Inoculum Density and Infection Efficiency of Conidia and Conidiophores of Isolates of *Pyrenophora tritici-repentis*

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ABSTRACT

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Glass slides and wheat leaves were inoculated with conidia and conidiophores of *Pyrenophora tritici-repentis* isolates to compare the density (no./cm^2) of propagules on nonhost and host surfaces. Regression functions of the density of each propagule form on glass slides, as a function of the inoculum concentration, overestimated the density of each propagule form on wheat leaves by three to four times. Subsequently, conidia and conidiophores of three isolates were inoculated at equal rates of propagule density on wheat cultivars TAM 105 (susceptible) and Red Chief (resistant) to compare lesion incidence resulting from the different forms of propagule. Conidia caused 26 times more lesions than did conidiophores, and differences among the isolates for lesion incidence were significant ($P \leq 0.05$). Finally, the infection efficiency of the three isolates was determined utilizing their conidiophores and conidia in separate inoculum suspensions. Infection efficiency was determined from the slope of the regression of lesion incidence as a function of the density of propagules per unit area of inoculated leaf. Infection efficiency for conidia of the isolates ranged from 0.91 to 0.55 whereas infection efficiency for their conidiophores was not significantly different from zero or was extremely variable. Results indicate that studies of epidemiological parameters of *P. tritici-repentis* are more precise when based on estimates of conidial density on host leaf surfaces, and when conidiophores are excluded from inoculum suspensions. The estimates of infection efficiency should prove useful in the identification of virulent isolates of *P. tritici-repentis* and should lead to improved identification of resistance to tan spot.

Ascospores, conidia, conidiophores, and aerial hyphae of *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.) Shoemaker) are infectious propagules capable of causing tan spot on wheat (*Triticum* spp.) and leaf spot on other gramineous hosts (9–11, 19,20). Studies relating inoculum density to the progression of tan spot have utilized different inoculum concentrations or focused on seasonal airborne spore populations (14,21,28,33) with no understanding of differences among *P. tritici-repentis* propagules that cause infection. Other studies of tan spot on wheat focused on the effects of temperature (13,27), postinoculation leaf wetness duration (12,13), leaf position or age (2,12,30), and the role of toxins in tan spot reactions (1,24–26,34). These studies have not considered effects due to the form of *P. tritici-repentis* propagule(s) being utilized as inoculum. Investigators have used wire loops (23,25,26), bent glass rods (13), or the edge of a glass slide (12) to scrape or dislodge inoculum from the surface of agar medium. Blended agar disks with conidia, conidiophores, and hyphae (34) have been utilized to prepare inoculum of *P. tritici-repentis* for subsequent inoculations onto wheat plants. All of these techniques result in inocula containing mixtures of conidia, conidiophores, and hyphal fragments that are difficult to quantify and reproduce between experiments (3). For example, Schilder and Bergstrom (32) reported that differences in sporulation and concentration of unquantified infective propagules, such as mycelial fragments and conidiophores, may have accounted for some of the variable results when the virulence of several isolates of *P. tritici-repentis* was compared. Francl and Jordahl (6) examined the effects of propagule type, inoculum dosage, and spatial interaction among lesions on infection phenotype, infections per unit area, and percent lesion incidence. They concluded that inoculum type and location of infection on host wheat leaves affected the infection phenotype. They recommended using uniform inoculum dosages, excluding conidiophores from inoculum, and collecting infection-type data from the middle of the uppermost leaf that was fully expanded at the time of inoculation to differentiate between resistant and susceptible wheat genotypes. Riaz et al. (30) described a procedure to quantify production of *P. tritici-repentis* conidia on wheat leaves. They showed that conidia are produced on susceptible wheat cultivars at a significantly greater rate than on wheat cultivars displaying intermediate or resistant reactions to tan spot. They also showed that conidia were produced less rapidly on younger leaves of the tan spot resistant cultivar Auburn than on older leaves. However, they said "it was not clear whether enhanced sporulation on older leaves was due to increased physiological reproduction capability of the fungus or to increased infection efficiency and tissue colonization"; distinguishing between these possibilities would require understanding infection efficiency of *P. tritici-repentis* conidia and conidiophores based upon propagule density (no./cm^2), inoculum composition, and inoculum concentration on wheat leaves.

We reported a procedure that facilitates the production of conidial inoculum of *P. tritici-repentis* nearly free of mycelial fragments and conidiophores (3). The objectives of this study were to utilize this procedure to quantify the density of conidia and conidiophores applied to defined areas of wheat leaves and glass slides, compare the lesion incidence of tan spot resulting from inoculation with conidiophores and conidia at equal inoculum density on wheat leaves, and estimate infection efficiency of *P. tritici-repentis* isolates using their conidia and conidiophores separately to infect resistant and susceptible wheat cultivars.

MATERIALS AND METHODS

Inoculum preparation. Three *P. tritici-repentis* isolates collected from wheat fields near Altus (91ALA1), Guymon (91GYA3), and Braman (91RBB6), OK, (3) were used in this study. Nearly pure suspensions of conidia from these isolates were produced following previously described methods (3). Conidiophore suspensions were produced using the same procedure except that cultures were not exposed to the 24 h of light needed for conidial formation. Conidiophores were removed from the surface of agar plates by first flooding the colony surface with 10 ml of distilled water containing 0.08 ml (two drops) of Tween 20 per 100 ml. Conidiophores were then dislodged by

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scraping the colony surface with a rubber spatula. The resulting suspension was decanted into an Erlenmeyer flask and agitated for 1 min, then filtered through two layers of cheesecloth. Concentrations of conidiophores or conidia were determined in a nematode counting dish and suspensions were adjusted to the desired concentration(s) (15). In all tests, the viability of conidia and conidiophores from each *P. tritici-repentis* isolate was assessed by inoculating each form of propagule onto water agar in petri dishes (100 × 15 mm). Following inoculation, petri dishes were maintained at 21°C in the dark. After 12 h, the percent germination of 100 conidia or conidiophores of each isolate was determined.

Assessment of inoculum density. The first experiment determined the density of conidia and conidiophores deposited on glass slides and wheat leaves. A device was assembled to deposit conidia or conidiophores onto various surfaces. A clear Pyrex glass cylinder, 20 cm long by 5 cm inside diameter, was attached vertically to a ring stand with an atomizing sprayer (Pulmo-Aide Model 5601D, DeVilbiss, Sommerset, PA) attached to the top of the cylinder. The top of the cylinder was covered with 25 µm Nitex nylon screen with a hole in the center for the sprayer nozzle. The cylinder was used to prevent air currents from affecting inoculations from sample to sample. Inoculum within the reservoir of the atomizer was agitated using a mini-stir bar and stir plate (Model S46415, Barnstead/Thermolyne, Dubuque, IA) on an adjustable stand to allow height adjustment. An inoculation stage 10 cm long by 7 cm wide was fabricated from a 1-cm-thick sheet of clear acrylic plastic. A clear plastic-film sheet, 0.15 mm thick, was attached to the stage and a 3 × 1 cm rectangular area was removed to expose a target area. Wheat leaves or glass slides were placed on the stage and covered with the plastic sheet so that only a 3-cm^2 area was inoculated when sprayed. Glass slides or leaves could then be inoculated by holding the stage in contact with the base of the inoculation cylinder, 20 cm below the orifice of the atomizing sprayer. Application of inoculum was regulated by attaching the atomizing pump to a timer (Gra-Lab Timer, Model 167, Dimco-Gray Co., Dayton, OH). Timed intervals for all applications were set for 1.5 s. The glass cylinder was swabbed between inoculations with a paper towel. The cylinder was sprayed with 95% ethanol using a hand-pump mist-sprayer between inoculations of each isolate. Samples of conidia that were washed from the cylinder with ethanol were plated on water agar and evaluated for germination after 12 h incubation in darkness. The cylinder was wiped with paper towels and allowed to dry (15 to 20 min) before proceeding with additional inoculations.

Suspensions of conidia and conidiophores prepared from isolate 91RBB6 were used in inoculum density assessments. Visual assessments of density of conidia and conidiophores on adaxial surfaces of wheat leaves were enhanced by adding 0.08 ml (2 drops/100 ml) of safranin to the inoculum suspensions. Conidia and conidiophores were collected separately on a 25 µm Nitex screen and washed with distilled water. Conidia and conidiophores were then resuspended in 100 ml of distilled water with 0.08 ml (two drops) of Tween 20. Inoculum concentrations were counted after the staining process and adjusted to the desired concentrations. The concentrations of conidia used in the first density assessment experiment were 2,000, 4,600, 6,100, and 10,700 conidia/ml and in the next density assessment experiment were 2,000, 4,500, 7,500, and 10,000 conidia/ml. The three concentrations of conidiophores used in the two assessments of conidiophore density were 10,000, 20,000, and 40,000 conidiophores/ml. For each concentration, inoculum was deposited onto a defined 3-cm^2 area on each of five glass slides (3 × 1 cm, L × W) and defined areas on the adaxial surface of five leaves of the wheat cultivar TAM-105. Eight seeds of TAM 105 were planted per pot. Plants were grown in Peters professional blend potting mix in KORD plastic pots (114 mm diameter, 102 mm depth) and fertilized with 1.6 g/pot of 14-14-14 Osmocote fertilizer. TAM 105 plants were grown in a controlled environment growth chamber under a 12-h photo period (610 $\mu E \cdot s^{-1} \cdot m^{-2}$ at 20 C and 12 h in the dark at 15°C. Relative humidity in the growth chamber was maintained at 30% and penultimate leaves were detached for assessment of propagule deposition at 5 or 6 weeks growth. A permanent fine-point felt-tip pen was used to mark the ends of the leaf area exposed to inoculum deposition. Propagules inoculated onto the surfaces of glass slides and wheat leaves were counted using a stereomicroscope (30×). Propagule density (no./cm^2) was derived by dividing the number of propagules observed by the area that was inoculated. All leaf portions that were exposed to inoculation were 3-cm long; however, their widths were always less than the width of the exposed stage window (<1 cm). Thus, the inoculated portion was excised at the marked points by cutting across the leaf perpendicular to the leaf axis. The inoculated leaf area was determined using a video imaging system consisting of a fluorescent background light source, television camera (Model ITC-48, Ikegami Tsushinki Co. LTD., Japan), picture monitor (Model PM-930, Ikegami, Tsushinki Co. LTD., Japan), and area meter (Delta-T Devices, Cambridge, England). All tests of inoculum density assessment were repeated. Data from the density assessment experiments for each propagule were combined and their density, on adaxial wheat leaf surfaces and defined areas of glass microscope slides, as a function of conidia or conidiophore inoculum concentration, was determined. Statistics were computed using the regression (REG) procedure of the SAS statistical program (SAS Institute, Cary, NC). Estimates of slope parameters and *y*-intercepts of the linear models were compared using the general linear models

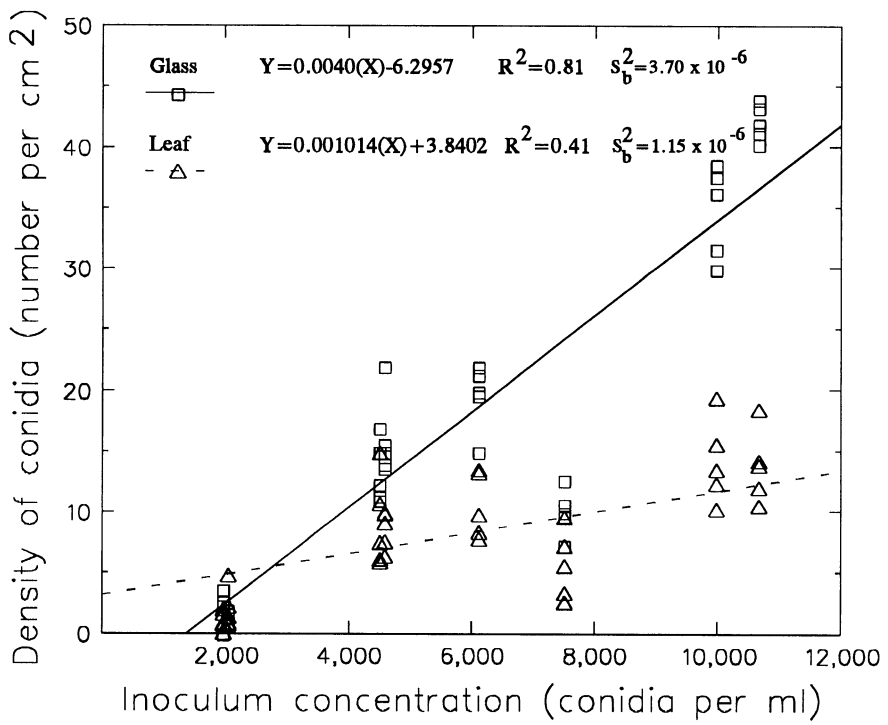

Fig. 1. Density of conidia on the surface of defined areas of glass (□) slides and adaxial surfaces of wheat cultivar TAM 105 leaves (Δ) as a function of conidial inoculum concentration.

(GLM) procedure of SAS. The regression functions of the estimates of the density of each propagule on wheat leaves (Figs. 1 and 2) were utilized in the second experiment to compare the lesion incidence resulting from each form of propagule at an equal density. In the third experiment the regression functions were utilized to estimate the infection efficiencies of conidia and conidiophores of the three *P. tritici-repentis* isolates.

Comparison of lesion incidence due to infections caused by conidia and conidiophores. The second experiment determined the lesion incidence on two wheat cultivars from inoculations with conidia and conidiophores of three *P. tritici-repentis* isolates. The inoculum concentrations were adjusted to standardize the density of conidia and conidiophores inoculated on separate wheat leaves. The design of the second experiment was a three-factor factorial in a randomized complete block with four replications. The three main effects were represented by two wheat cultivars (TAM 105 and Red Chief), three *P. tritici-repentis* isolates (91ALA1, 91GYA3, and 91RBB6), and two forms of propagule (conidia versus conidiophores). The fungal isolates each produced necrotic lesions with chlorotic halos on TAM 105 and Red Chief grown in the field and under greenhouse conditions. Eight seeds of TAM 105 or Red Chief were planted per pot (replication) and later thinned to five seedlings after establishment. One plant per pot was used as a control. Plants were grown in Peters professional blend potting mix in KORD plastic pots and fertilized with Osmocote fertilizer as described earlier. Wheat plants were grown in a controlled environment growth chamber under photoperiod, temperature, and relative humidity conditions described earlier for growing TAM 105 plants. Plants were inoculated after 5 or 6 weeks growth. The middle 3 cm of the uppermost fully expanded leaf (penultimate leaf) of four plants in each pot (replication) was inoculated with each form of propagule of each isolate on the two wheat cultivars. Inoculated leaves remained attached to the host plants until lesion counts and leaf area measurements were conducted. Based on the regression functions (Figs. 1 and 2) for the respective propagules, concentration of conidia was adjusted to 10,000/ml and concentration of conidiophores was adjusted to 15,000/ml to result in comparable numbers of each form of propagule inoculated per cm^2 as described earlier for TAM 105. Leaves of the control plants were inoculated with a Tween 20 solution (0.08 ml of Tween 20 per 100 ml of distilled water). Following inoculation, plants were allowed to air dry for 1 h and then placed in a mist chamber providing >95% relative humidity for 12 h in light (46 $\mu E \cdot s^{-1} \cdot m^{-2}$) at 22 to 24°C followed by 12 h in darkness at 18 to 20°C. A hygrothermograph was used to monitor relative humidity during the postinoculation wet period. During the postinoculation wet period visible moisture was apparent on wheat leaves as a very fine dew. After the 24-h postinoculation wet period, misting was stopped and plants were maintained in the mist chamber. The number of lesions in the length of inoculated wheat leaf was counted 4 to 6 days following the postinoculation wet period. After the lesions were counted, the inoculated leaf area was excised at the points marked with a permanent marker pen and measured as described earlier. Inoculum concentrations for the respective propagules were entered into their corresponding regression functions (Figs. 1 and 2) to estimate the propagule density on wheat leaves. The propagule density value was then multiplied by the measured leaf area to estimate the number of propagules that were deposited on the inoculated leaf portion (no./leaf). Analyses were conducted on the mean of four leaves (penultimate leaf of each of four plants) per pot for inoculated leaf area, the estimate of the number of propagules deposited on the defined wheat leaf area, and the number of lesions that were observed within the defined area (lesion incidence). Statistical analyses were conducted on the combined data of the repeated tests following the SAS analysis of variance (ANOVA) procedure. Main effect means were separated by means of Fisher's least significant difference (LSD) test computed at $P \leq 0.05$.

Infection efficiency of conidia and conidiophores. Experiments in the third portion of these studies were designed to estimate infection efficiencies of three isolates of *P. tritici-repentis* as influenced by the form of propagule used to cause infection. Infection efficiencies of conidia and conidiophores of the isolates were based on the regression of the number of lesions (lesion incidence) observed on inoculated wheat leaves as a function of the estimated propagule densities on the defined leaf areas. Estimates of propagule density were based on inoculum concentration and measured inoculated leaf area as described earlier. Wheat plants were grown and inoculated as previously described.

The experimental design was a two-factor factorial in a randomized complete block with four replications. The main effects and their levels consisted of the two wheat cultivars and the three *P. tritici-repentis* isolates (six treatments). Leaves of TAM 105 and Red Chief were inoculated with nearly pure conidial suspensions of the three *P. tritici-repentis* isolates. The experiment was conducted twice for each inoculum concentration utilized. Four concentrations of conidial suspensions containing 4,000, 5,500, 7,500, and 10,000 conidia/ml were utilized to inoculate

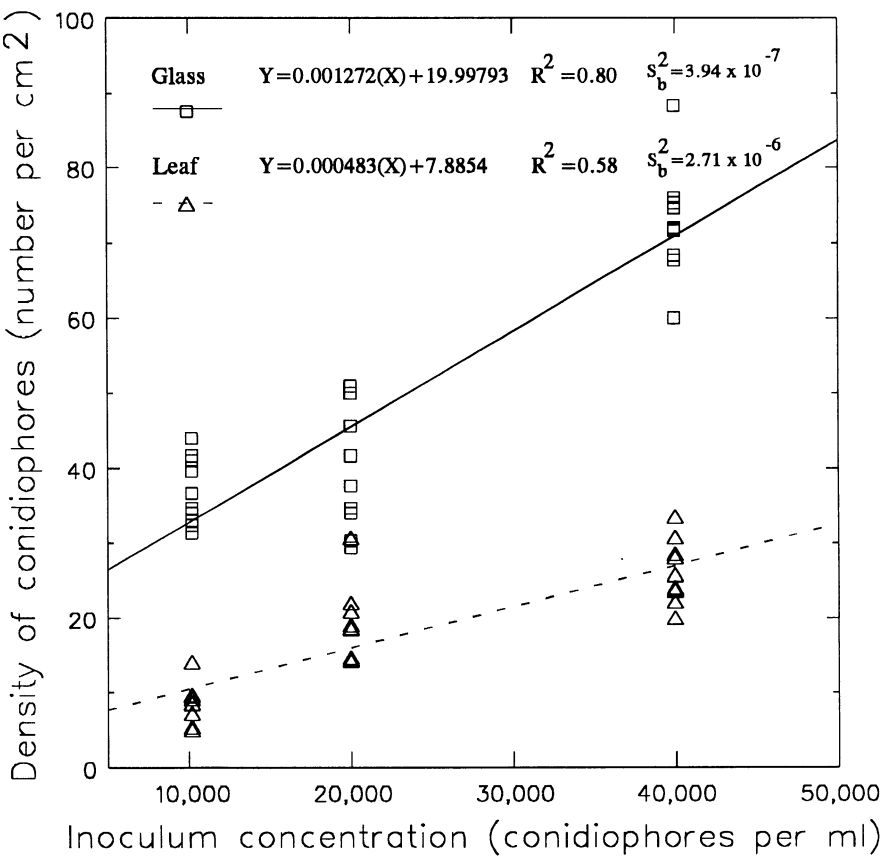

Fig. 2. Density of conidiophores on the surface of defined areas of glass (□) slides and defined adaxial surfaces of wheat cultivar TAM 105 leaves (Δ) as a function of conidiophore inoculum concentration.

leaves. Eight experiments were conducted to determine the infection efficiency of the conidia of the *P. tritici-repentis* isolates. Data from the repeated tests were combined and regression analysis of number of lesions/cm^2 as a function of inoculum density was conducted averaged over the two wheat cultivars (susceptible and resistant) to provide an average estimate of the infection efficiency of conidia of the *P. tritici-repentis* isolates (Fig. 3).

Three concentrations of suspensions of conidiophores were utilized, in the same fashion as described for conidia, to assess the infection efficiencies of the three *P. tritici-repentis* isolates. The experimental design was a two-factor factorial in a randomized complete block with four replications. The main effects and their levels consisted of the same two wheat cultivars and the three *P. tritici-repentis* isolates (six treatments). The experiment was conducted twice for each inoculum concentration. Wheat leaves of TAM 105 and Red Chief were inoculated with suspensions of conidiophores containing 10,000, 20,000, and 40,000 conidiophores/ml. Six experiments were conducted. Infection efficiency of conidiophores of the *P. tritici-repentis* isolates was determined from the regression of lesion incidence as a function of the density of conidiophores on wheat leaf surfaces averaged over the two wheat cultivars as described previously.

Residual analyses (5) were conducted for lesion incidence data. The lesion incidence values were weighted for both propagules using the weight option described in the GLM procedure of the SAS program to provide the best linear unbiased estimators for the regression. The slope and intercept of the regression functions were tested for significance with standard t tests. A comparison of slope parameters and y-intercepts of the three isolates were conducted as described earlier for the assessment of inoculum density. The slope of the regression function for each isolate and propagule represents the estimate of their respective infection efficiencies (%) over the range of inoculum concentrations that were utilized. Estimates of slope parameters and y-intercepts of the linear models of infection efficiency of the isolates conidia and conidiophores were compared using the regression (REG) procedure of SAS.

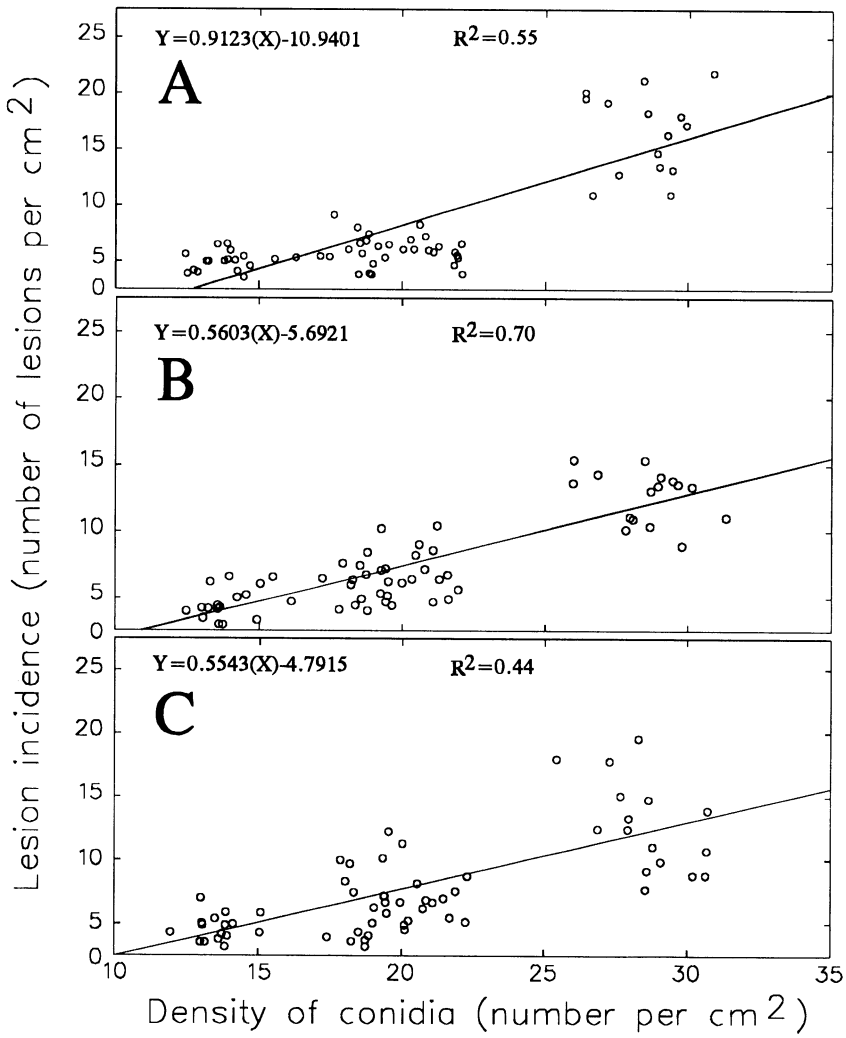

Fig. 3. Relationship between lesion incidence and estimated density of conidia on adaxial surfaces of wheat cultivars TAM 105 and Red Chief leaves with three *P. tritici-repentis* isolates. Linear equations for the isolates **(A)** 91ALA1, **(B)** 91GYA3, and **(C)** 91RBB6, respectively.

RESULTS

The three *P. tritici-repentis* isolates were dark green on potato dextrose agar (3). Conidiophores and conidia were produced prolifically on clarified V8 juice agar. Inoculation with conidia from these isolates onto leaves of TAM 105 and Red Chief resulted in lesions that had a small, black, necrotic spot surrounded by a tan necrosis with associated chlorotic halos. Red Chief (resistant) and TAM 105 (susceptible) were utilized based on lesion size and reaction to tan spot in previous studies (2, 30). In other studies of resistance to tan spot, lesions that developed on Red Chief were shorter than those observed on TAM 105 (4).

Inoculum preparations. All conidial suspensions had less than 5% other infective propagules (conidiophores and hyphal fragments). All suspensions of conidiophores had less than 6% hyphal fragments present and no conidia after final adjustment to the desired concentration. Germination of conidia on water agar was greater than 98%, and germination of conidiophores on water agar was greater than 97%. Conidiophores and conidia washed from the inoculation apparatus after treatment with 95% ethanol did not germinate when plated on water agar.

Inoculum density assessment. The first experiment demonstrated that inoculating propagules of *P. tritici-repentis* onto glass slides overestimated their density on the surfaces of wheat leaves by a factor of three to four times (Figs. 1 and 2). The density of conidia and conidiophores on the surface of glass slides and adaxial surfaces of wheat leaves increased linearly in relation to inoculum concentration. The y-intercepts of conidial density on glass slides and wheat leaves were significantly different from zero (P = 0.0046 and P = 0.0189, respectively). The y-intercepts of conidiophore density on glass slides and adaxial wheat leaf surfaces were also significantly different from zero (P = 0.0001). Slopes of regression functions for the density of conidia and conidiophores on glass slides and adaxial wheat leaf surfaces were significantly different from zero (P = 0.0001). Conidial inoculum concentration described 81% of the variation of conidia density on glass slides whereas it described 41% of the variation of their density observed on adaxial surfaces of wheat leaves. Conidiophore inoculum concentration described 80% of the variation of conidiophore density on glass slides and described 58% of the variation of conidiophore density on adaxial surfaces of wheat leaves. Comparison of the slope parameters indicated the density of conidia inoculated on glass was four times greater than the density of conidia inoculated on adax-

ial surfaces of wheat leaves ($P = 0.0001$). However, at inoculum concentrations of 2,000, 4,000, 6,000, and 10,000 conidia per ml, the ratios of the density of conidia on glass slides compared with the density observed on leaves were 0.29, 1.23, 1.78 and 2.41, respectively. The *y*-intercept of the model of the density of conidia inoculated on adaxial leaf surfaces was significantly greater ($P = 0.007$) than the intercept of the model for the density of conidia on glass (Fig. 1). The comparison of the slope parameters indicated the density of conidiophores inoculated on glass was three times greater than the density of conidiophores inoculated on adaxial surfaces of wheat leaves ($P = 0.0001$). At conidiophore inoculum concentrations of 10,000, 20,000, and 40,000, the ratio of their deposition on glass slides to their deposition on leaf surfaces was 2.57, 2.59, and 2.60, respectively. The *y*-intercept of the model of the density of conidiophores inoculated on adaxial leaf surfaces was significantly less ($P = 0.0001$) than the intercept of the model for the density of conidiophores on glass (Fig. 2). In both inoculation tests, the density of conidia or conidiophores on glass slide surfaces overestimated each propagule's density on adaxial surfaces of wheat leaves as indicated by significant differences between the slopes and intercepts of the regression functions.

Comparison of lesion incidence due to infections caused by conidia and conidiophores. The second set of experiments demonstrated conidia are much more infective than conidiophores when compared at equivalent inoculum density (no. of propagules/cm^2) on wheat leaves. The combined analysis indicated there was no significant difference between tests for inoculated leaf area, estimated propagule deposition, and lesion incidence (Table 1). There was a significant difference between propagules for their estimated propagule deposition (no./leaf) when averaged over the cultivars and isolates. Slightly fewer conidia were deposited per adaxial wheat leaf surface compared with the deposition of conidiophores per wheat leaf surface (Table 2). There was a significant difference between wheat cultivars, among *P. tritici-repentis* isolates, and between propagules in regard to their effect on lesion incidence (Table 1). Mean separations of the two wheat cultivars for lesion incidence confirmed that Red Chief was more resistant to tan spot, having fewer lesions/cm^2 on inoculated leaf portions than TAM 105 (Table 2). Mean separation of the three *P. tritici-repentis* isolates for lesion incidence revealed that isolate 91ALA1 produced more lesions/cm^2 on inoculated wheat leaf portions than the other two *P. tritici-repentis* isolates (Table 2). Mean separation of the two propagules for lesion incidence was dramatic. Conidia were 26 times more effective at causing infection than conidiophores (Table 2). This extreme difference in lesion incidence at a comparable inoculum density indicated that infection efficiency of each propagule would need to be studied over a separate range of inoculum concentrations.

There was a significant cultivar by propagule interaction for inoculated leaf area, estimated propagule deposition, and lesion incidence (Table 1). The interactions are presented in the following text. The mean leaf area of Red Chief that was inoculated with conidia was 2.08 cm^2 (standard error [*se*] = 0.07) whereas the mean area inoculated with conidiophores was 1.97 cm^2 (*se* = 0.08). The mean leaf area of TAM 105 that was inoculated with conidia was 2.00 cm^2 (*se* = 0.13) and the mean area inoculated with conidiophores was 2.07 cm^2 (*se* = 0.08). This interaction was primarily due to uncontrolled variation of the inoculated leaf area that occurred from plant to plant averaged over the level of the main-effect of isolates. The estimated number of conidia deposited per leaf portion of Red Chief was 29.04 (*se* =

Table 1. Analysis of variance of three main effects in a 2 × 3 × 2 factorial that was repeated to determine the influence of wheat cultivar, *Pyrenophora tritici-repentis* isolate, and propagule form, respectively, affecting inoculated leaf area, estimated propagule deposition, and lesion incidence

		Mean square values		
Source	df	Inoculated leaf area (cm^2)	Estimated propagule deposition (no./leaf) (× 10^{-3})[u]	Lesion incidence (no./cm^2) (× 10^{-2})[v]
Test	1	17.33 NS[w]	3.31 NS	20.60 NS
Replication (test)	6	6.52 NS	1.36 NS	5.37 NS
Cultivar[x]	1	5.55 NS	1.65 NS	133.57**
Isolate[y]	2	10.05 NS	2.04 NS	51.55**
Propagule[z]	1	9.80 NS	101.06**	3,108.33**
Cultivar × isolate	2	6.55 NS	1.32 NS	5.28 NS
Cultivar × propagule	1	186.38**	40.02**	96.96**
Isolate × propagule	2	0.48 NS	0.14 NS	54.16**
Cultivar × isolate × propagule	2	6.32 NS	1.22 NS	6.37 NS
Error	77	9.59	2.02	2.80

[u] Propagule deposition derived using inoculum concentrations with regression functions (Fig. 1) corresponding to each propagule's deposition onto adaxial wheat leaf surfaces. Resulting value multiplied by measured leaf area to arrive at estimated propagule deposition value per inoculated leaf section.

[v] Lesion incidence assessed by counting number of visible lesions on a 3-cm length of inoculated area in the middle of a wheat leaf.

[w] NS = not significant, * = significant at $P \leq 0.05$, and ** =significant at $P \leq 0.01$.

[x] The two wheat cultivars were Red Chief (resistant) and TAM 105 (susceptible).

[y] The three isolates were 91ALA1, 91GYA3, and 91RBB6 from Altus, Guymon, and Braman, OK, respectively

[z] Propagules consisted of conidia and conidiophores of the *P. tritici-repentis* isolates.

Table 2. Mean separations of levels of main effects in a 2 × 3 × 2 factorial to demonstrate how they affect inoculated leaf area, estimated propagule deposition, and lesion incidence

Main effect/levels[w]	Inoculated leaf area (cm^2)	Estimated propagule deposition (no./leaf)[x]	Lesion incidence (no./cm^2)[y]
Cultivar			
Red Chief	2.02[z]	29.42	4.95 b
TAM-105	2.03	29.68	7.31 a
LSD[z]	0.03	0.57	0.68
Isolate			
91ALA1	2.01	29.27	7.59 a
91GYA3	2.03	29.62	5.35 b
91RBB6	2.04	29.76	5.43 b
LSD	0.04	0.70	0.83
Propagule			
Conidia	2.04	28.52 b	11.82 a
Conidiophores	2.02	30.57 a	0.44 b
LSD	0.03	0.57	0.68

[w] Three main effects consisted of two wheat cultivars, three *Pyrenophora tritici-repentis* isolates, and two propagules of same pathogen.

[x] Propagule deposition derived using inoculum concentrations with regression functions (Fig. 1) corresponding to each propagule's deposition onto adaxial wheat leaf surfaces. Resulting value multiplied by measured leaf area to arrive at estimated propagule deposition value per inoculated leaf section.

[y] Lesion incidence assessed by counting number of visible lesions on a 3-cm length of inoculated area in the middle of a wheat leaf.

[z] Means within column of levels within each main effect followed by the same letter are not significantly different according to the least significant difference (LDS) test ($P = 0.05$).

1.04) and the number of conidiophores deposited per leaf portion was 29.80 (*se* = 1.32). There was a significant difference between the estimated number of conidia and conidiophores on adaxial surfaces of TAM 105 leaf portions. The estimated number of conidia per leaf portion of TAM 105 was 28.01 (*se* = 1.82) whereas the number of conidiophores per leaf portion was 31.35 (*se* = 1.23). The cultivar by propagule interaction for lesion incidence revealed that Red Chief and TAM 105 developed fewer lesions from conidiophores than they did from infection with conidia. The number of lesions/cm^2 from infection by conidia on Red Chief was 9.63 (*se* = 2.94) and from conidiophore infection was 0.26 (*se* = 0.27). The number of lesions/cm^2 from infection by conidia on TAM 105 was 14.01 (*se* = 3.59) and from conidiophore infection was 0.61 (se = 0.48). Averaged over isolates, conidia are much more infective than conidiophores on the two wheat cultivars (Table 2).

The significant isolate by propagule interaction for lesion incidence revealed that each isolate's conidia were more infective than their conidiophores. Conidia of the 91ALA1 isolate caused 14.78 lesions/cm^2 (*se* = 4.18) whereas conidiophores of 91ALA1 caused 0.40 lesions/cm^2 (*se* = 0.48). The number of lesions/cm^2 caused by conidia of the 91GYA3 isolate was 10.29 (*se* = 1.95) whereas conidiophores of 91GYA3 caused 0.42 lesions/cm^2 (*se* = 0.33). The conidia of the 91RBB6 isolate caused 10.37 (*se* = 3.60) lesions/cm^2 whereas conidiophores of 91RBB6 caused 0.49 lesions/cm^2 (*se* = 0.46). Averaged over cultivars, conidia of each isolate are much more infective than their conidiophores.

Infection efficiency of conidia and conidiophores. Data from the two wheat cultivars were combined to demonstrate differences of lesion incidence due to infection of the three *P. tritici-repentis* isolates. Lesion incidence of the three isolates increased in a linear fashion with increasing density of conidia (Fig. 3). The *y*-intercepts of the regression functions of lesion incidence caused by conidia (Fig. 3) were significantly different from zero (91ALA1 and 91GYA3 P = 0.0001, 91RBB6 P = 0.0182). Slopes of the regression functions (estimated infection efficiencies) of lesion incidence due to infection from conidia of the three isolates were significantly different from zero (P = 0.0001). The density of conidia on adaxial surfaces of wheat leaves explained 44 to 70% of the variation of lesion incidence (Fig. 3A–C). The comparison of regression functions among the three isolates revealed that the intercept and beta-coefficient of isolate 91ALA1 was significantly different ($P > 0.07$ and $P > 0.003$, respectively) from those of the other two isolates (Fig. 3A). There was no significant difference between intercepts and beta-coefficients of isolates 91GYA3 and 91RBB6 (Fig. 3B, C). Lesion incidence due to infection from conidiophores was much lower than infections caused by conidia (Fig. 4). The *y*-intercepts of the regression equations of lesion incidence due to infections by conidiophores of 91ALA1 and 91GYA3 were not significantly different from zero (P = 0.07). The *y*-intercept of the regression equation of lesion incidence due to infection by conidiophores of 91RBB6 was significantly different from zero (P = 0.0001). The slopes of the regression equations of lesion incidence due to conidiophore infections of 91ALA1 and 91GYA3 were not significantly different from zero (P = 0.10 and P = 0.82, respectively). The comparison of slopes (P = 0.53) and *y*-intercepts (P = 0.74) of the regression equations of lesion incidence due to conidiophore infections of 91ALA1 and 91GYA3, respectively, revealed there was no difference between the models estimating their infection efficiencies. The comparison of the slope and *y*-intercepts of the regression of lesion incidence due to infection by conidiophores revealed that isolate 91RBB6 was significantly different from isolates 91ALA1 ($P > 0.001$ and $P > 0.001$, respectively) and 91GYA3 ($P > 0.003$ and $P > 0.009$, respectively). However, residual analysis (4) demonstrated that the variance of lesion incidence was proportional to the increase in deposition of conidiophores of isolate 91RBB6 (Fig. 4C). Thus, there appears to be little or no relationship between conidiophore density and lesion incidence (Fig. 4A–C).

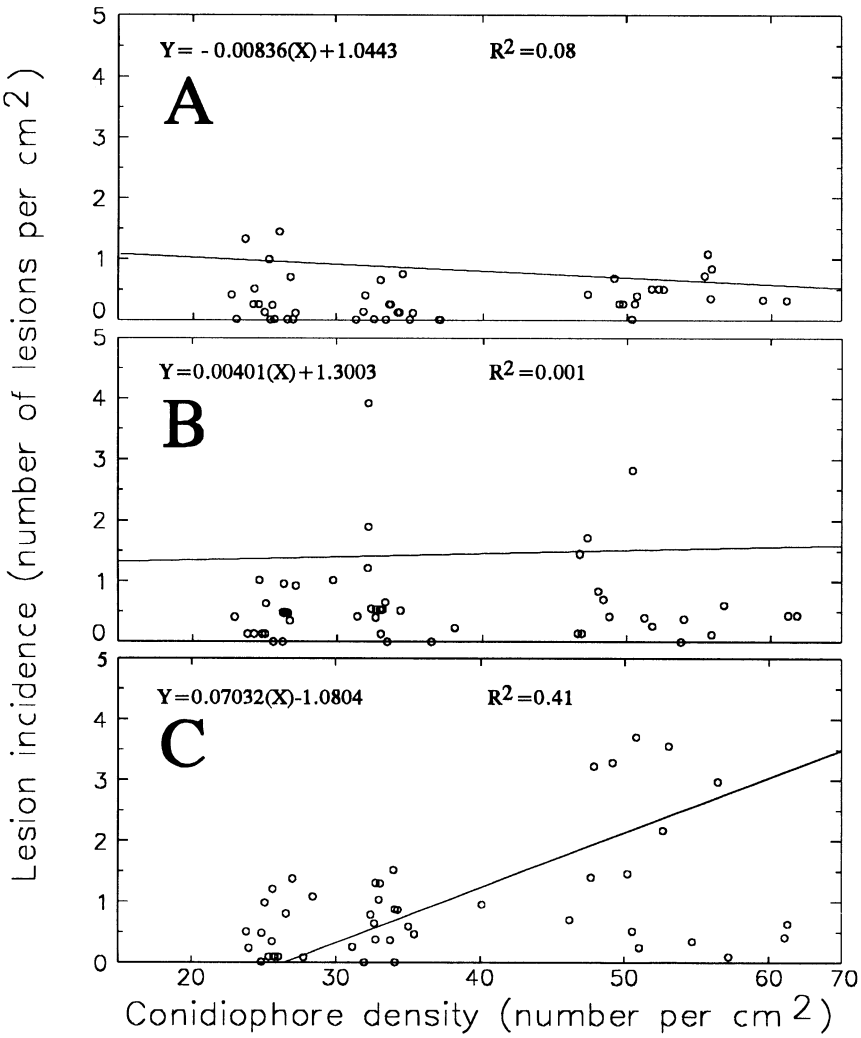

Fig. 4. Relationship between lesion incidence and estimated density of conidiophores on adaxial surfaces of wheat cultivars TAM 105 and Red Chief leaves with three *P. tritici-repentis* isolates. Linear equations for the isolates **(A)** 91ALA1, **(B)** 91GYA3, and **(C)** 91RBB6, respectively.

DISCUSSION

Determining the density of propagules on wheat leaf surfaces allowed direct estimation of the infection efficiency of several isolates of *P. tritici-repentis*. Experience has shown that inoculating wheat plants with *P. tritici-repentis* in a quantitative fashion is nearly impossible due to the architectural orientation of the leaves on wheat plants. Wheat leaves are slender and often are twisted so that neither abaxial or adaxial surface is uniformly exposed to inoculum. Schein (31) and others (6,7,22, 29) have developed specialized equipment

and different techniques of inoculating and measuring infection efficiency of plant pathogens. We felt their techniques were useful and incorporated many aspects for determining infection efficiency in our host and pathogen combination. Our intent was to develop a simple, inexpensive apparatus from materials in our lab so that we could deposit propagules of *P. tritici-repentis* onto defined areas of wheat leaves in a quick and repeatable manner. We employed an inoculation stage and a glass cylinder so that the adaxial surface of a 3-cm-long portion of leaf could be targeted. This was done in the middle of an upper-most developed wheat leaf on individual plants as recommended by Francl and Jordahl (6). Measurement of the leaf area inoculated was necessary because all the wheat leaves that were inoculated were narrower than the 1-cm width of the 3 × 1 cm window of the inoculation stage. Since leaf area was not controlled, analyses were necessary to demonstrate how inoculated leaf area affected estimates of infection efficiency.

Our tests involved deposition of propagules onto one wheat cultivar (TAM 105). Our focus was to demonstrate the differences of inoculum density that occur when the two propagules are deposited onto glass (nonhost) surfaces and wheat leaves. An earlier assessment of propagule deposition and density was conducted by inoculating glass surfaces, absorbent paper, and wheat leaves (16). Conidial density on wheat leaves was indirectly assessed by counting the number of conidia within a defined area of inoculated glass surface and relating this with the number of lesions observed on inoculated wheat leaves, but direct counts of propagule density on wheat leaves were not reported. We found that increasing inoculum concentration did not result in an equivalent inoculum density on glass slides or wheat leaves. This may have been due to increasing resistance of inoculum passing through the atomizer as inoculum concentration was increased, thus reducing the volume of inoculum applied. Electrostatic charges on fungal spores have been documented (8), and this may affect deposition, but we did not investigate this possibility. We did not quantify differences of propagule density that may occur among wheat cultivars due to trichomes or epicuticular waxes; this remains to be examined. The extent propagules overlap (e.g., a conidium or conidiophore adjacent to or overlapping another respective propagule) on leaf surfaces in the first experiment was not determined as well; this probably does occur, but how this affects estimates of infection efficiency is unknown and should be resolved in future studies. In order to achieve increased precision we recommend that future studies of the response of wheat to infection by *P. tritici-repentis* should be based on estimates of propagule density on wheat leaf surfaces.

The results of lesion incidence from infection by conidia and conidiophores on wheat confirm and strengthen those of Francl and Jordahl (6,17). They reported that conidia were 25 times more infectious than conidiophores (17) and later reported that tan spot severity ratings and reaction phenotype from infection by *P. tritici-repentis* conidia were higher than those resulting from infection by conidiophores (6). We determined that lesion incidence on wheat from infection by conidia of *P. tritici-repentis* was 26 times greater than infection by conidiophores (Table 2). In addition, we found that the 91ALA1 isolate caused more lesions to form per unit of conidial inoculum than did the conidia of the other isolates used. There was a lack of interaction between pathogen isolates and wheat cultivars in this test although the number of each was limited. Krupinsky (18) reported a lack of interaction of 84 *P. tritici-repentis* isolates inoculated on six cultivars of wheat in 91% of his analyses and was able to differentiate among isolates based upon their aggressiveness. Our inoculum production and inoculation results enable us to quantitatively identify isolates with high levels of infection efficiency to screen for improved resistance to tan spot of wheat.

The focus of the third study was to investigate differences in infection efficiency among *P. tritici-repentis* isolates, utilizing their conidiophores and conidia separately. In part, this was appropriate due to a lack of cultivar by isolate interaction for lesion incidence in the second test. The regression of lesion incidence as a function of inoculum density, averaged over TAM 105 and Red Chief, provided a conservative estimate of the infection efficiency among the isolates that was equally influenced by the susceptible and resistant host cultivar. The regression functions of the isolates' lesion incidence as a function of conidiophore density revealed little or no relationship between their density and lesion incidence. These experiments demonstrate the importance of excluding conidiophores from P. *tritici-repentis* inoculum when assessing infection efficiency among several isolates. A comparison of separate isolates using inoculum suspensions containing proportionate or disproportionate ratios of conidia and conidiophores could easily be influenced by inoculum composition, as demonstrated by the infection efficiency among the isolates utilizing their conidiophores to infect wheat leaves (Fig. 4A–C).

Isolate 91ALA1 had the greatest infection efficiency of the three that were studied as determined by the slope of its regression function from infection with conidia. Francl and Jordahl (6) reported that slope coefficients of infection of wheat by *P. tritici-repentis* conidia ranged from 0.013 to 0.037. However, their slope coefficients were derived from lesion incidence ratings (%) as a function of inoculum concentration. The slope coefficients of the isolates we studied were much higher but were derived from lesion incidence as a function of estimated propagule density on wheat leaf surfaces. Investigators (12,18) reported that Hunger and Brown (15) differentiated single-ascospore isolates of *P. tritici-repentis* based, in part, on their infection efficiency on TAM 101. In that study, Hunger and Brown inoculated wheat seedlings with agar rings colonized with isolates of *P. tritici-repentis*. The agar rings were placed "at the base of the second leaf of [wheat] seedlings" and infection was reported as the percentage of 30 seedlings that were infected (incidence). This can be interpreted as a percentage of infection but does not compare with the technique of inoculating wheat with conidia or conidiophores of *P. tritici-repentis* to simulate infection as it occurs under natural conditions. This paper presents the first direct estimates of infection efficiency of conidia and conidiophores of *P. tritici-repentis*. Future studies should focus on the effects of temperature, leaf wetness duration, host genotype, and infection efficiency of ascospores of *P. tritici-repentis* on tan spot development as well. Evaluation of these effects is needed in order to develop predictive models for field based epidemiological studies of tan spot.

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