue-borne ascocarp numbers, were applied to wheat (*Triticum aestivum*) plots in the fall. After the appearance of initial infections in the spring, tan spot severity was rated every 5–8 days until leaf senescence, and disease progress curves were constructed. There were significant positive relationships between the area under the disease progress curves and the level of primary inoculum for both years, even though weather conditions were very different, 1987 being very wet and 1988 very dry. Statistical analysis indicated, however, that the quantitative effect of local primary inoculum on epidemic development differed between the two years; thus, the importance of local primary inoculum, relative to incoming secondary inoculum, is likely to depend on the particular conditions of the epidemic. The highest level of local primary inoculum significantly reduced yield, but confounding factors (rust infection and drought) made the overall relationship difficult to demonstrate. The experiments indicated that pathogen control measures that reduce residue-borne primary inoculum can decrease epidemic development and crop damage, despite multiple infection cycles caused by wind-disseminated secondary inoculum.

Additional keywords: reduced tillage, yellow leaf spot.

Pyrenophora tritici-repentis (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.) Shoemaker) is the causal organism of tan spot disease on wheat (*Triticum aestivum* L.) (6), epidemics of which can cause up to 49% yield loss (11). Tan spot is increasing in importance with the growing emphasis on conservation tillage practices for erosion control (3,14). This is because *P. tritici-repentis* can survive saprophytically between cropping seasons in infested residue on or above the soil surface (5,8).

Ascocarps that are formed on infested residue release ascospores (primary inoculum) in the spring. Following primary infection, conidia are produced on lesioned tissue and are disseminated by wind to cause secondary infection cycles. Observations of spore types associated with tan spot lesions on winter wheat in Kansas (W. W. Bockus and P. J. Raymond, *personal communication*) indicate that ascospores are responsible for primary infections but become unimportant sometime in early May, as conidia (secondary inoculum) become the predominant propagules in the epidemic. Rees and Platz (10) came to the same conclusion from a study of propagules associated with tan spot epidemics in

Australia.

Other researchers have shown that increased amounts of residue infested with *P. tritici-repentis* can increase disease intensity (4,11), with subsequent reductions in yield (11). However, the quantity of inoculum itself (number of ascocarps per gram of infested straw) was not measured in these studies. Knowledge of the concentration of ascocarps necessary to reduce yield is important in evaluating control methods designed to reduce the number of ascocarps produced on residue. In using wheat residue for erosion control in a monoculture, it would be desirable to lower the inoculum potential to a level at which there is no significant risk of yield loss from tan spot while still retaining sufficient residue for erosion control.

The purpose of this field experiment was to determine the number of ascocarps per square meter necessary to significantly increase disease severity, as measured by the area under the disease progress curve (AUDPC), and reduce yield compared to plots containing no primary inoculum.

MATERIALS AND METHODS

In 1986 and 1987, straw naturally infested with *P. tritici-repentis* was collected in early July and stored indoors (temperature 24–32 C, relative humidity 40–85%) in burlap bags during the summer. From 8 September to 14 November, the straw was kept outdoors on a wire net frame 5 cm above the ground where it would be exposed to light and moisture early in the fall to initiate ascocarp development. The heads, sheaths, and leaves were then discarded, and the amount of inoculum on the straw was measured by counting the number of mature ascocarps (those longer than 300 μm) on the culm tissue. Previous work (7) had suggested that smaller ascocarps contain few or no ascospores.

In the 1986–1987 cropping year, field plots at Rocky Ford Experimental Farm in Manhattan, KS, were covered with 97, 9.7, 0.97, or 0 g of infested straw per square meter; these residue levels are similar to those reported by other investigators (4,11). Because the residue averaged 260 ascocarps per gram, the treatments were equivalent to approximately 25,000, 2,500, 250, and 0 ascocarps per square meter, respectively.

For the second field experiment (1987–1988 cropping year), the levels of infested residue used were 95.2, 5.5, 0.5, and 0 g/m^2. The straw supported 1,050 ascocarps per gram of culm, so the ascocarp levels per square meter were 100,000, 5,000, 500, and 0, respectively.

The total amount of residue in each plot (including checks) was brought to 100 g/m^2 using oat straw not infested with the pathogen. The oat straw and infested wheat straw were spread on the plots in the first half of December in both years. Microscopic examination of 100 ascocarps sampled from the straw in March of each year showed that approximately 85 and 65% of the ascocarps contained ascospores in 1987 and 1988, respectively.

The plots were arranged in a randomized complete block design with three replications. One block did not have a control treatment the first year because of lack of space. In 1988, a low-inoculum plot was damaged by animals and was not included in the analyses. Because of these missing values, the data were analyzed by using the GLM procedure of SAS (SAS Institute Inc., Cary, NC), in which marginal means are unweighted.

The plots measured 1.5 × 1.5 m (six rows of wheat) the first year and 1.2 × 1.8 m (five rows of wheat) the second. A 3-m border of wheat (same cultivar as within the plot) immediately surrounded each plot. More than 13 m of fallow ground separated these bordered plots from each other. The winter wheat cultivar Arkan (PI 475771) was planted the first year. Rohm and Haas 7837 (Rohm and Haas Seed, Inc., Mt. Hope, KS) was planted the second year for improved resistance to leaf rust (caused by *Puccinia recondita* Rob. ex Desm. f. sp. *tritici*). These two cultivars are similar in stature, maturity, and susceptibility to tan spot (13). Plots were planted 25 September 1986 and 6 October 1987 with a five-row hoe drill at 23-cm row spacing.

Disease was rated every 5–8 days after the appearance of initial tan spot infections in the spring. The rating system assessed disease severity based on both the quality (necrosis, chlorosis) of the lesion and the area of infected tissue per leaf (9): 0 = no disease; 1 = very small lesions or flecks, less than 10% of leaf area infected; 2 = necrosis with yellow halo, less than 10% of leaf area infected; 3 = lesions coalesced, 10–50% of leaf area infected; 4 = 50–100% of leaf area infected, with some green tissue left; 5 = total leaf senescence. Isolations from individual lesions were made periodically to confirm that lesions were caused by *P. tritici-repentis*.

For sampling, each row was divided into sections 7 cm long, and two sections were randomly selected from each of five rows in a plot. Two plants from each section were randomly chosen for severity ratings, providing a total of 20 plants per plot at each sampling date. The top two leaves present at each sampling date were rated. Thus, the severity rating at each date indicates the degree of damage to the leaves most active in photosynthesis at the time; the third leaf from the top at any date was generally somewhat senescent. Severity ratings were taken from 19 March (wheat growth stage 3 [15]) to 12 May (stage 10.5.4) the first year and from 5 April (stage 4) to 26 May (stage 10.5.4) the second year. In the first year, an epidemic of leaf rust caused early senescence of the leaves; after 12 May it was difficult to distinguish tan spot infections from other chlorotic symptoms.

To estimate the leaf area colonized by *P. tritici-repentis* near the end of the season, five penultimate and five flag leaves were randomly sampled at mid-dough stage (stage 11.2 [15]) from each replicate plot on 6 and 7 June 1988, respectively, about 10 days after the final disease severity rating for these leaves. Each leaf was surface-sterilized, cut into 1-cm lengths (10 segments alternately selected), and plated onto modified *Septoria* symptomless agar (1) (10 g of agar, 200 mg of chloramphenicol, and 0.42 mg of triphenyltin hydroxide per liter). This medium stimulates conidium production in *P. tritici-repentis* and reduces interfering mycelial growth of other fungi (E. A. Adee and W. F. Pfender, *unpublished observation*). After 1 wk of incubation at 23 C (12-hr photoperiod), leaf segments were observed under a dissecting microscope at 50× for the presence of conidia and/or ascocarps of *P. tritici-repentis*. The percentage of segments bearing the pathogen was taken as an indicator of the relative extent of colonization of the leaf.

Individual plots were harvested by hand on 15 June 1987 and 13 June 1988. Total grain weight and 1,000-kernel weight were recorded for each plot. The ratings for disease severity on the top two leaves were plotted against time for the duration of the rating period, and the AUDPC values for leaves 1 and 2 were averaged. The AUDPC values, yields, and 1,000-kernel weights were analyzed by analysis of variance. Relationships of inoculum levels to AUDPC, yield, and 1,000-kernel weight were also analyzed using linear regression (SAS Institute) for each year individually. To obtain a linear relationship amenable to linear regression analysis, the values for inoculum level and AUDPC were transformed to their logarithm values. Scatterplots of residuals against predicted values were inspected to verify that there were no systematic patterns to the residuals.

RESULTS

Disease progress curves for 1987 (Fig. 1A) and 1988 (Fig. 1B) demonstrate the influence of the initial inoculum level on tan spot development and also show that the disease was more severe in 1987 than in 1988. In 1987 the AUDPC values for the medium- and high-inoculum treatments were significantly greater than that for the control treatment (Table 1). In 1988, only the high-inoculum treatment had a significantly greater AUDPC value than the control level of inoculum. Average yields for the medium-, low-, and control-inoculum treatments in 1987 were similar; yield from the high-inoculum treatment was significantly less than that in the check plot (Table 1). In 1988, the average yield in control plots was greater than in the medium- and high-inoculum treatments but was not significantly different from the low-inoculum treatment. No significant difference in 1,000-kernel weights was found among treatments in either year (data not

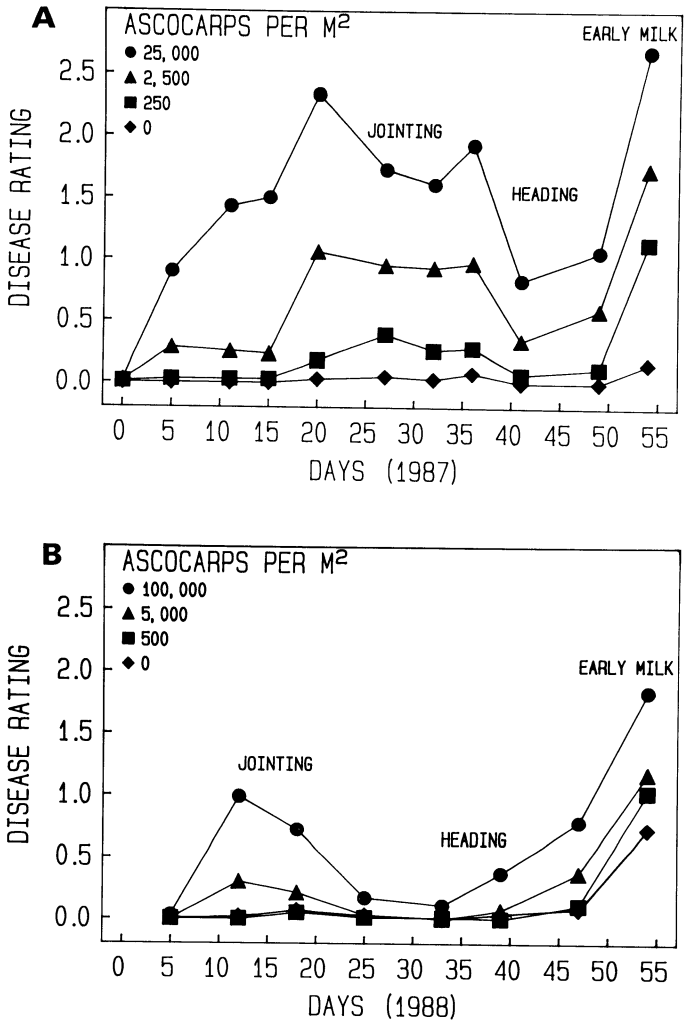

Fig. 1. Tan spot epidemic development in **A,** 1987 and **B,** 1988 as affected by primary inoculum level. Average rating (on a scale of 0 to 5, where 0 = no disease and 5 = leaf senescence) of top two leaves of winter wheat plants at each rating period. Wheat growth stages during the epidemic were jointing (stages 6–7) at days 27–32 (1987) and days 12–18 (1988), heading (stages 10.1–10.5) at days 41–49 (1987) and days 33–39 (1988), and early milk (stage 11.1) at day 54 (both years).

TABLE 1. Area under the disease progress curve (AUDPC) and yield of wheat plots inoculated with primary inoculum of *Pyrenophora tritici-repentis*

Primary inoculum level[a]	AUDPC[b]	Yield (kg/ha)[b]
1987		
25,000	76.5*	861*
2,500	34.6*	1,264
250	10.8	1,231
0	1.3	1,177
1988		
100,000	24.0*	3,941*
5,000	11.0	4,001*
500	5.2	4,304
0	4.9	4.385

[a] Ascocarps per square meter applied to plots.
[b] Numbers followed by an asterisk are significantly ($P = 0.05$) different from the uninoculated treatment according to Dunnett's procedure.

shown).

The logarithm of AUDPC (LAUDPC) and the logarithm of primary inoculum (LINOC) were linearly related in both years (Fig. 2). The regressions explained 99 and 73% of the variability for 1987 and 1988, respectively. The two regression lines differed significantly ($P = 0.01$) in both slope and intercept. The relationship between yield (kg/ha) and LAUDPC was not significant in 1987 but was significant ($P = 0.08$) in 1988. There was no significant relationship between yield or 1,000-kernel weight and LINOC in either year. Combining the data from both years for analysis did not improve any of the relationships.

The estimated percentage of wheat leaf tissue colonized by *P. tritici-repentis* near the end of the season in 1988 varied directly with the initial inoculum level (Table 2). The medium- and high-inoculum treatments resulted in significantly higher infection levels on leaf 1 than the control treatment. On leaf 2, only the high-inoculum treatment resulted in a significantly higher percentage of colonization than the uninoculated treatment. The linear regressions of percent colonization on logarithm of initial inoculum level were significant for each leaf position (Table 2). The estimated percentage of colonization was correlated also with the final disease severity readings (for leaf 1, $r^2 = 0.39$, $P = 0.04$; for leaf 2, $r^2 = 0.50$, $P = 0.016$), which supports the validity of the visual assessment of disease severity.

TABLE 2. Colonization of winter wheat leaf tissue by *Pyrenophora tritici-repentis* at growth stage 11.2 in 1988

Primary inoculum level[a]	Colonization intensity[b]	
	Flag leaf	Penultimate leaf
100,000	70*	87*
5,000	68*	48
500	66	44
0	39	25
Regression[c]		
Adjusted r^2	0.57	0.64
Probability $> F$	0.007	0.003

[a] Ascocarps per square meter applied to plots.
[b] Average percentage of leaf segments yielding *P. tritici-repentis* when plated on agar medium. Numbers followed by an asterisk are significantly ($P = 0.05$) different from the uninoculated treatment according to Dunnett's procedure.
[c] Linear regression analysis of colonization intensity versus the logarithm of the primary inoculum level for each leaf position.

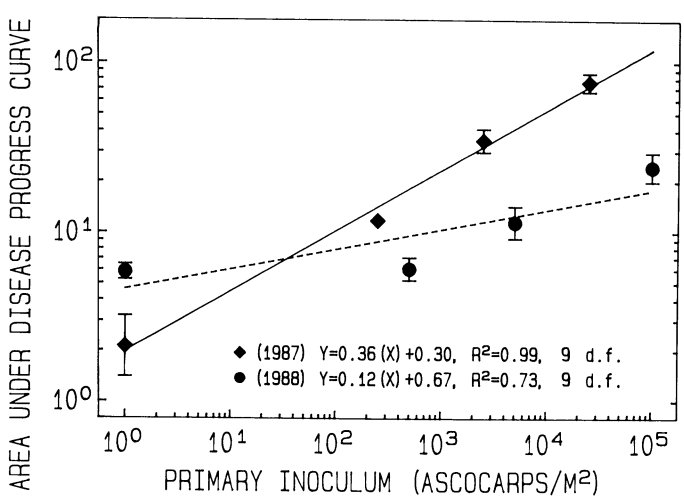

Fig. 2. Regression analysis of the logarithm of the area under the disease progress curve on the logarithm of primary inoculum for two epidemics of wheat tan spot.

DISCUSSION

A major goal of these experiments was to determine whether a reduction in local primary inoculum (for example, by means of cultural or biological control measures) would ultimately reduce disease severity, or alternatively whether infections caused by windborne conidia from outside sources would eventually negate any benefit from reduction of local primary inoculum. We found that the amount of primary inoculum of *P. tritici-repentis* did have a significant effect on tan spot epidemic development (AUDPC) in both favorable (1987, rainy) and unfavorable (1988, hot and dry) seasons for disease development (Fig. 2).

The influence of primary inoculum level on disease development was evident not only in cumulative disease severity (AUDPC) but also was present even at the end of the season, as indicated by the correlation between initial inoculum level and estimated percentage of leaf colonization (both flag and penultimate leaves) at growth stage 11.2 in 1988 (Table 2). Thus, the local primary inoculum level has an impact that can persist throughout an epidemic of this disease, despite the important part played by windborne secondary inoculum. Because ascospores are probably not involved in infections occurring after mid-spring, their impact on season-long disease development is via the amount of infectious (conidia-producing) leaf tissue they initially incite.

The importance of primary inoculum may in fact be underestimated in this study. Greater differences in epidemic development might have been shown between inoculum levels had the plots been farther apart, reducing interplot interference (2). Such interference tends to increase disease in the control plots relative to the amount of disease that would be seen in a clean-tilled field. Thus, the effects we observed were considered a conservative estimate of the epidemiological importance of local primary inoculum (and the secondary inoculum derived from it).

The level of primary inoculum at which epidemic development was significantly ($P = 0.05$) greater than that in the check plots differed in the two years of our experiments (Table 2). In 1987, this level was below 2,500 ascocarps per square meter, whereas in 1988 it was above 5,000 ascocarps per square meter. Although the statistical test for significant differences is clearly a function of the particular experimental observations (sample size, error variance, choice of experimental inoculum levels), this result suggests that the two years differed in the level of local primary inoculum required to produce a level of disease greater than that caused by influx of secondary inoculum alone. This difference in the influence of local primary inoculum is also evident in the different slopes of the respective regression lines (Fig. 2).

This difference could have been caused by weather, as indicated previously; it seems likely to us that the 1988 AUDPC values would have been higher, and the differences in disease development among inoculum levels greater, had conditions been more conducive to tan spot. The different wheat cultivars used in the two years are unlikely to be responsible for the difference because they have responded similarly to tan spot in statewide trials and are similar in maturity date and stature (13).

More years of experiments are needed to adequately test the relationship between weather and the primary inoculum levels required to cause a given level of disease severity. However, it appears that the relative importance of local primary inoculum and incoming secondary inoculum can differ in different years. In some years, crop surveys indicate obvious differences in tan spot severity in fields with primary inoculum and clean-tilled fields, whereas in other years there are no obvious differences in the level of disease in fields with or without local primary inoculum (W. G. Willis, *personal communication*). Also, the relative importance of the two types of inoculum could vary depending on the proximity of another source of secondary inoculum. The closer such a source, the greater the relative importance of secondary inoculum to the final amount of disease. Proximity of external inoculum could have been influential in these experiments because of the relative closeness of the plots to each other and to other fields in which tan spot developed.

The relatively low influence of primary inoculum on yield could be the result of the influence of factors other than tan spot severity on yield. In 1987, a severe epidemic of leaf rust at the dough stage (stage 11.2 [15]) of grain fill greatly reduced yields in all plots by causing early senescence of all leaves. The control and low-inoculum plots had more rust pustules per leaf than the medium- and high-inoculum plots (data not shown), probably because of higher humidity in the less-damaged stands and/or more healthy tissue available for the rust fungus. Therefore, yields of the control and low-inoculum plots were probably affected proportionally more by the rust epidemic than were yields of the medium- and high-inoculum plots, and thus the rust epidemic masked yield differences caused by tan spot.

The greater high-inoculum level in 1988 (100,000 ascocarps per gram, compared to 25,000 ascocarps per gram in 1987) did significantly lower the yields from the other inoculum levels, as the high-inoculum level had in 1987, but the yield loss might have been greater had the conditions been more favorable for a tan spot epidemic. Moreover, the 1,000-kernel weight was not significantly related to primary inoculum or AUDPC in either year because of the conditions mentioned previously. Tan spot that occurs during the boot to early-milk stages of wheat development (stages 10 to 10.5.4 [15]) has been shown to reduce kernel weight significantly (12); but the rust epidemic and drought during this time period in 1987 and 1988, respectively, likely masked the effect of tan spot on this yield parameter.

In conclusion, these experiments have shown that the level of tan spot disease was related to the amount of primary inoculum in two years with very different weather conditions. Disease progress during the season, as well as severity ratings (and estimated proportion of leaf area colonized) near the end of the season, were correlated with primary inoculum levels. More severe disease at the time of kernel development would be expected to reduce yield. Furthermore, the more severe disease levels observed in high-inoculum plots early in the season may be an important yield determinant; Shabeer and Bockus (12) showed that up to 17% of the total tan spot yield loss is the result of primary infections before jointing of the wheat. However, because many factors besides tan spot severity determine the yield of a crop, the relationships of yield to AUDPC and primary inoculum are more difficult to demonstrate in field plots than is the relationship of disease development to primary inoculum.

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