INFECTION EFFICIENCY OF PROPAGULES OF *PYRENOPHORA TRITICI-REPENTIS* ISOLATES AND EVALUATION OF WHEAT FOR REACTION TO TAN SPOT

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I thank my fellow graduate students for their care, support, humor, and friendship throughout this study. Without them science would be less interesting. Finally, I dedicate the following works to further the understanding of tan spot of wheat and hope that my efforts will ultimately benefit those who provide mankind with bread.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

Table Page

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The cash value of wheat grain, from the years 1987 through 1989, represented a net value of 1.5 billion dollars to Oklahomas' economy. Thus, wheat represents one of the important agricultural crops grown in the state. In the 1970's and early 1980's wheat farmers experienced a decline in margins of profit due to falling land values, unpredictable grain markets and increasing interest rates. Additionally, factors such as increased labor, machinery, fuel, pesticide, and fertilizer costs have compounded the situation. Wheat producers have attempted to reduce costs by minimizing the inputs they had previously practiced for weed control and tillage practices in preparation for planting. Wheat growers have increasingly turned to low tillage systems that reduce energy inputs and machinery maintenance costs. Reduced tillage practices also benefit the land by maintaining more crop residue on the soil surface, thus reducing soil erosion, improving the rate of water infiltration, and increasing the levels of moisture retained in the soil (43). The combined factors of increased crop residues on the soil surface and the presence of the tan spot pathogen on native grass hosts and wheat, set the stage for tan spot to become an economically significant disease of wheat in Oklahoma and other wheat producing regions.

Crop rotations and effective chemical control strategies, although risky and expensive, have provided adequate tan spot control. However, I believe that energy costs will likely increase in the future, thus, forcing wheat producers to utilize cultural

practices, such as reduced tillage, that will tend to increase the prevalence of tan spot on wheat in Oklahoma and the Southern Great Plains Region. Also, federal environmental policies and the reevaluation of fungicides may bring about restrictions of the available chemicals that growers presently use to control tan spot.

Investigators concerned with tan spot on wheat, have conducted symposia in 1981 and in 1992 to discuss findings and information about strategies to control tan spot of wheat. Since its recognition as a major pathogen on wheat in 1971, much research has been conducted on wheat hosts (*Triticum* spp.), the pathogen *P. tritici-repentis* and various alternative gramineous hosts and host-pathogen interactions, resulting in and improved understanding of alternative grass hosts, epidemiological factors, and the biology of the tan spot pathogen. Quantitative evaluations of genetic resistance and epidemiological factors of tan spot have been limited and it seems that investigations of host resistance, epidemiologic factors, and infection processes of *P. tritici-repentis*, utilize methodology and techniques that make comparative studies difficult to conduct or repeat. Hence, the objectives of my research were to: 1) develop a technique that facilitates the production of relatively pure suspensions of conidia and relatively pure suspensions of conidiophores of *P.triticipentis*, 2) study the inoculum density and infection efficiency of conidiophores compared to conidia on susceptible and resistant wheat cultivars and, 3) evaluate selected wheat genotypes for their reaction to tan spot in laboratory and field conditions.

As a courtesy to the reader the author should mention that chapters two through four and all appendixes have been prepared in manuscript format for publication in various scientific journals. Chapter two has been published in the journal Plant

Disease. Chapter three of this thesis has been submitted to the journal Plant Disease, and chapter four is in preparation and will be submitted for publication in a suitable journal at the completion of this writing. All three appendixes have been published in the journal Biological and Cultural Tests for Control of Plant Disease.

HISTORY OF TAN SPOT

Pyrenophora tritici-repentis (Died.) Drech. (anamorph: *Drechslera tritici-repentis* (Died.) Shoem. [syn: *Pyrenophora trichostoma*]) has been identified in many of the major wheat (*Triticum* spp.) producing regions of the world. *P. tritici-repentis* is a homothalic ascomycete that incites the foliar fungal disease of wheat (*Triticum aestivum* L.) called tan spot and is known by other synonyms such as yellow leaf spot, yellow leaf blotch, leaf blotch, wheat leaf blight, and eyespot (17). The taxonomic position of *P. tritici-repentis* is presented in Table I (2). The first report of this pathogen was on grasses in Germany in 1902 (9), and was first identified on wheat (*Triticum* spp.) in Japan in 1928 (38). Investigators were aware of the pathogens presence on grasses in the United States as early as 1923 (11) and occasionally on wheat in the following years (4,6). However, it was not until 1971 that tan spot was recognized as a serious pathogen to wheat (17). Since 1971 researchers in the United States and other wheat producing countries have recognized that tan spot can cause widespread economic losses (17,20,39,46) with reported reductions of 20 to 50% in grain yields and 9 to 20% reductions in 1000 kernel weight possible (46,52,53). This recognition was brought about by the increased practice of conservation tillage in wheat producing regions of the world and the United States beginning in the early 1960's (43). Thus, tan spot increasingly became a disease that wheat producers had to deal with (10) when

practicing reduced or no tillage farming in wheat-after-wheat grain production systems.

EPIDEMIOLOGY

P. tritici-repentis persists as a saprophyte on host debris (17). Pseudothecia develop and mature on wheat straw during the fall and winter and release ascospores which serve as the primary inoculum in the spring and throughout the growing season (1,47,57). The conidia, which appear in spring through late summer, are dispersed primarily by the wind and serve as secondary inoculum (17). Liberation of conidia from conidiophores and their subsequent dispersal is accomplished in wind speeds as low as 0.7 m s^{-1} with over 60% of conidia being liberated at a relative humidity of 35%. Conidia are liberated at a level of 100% at all relative humidities when a windspeed of 3.3 ms^{-1} occurs. Conidia can even be liberated in still air by changes in relative humidity which causes dehydration of conidia and conidiophores, thus inducing liberation of conidia through flicking movements of each propagule (44).

Krupinsky has identified over 30 gramineous species which can serve as alternative hosts for *P. tritici-repentis* (24-26,28). Shoemaker (54) reported that *P. trichostoma* has the widest host range of all species in *Drechslera*. Thus sources of inoculum other than wheat residue exist for tan spot of wheat. Schilder and Bergstrom (51) measured ascospore and conidia dispersal from a point source located in a study area isolated from known sources of *P. tritici-repentis* inoculum. They found that major conidial dispersal events were usually preceded by rainfall events and windborne conidia were detected, in a decreasing logarithmic pattern, as far as 100 m from the source. Ascospore dispersal counts were much lower and were detected as far away as 11 m.

Four factors and the interactions of these factors are the primary determinants of

losses in wheat due to tan spot. The first is the inoculum level of *P. tritici-repentis* (46), second is the effect of post inoculation wet period (21), third is the host genotype (45), and fourth is the growth stage at which a wheat plant is infected (47). Rees (46) described a logarithmic relationship between the amount of wheat residue on the soil surface and loss in wheat grain yields. Infection on wheat was most severe when in close proximity to wheat straw debris infested with pseudothecia. The potential for inoculum build-up occurs during seasons when there is adequate moisture associated with the cultural practice of retaining wheat straw residue on the soil surface to prevent soil erosion (18). The tan spot disease spreads upwards through the wheat foliar canopy under moist conditions. Infections appear as necrotic spots often with chlorotic halos which frequently coalesce and ultimately cause the infected leaves to die (19). Depending upon wheat genotype, a post inoculation wet period of 6-48 hr is required for infection to occur, and dramatic yield losses result when infections occur during the boot to heading stage when flag leaves are severely damaged (7,17,18,23,56). "Thus, severity of tan spot tends to be higher with reduced tillage cropping and is favored by prolonged periods of rain or dew" (52).

No wheat genotypes have been found which are immune to *P. tritici-repentis*. When post inoculation wet period is sufficiently long, all inoculated wheat genotypes become infected and express tan spot symptoms (20). The fungus can infect green leaves of wheat at all growth stages (17), and causes a leaf spotting which varies in severity according to where the infection occurs on a leaf (22), the leaf position, inoculum levels, climatic conditions, and pathogen isolate and varietal interactions (7,8,22,26,27,29,31,32,36,50). When *P. tritici-repentis* infects a so-called resistant

wheat genotype, intracellular hyphal growth and development of necrotic lesions proceed slower than in susceptible genotypes (29,35). Lee and Gough (34) suggested a single gene characterizes resistance in the cultivar 'Carifen 12'. However, Nagel *et al.* (37) reported tan spot resistance to be a quantitatively inherited trait. Nonetheless, Lamari and Bernier (31) have identified *P. tritici-repentis* isolates on specific wheat genotypes that interact to produce necrosis + chlorosis +, necrosis + chlorosis -, necrosis - chlorosis +, and necrosis - chlorosis - symptoms. They suggested the involvement of two independent host genes, one controlling the development of tan necrosis, the second controlling the development of extensive chlorosis.

The infection process of *P. tritici-repentis* by conidia has been described by Loughman and Deverall (35) and Larez et al. (33). Conidial germination, appressorial formation, penetration, and colonization occur in 6 to 12 hours depending upon the level of resistance of the cultivar. An average of 2.5 germ tubes per conidium emerge within 6 to 72 hrs from polar and intercalary cells. The majority of appressorial attachments form over junctions of epidermal cell walls, fewer attachments form over epidermal cell walls, and only rarely do attachments form over stomatal structures. Infection pegs grow from appressorial attachments and penetrate the epidermal wall, which is followed by papilla formation and cellular colonization. After penetration the fungus often produces an intracellular vesicle from which one or more secondary hyphae are formed. Occasionally, no vesicles are formed and the hyphae ramify in two or more directions inside the epidermal cell. By 6 to 12 hrs after inoculation hyphae grow through cell walls into the mesophyll. From 12 hrs to four days after infection, hyphae were observed growing beyond the lesion margin into non-

symptomatic cellular tissue. After five days no hyphae were found beyond the lesion margin in non-symptomatic tissue. Thereafter, lesion expansion ahead of hyphae was thought to be due to the secretion of a toxin(s) (30,55).

Resistant wheat genotypes have been identified which require longer post inoculation wet periods for *P. tritici-repentis* infection than susceptible genotypes (23). Sources of resistance in bread wheats and species used in interspecific breeding efforts have been identified as well (3,5,48,49). However, accessions of *Triticum tauschii* (the donor of the D genome of hexaploid wheat) have not yet been evaluated as a potential source of resistance to *P. tritici-repentis* (5).

CONTROL

Removal of infested wheat straw from the soil surface by tillage or burning, or utilizing crop rotations eliminates the primary inoculum source (56). However, elimination of residues can result in an increased potential for soil erosion (43). Wheat grain producers may choose to use chemical(s) to control tan spot; however, propiconazol (Tilt®) is the only chemical currently labeled specifically for tan spot control in Oklahoma. Biological control has been suggested as a means of reducing levels of inoculum and infection of *P. tritici-repentis*. Ghazanfari and Gough (16) identified several microorganisms which reduced infection, mycelial growth, and ascocarp production on wheat seedlings and straw under lab conditions. Pfender (40) reported that *Limonomyces roseipellis*, a basidiomycete which causes pink patch on turf grass, reduced ascocarp and ascospore production of *P. tritici-repentis* by 50 to 99% under laboratory conditions and suggested that the suppression may involve mycoparasitism due to its chitinolytic ability. Recently Pfender et al. (41) utilized

bacteria that were capable of producing pyrolnitrin to suppress ascocarp production by *P. tritici-repentis* under laboratory conditions, however the bacteria were ineffective when tested under field conditions. Continued efforts to identify effective field biological control organisms are ongoing as indicated by their inability to significantly reduce levels of tan spot inoculum under field conditions (42).

Recently, investigators have reported the results from screening promising wheat genotypes and genetic stocks for resistance to tan spot (12-15,49,50). However, no wheat genotypes have been reported to be immune to the pathogen and continuing efforts are needed to identify improved resistance to tan spot in wheat germ plasm stocks.

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Table I. Taxonomy of *Pyrenophora tritici-repentis* (2)

Division:	Eumycota (eumycetes)- Produce mycellium.
Subdivision:	*Ascomycotina* (ascomycetes)- Produce sexual spores, called ascospores, generally in groups of eight within an ascus.
Class:	*Loculoascomycetes* (the ascostromatic fungi)- Produce pseudothecia, i.e., perithecium-like stromata with asci in separate or single large cavities.
Order:	*Pleosporales* - Cavities arranged in a basal layer and containing many asci. Pseudothecia have pseudoparaphyses.
Genus:	*Pyrenophora* - Causing leaf spots of cereals and grasses.

CHAPTER II

ENHANCED PRODUCTION OF *PYRENOPHORA TRITICI-REPENTIS* CONIDIAL SUSPENSIONS

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ABSTRACT

Evans, C. K., Hunger, R. M. and Siegerist, W. C. 1993. Enhanced production of *Pyrenophora tritici-repentis* conidial suspensions. Plant Dis. 77:981-984.

A technique that yields conidial suspensions of *Pyrenophora tritici-repentis* nearly free of other propagules involves growing the fungus in potato-dextrose broth, followed by removal, comminution, and inoculation into liquid clarified V-8 juice agar (CV8). After 48 hr of growth in the dark, the fungus is exposed to alternating periods of light and dark that stimulate the production of conidiophores and conidia in a lawn covering the entire surface of the colony. In two tests using 24 isolates of *P. tritici-repentis*, inoculum suspensions were obtained with an average of 1,386 and 1,692 propagules (conidia, conidiophores, and hyphal fragments) per milliliter, in which 92 and 100%, respectively, of the propagules were conidia. In a third test, the use of a directed stream of water to remove inoculum resulted in a suspension in which 98% of the total propagules were conidia. Use of a glass microscope slide or a rubber spatula to scrape inoculum from agar surfaces resulted in suspensions in which only 67% of the total

propagules were conidia. A fourth test demonstrated that conidial lawns resulted in inoculum yields two to three times greater than those obtained from conidial rings formed on the periphery of fungal colonies grown on CV8 medium. The technique enhances conidial yield and facilitates the production of conidial suspensions nearly free of other *P. tritici-repentis* propagules. The technique will promote more precise and repeatable genetic and epidemiological studies of tan spot of wheat.

Pyrenophora tritici-repentis (Died.) Drechs. [Anamorph: *Drechslera tritici-repentis* (Died.) Shoemaker.], the causal agent of tan spot of wheat (*Triticum aestivum* L.), has been the focus of genetic (9,10,12), epidemiological (6,11,16) and other studies. Although in vitro production of conidia by *P. tritici-repentis* (PTR) has been reported (13), obtaining conidial suspensions unadulterated with other infective propagules (OIP), i.e., conidiophores and hyphal fragments, is difficult. The basic technique previously used to produce conidia involves growing the fungus sequentially on potato-dextrose agar in darkness, followed by growth on V-8 juice agar exposed to alternating periods of light and dark (14). Exposure to light induces *P. tritici-repentis* to produce conidiophores near the perimeter of the fungal colony. A subsequent dark period induces the fungus to produce conidia in a ring near the fungal colony periphery. This results in rings of conidia associated with the period(s) of darkness.

Conidia produced by cultures usually are removed from agar surfaces by flooding petri plates with water and gently scraping the colony surface with a rubber policeman, metal spatula, glass slide, bent glass rod, or wire loop (5,8,15,16,18). Although these techniques are used to produce conidial suspensions, they are not efficient in producing

conidia. For example, the entire surface of the V-8 juice agar is not used for conidial production, and several periods of alternating light and dark are required to produce multiple conidial bands. Most important, removal of conidia from agar surfaces results in suspensions of inocula composed of a conglomerate of conidia, conidiophores, and aerial hyphae (1-3). Quantifying inocula of such a conglomerate is difficult, and reproducing the composition (conidia, conidiophores, and hyphal fragments) of inocula between experiments is nearly impossible. This paper describes a technique that results in high yields of conidia that are produced in lawns over the entire surface of V-8 juice agar in petri dishes, and in conidial suspensions that are relatively free of OIP (4).

MATERIALS AND METHODS

Twenty-four *P. tritici-repentis* isolates obtained from three asci (eight single ascospore isolates [SAI] per ascus) were used in the first two tests. Each set of eight SAI was obtained from pseudothecia that formed on straw infested with *P. tritici-repentis* collected during the summer of 1991 from wheat fields near Altus, Guymon, and Braman, Oklahoma. Isolates were characterized for growth and appearance *in vitro* and stored for later use in cryogenic tubes with one drop of 10% dimethyl-sulfoxide at -70 C (6). The third test consisted of three of the SAI (one each from Altus, Braman, and Guymon) used in the first two tests and three single conidial isolates obtained from infested straw collected from wheat fields located near Clinton, Goltry, and Ponca City, Oklahoma. The three SAI used in the third experiment were used in a fourth test.

PRODUCTION OF CONIDIAL INOCULUM. Isolates of *P. tritici-repentis* were grown on freshly prepared potato-dextrose agar (PDA: 200 g potatoes, 20 g dextrose

and 15 g agar per liter of medium). After 3 days of growth on PDA at 21 C in darkness, five plugs (5 mm dia) were excised from the periphery of each colony and transferred to 100 ml of potato-dextrose broth (PDB; PDA without agar) in a 250-ml Erlenmeyer flask. Flasks were incubated at room temperature (20-25 C), and exposed to room lighting for 12-15 hr on an orbital shaker table at 1200 rpm. After 5 days, the mycelial mass was poured onto a sterile nylon screen and squeezed with a sterile microspoon to remove excess PDB. Then, 10 grams of fresh weight from the mycelial mass was placed in 20 ml of sterile distilled water in a 50-ml beaker and comminuted for 30 sec at 13,500 rpm using a Tissumizer Mark II (Tekmar Co., Cincinnati, OH) with the S25N-10G dispersing tool. Following comminution of each isolate, the dispersing tool was sequentially rinsed once in 1% sodium hypochlorite solution, once in a 95% ethanol solution, and twice in sterile distilled water.

Following comminution, each isolate was set aside until all were prepared within approximately 20 min. First, 2 ml of the comminuted mycelial suspension was added to 100 ml of liquid clarified V8 juice agar (CV8; 3 g $CaCO_3$ and 150 ml V8 juice centrifuged for 10 min at 2000 rpm, plus 15 g of agar per liter of medium) in a 250-ml Erlenmeyer flask maintained at 48 C. The flask was swirled to distribute the comminute in the CV8, which was poured into plastic petri dishes (100 $\times$ 15 mm). Four plates (replications) were poured for each *P. tritici-repentis* isolate. After the plates were incubated for 48 hr at 24 C in darkness, aerial hyphae were appressed to the agar surface using three drops (0.04 ml per drop) of sterile distilled water and a sterile bent-glass rod. Plates were incubated for 24 hr in light (51 $\mu E \cdot m^{-2} \cdot s^{-1}$) and then for 24 hr in darkness at 24 C to induce conidiophore and conidial formation.

Following the final incubation period, plates were examined using a stereomicroscope to verify conidial production.

Conidia were removed from the agar surface with a stream of distilled water (containing one drop of Tween 20 per 100 ml) from a plastic wash bottle. This was done carefully to minimize disruption of the agar medium surface. Each plate was washed with 100 ml of solution, which was sufficient to remove conidia. Plates were examined with a stereomicroscope after washing to determine the effectiveness of conidia removal. One drop of safranin stain was added to a nematode counting dish prior to addition of the conidial suspension to facilitate counting of conidia with a stereomicroscope (7). After conidia were counted, 1 ml of suspension was pipetted onto water agar and observed after 2 hr to determine the percent germination of conidia. Concentrations of conidia and OIP in the suspension were determined, and the percentages of conidial propagules were derived by dividing the number of conidia per milliliter by the sum of conidia and OIP per milliliter. The percent values were transformed to arcsin square root values, analyses were conducted using the SAS GLM procedure, and mean separations were computed following the Waller-Duncan Bayesian *k*-ratio *t* test option with a specified *k*-ratio of 100 to compute the minimum significant difference value (17). The statistical design was a randomized complete block with four replications.

Procedures in a repeated test were the same as in the first test except that 2 ml of comminute was added immediately into the liquid CV8. This was done to minimize the time isolates were left in sterile distilled water. Determination of conidia and OIP concentration and statistical analyses were conducted as in test 1.

REMOVAL OF INOCULUM. Three methods of removing inoculum from the surface of agar medium in petri plates were evaluated using six *P. tritici-repentis* isolates in two experiments. The experimental design was a 3 × 6 factorial with four replications; each test was conducted twice. Procedures in the third test were the same as those in the second test except that an incubation temperature of 21 C was used instead of 24 C to improve conidial production (14).

The first method of inoculum removal involved washing inoculum from the surface of petri plates with a directed stream of distilled water with one drop Tween 20 per 100 ml as described previously. The second method followed a reported technique (16) in which 10 ml of water, with one drop Tween 20 per 100 ml, was added to the surface of a *P. tritici-repentis* colony growing on CV8. Inoculum was gently dislodged by scraping the surface of the colony with a rubber spatula. Inoculum was poured into a 250-ml Erlenmeyer flask, and the plate was rinsed with an additional 10 ml of distilled water with one drop Tween 20 per 100 ml, which was added to the 250-ml flask. The final volume was adjusted to 100 ml, and inoculum composition was determined. The third method of inoculum removal was based on a previous report (5) and is similar to the rubber spatula method except that a glass microscope slide was used to gently scrape the surface of the colony. The volumes used per plate for the initial scraping and rinse were the same as those used in the rubber spatula method. The final volume was adjusted to 100 ml, and inoculum composition was determined. The percent conidia values were transformed to arcsin square root values, and analyses were conducted using the SAS GLM procedure. Mean separations for method of inoculum removal were computed following the Waller-Duncan Bayesian *k*-ratio *t* test option

with a specified *k*-ratio of 100 to compute the minimum significant difference value (17).

CONIDIAL INOCULUM YIELD. The yields of inoculum resulting from two methods of conidial production were evaluated using *P. tritici-repentis* isolates 1, 11, 22 (Table II) in two experiments. The experimental design of each test was a 2 × 3 factorial with four replications; each test was conducted twice.

Two methods were used to produce conidia. One method followed the procedures described earlier resulting in conidial lawns grown over the entire surface of CV8 medium. The second method followed a technique in which more than one alternating light and dark period (2,9) was used to produce conidia on CV8. After 3 days of growth on PDA at 21 C in darkness, four plugs (5 mm diameter) were excised from the periphery of each colony and transferred to the surface of CV8 medium in petri dishes (replications). After the plates were incubated for 72 hr at 21 C in darkness, aerial hyphae were appressed to the agar surface using three drops (0.04 ml per drop) of sterile distilled water and a sterile bent-glass rod. Plates were incubated for 24 hr in light (51 $\mu E \cdot m^{-2} \cdot s^{-1}$) and then for 24 hr in darkness at 21 C to induce conidiophore and conidia formation. Conidia were produced so that inoculum from each method of production was harvested at the same time.

In a second test, each method of conidial production was performed as described except that after hyphae were appressed to the agar, plates were incubated for 24 hr in light followed by 24 hr in darkness, 24 hr in light, and 24 hr in darkness. This resulted in the formation of two conidial rings on the agar surface.

In both experiments, conidia were removed from agar surfaces with a stream of

distilled water, and volumes were adjusted to 100 ml as described previously. Conidia and OIP were counted in a nematode counting dish, and yields of conidia and OIP per petri dish for each method of inoculum production were derived by multiplying the counts by 100. Sporulation density was determined by dividing the number of conidia by the surface area of the agar medium in a 100 × 15 mm petri dish. Both experiments were conducted twice, and separate analyses were made following the SAS ANOVA procedure (17). Mean separations were conducted using an LSD computed at $P=0.05$.

RESULTS

All isolates used in the four tests were dark green when grown on PDA and typically produced conidiophores and conidia at their colony edges when grown on CV8 as described previously (7). In all tests, the viability of conidia, as indicated by germination on the surface of water agar, was greater than 98%.

PRODUCTION OF CONIDIAL INOCULUM. The statistical analyses with tests one and two indicated there was an isolate by test interaction for conidial production. Hence, data from tests one and two were analyzed and are presented separately (Table III).

In tests one and two, significant differences in conidial production among isolates were detected. The SAI from Altus (isolates 1-8) and Braman (isolates 17-24) produced more conidia in the second test. With the exception of isolate 11, production by SAI from Guymon (isolates 9-16) was stable over tests

In tests one and two, there were no significant differences among isolates for the percentage of conidia in suspension; this ranged from 92 to 100% in test 1, and from 95-99% in test 2 (Table II). There were significant differences among isolates with

regard to OIP in the first test but not in the second test. In both tests, suspensions of conidia nearly free of OIP were obtained for all isolates.

METHODS OF INOCULUM REMOVAL. The analysis indicated that there were significant differences in inoculum yields between tests, among inoculum removal methods, and among isolates for number of conidia, number of OIP, and percent conidia per milliliter (Table III). For each of these variables, the inoculum removal method explained the greatest magnitude of the variation observed. The analysis also indicated there was no significant interaction between inoculum removal method and *P. tritici-repentis* isolate. Mean yields of inocula were higher in the second test. Data were presented from the tests as means for the inoculum removal method averaged over the six *P. tritici-repentis* isolates to show the effects of using different methods of inoculum removal (Table IV). The percent conidia of the total propagules in suspension was significantly higher when a directed stream of water from a plastic wash bottle was used than when the other two methods were used (Table IV). The percent conidia was identical with the rubber spatula and a microscope slide methods of removal.

CONIDIAL INOCULUM YIELD. In both experiments comparing the methods of inoculum production, significant differences were observed among isolates and between methods of inoculum production. Significant method × isolate interactions were observed in both experiments as well. There were no significant differences between tests that were repeated.

Mean yield of conidia, induced by exposure of *P. tritici-repentis* isolates to one photoperiod, was significantly higher when produced over the entire surface of the

medium in petri dishes in conidial lawns (Table V); the yield of conidia obtained by growing conidia in a ring was approximately one-third that of the yield obtained from conidial lawns. Mean yield of conidia induced by exposure of *P. tritici-repentis* isolates to two photoperiods was also significantly higher when produced in a conidial lawn. Again, spore density of conidia produced in a lawn was significantly greater than that of conidia grown in two conidial rings.

DISCUSSION

We have described a technique that consistently results in suspensions of conidia relatively unadulterated with OIP when using an array of SAI and single-conidium isolates of *P. tritici-repentis* collected from different geographic regions of Oklahoma. By comparison, scraping *P. tritici-repentis* colonies on agar surfaces with an instrument such as a rubber spatula or a glass microscope slide results in conidial suspensions that contain a high proportion of OIP. We specifically demonstrated that using a directed stream of water to remove conidia results in suspensions with significantly lower concentrations of OIP. Suspensions with high numbers of OIP may be appropriate in some studies but may not accurately reflect natural inoculum when used in genetic and epidemiological studies of tan spot. Additionally, production of conidial suspensions free of OIP minimizes conglomeration of conidiophores, hyphal fragments, and conidia that typically occurs when large amounts of OIP are present in the suspension (*unpublished*). Also, the concentration of conidia in a suspension nearly free of OIP is easily adjusted by collecting conidia on Nitex nylon screen (25 μm) with resuspension in a smaller volume of water. Conidia readily become resuspended rather than conglomerate with the OIP because the amount of OIP is extremely low.

This technique also allows production of conidia that are ontogenetically similar, in contrast to those produced from multiple conidial rings. Additionally, we have demonstrated that inoculum yields can be significantly increased by utilizing the entire surface of agar medium in petri dishes to produce conidial lawns. We used an excessive volume of water to remove conidia from the surface of agar medium mainly to standardize our procedures and simplify counting conidial concentrations. Lesser volumes of water could be used to remove conidia from agar surfaces and thus increase the concentration of conidia in the suspensions. Furthermore, this technique may be adaptable for inoculum preparation using fungi in similar or related groups.

We should note that the technique did not consistently differentiate the abilities of our *P. tritici-repentis* isolates to produce conidia, and other methods may be more appropriate for this determination. However, our experiments were specifically designed to demonstrate a technique that efficiently produces higher yields of *P. tritici-repentis* conidia and conidial suspensions nearly free of OIP, rather than to differentiate the abilities of fungal isolates to produce conidia. We feel that the reported technique will facilitate precise, quantifiable, and repeatable studies that address the infection kinetics of *P. tritici-repentis* conidia as well as other genetic and epidemiological studies where precise, uniform inoculum of *P. tritici-repentis* conidia is required.

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Table II. Asexual inoculum production and composition by isolates of *Pyrenophora tritici-repentis* produced by directed washing of cultures grown in clarified V-8 juice medium

	Inoculum composition per milliliter					
	Test 1			Test 2		
Isolate[v]	Conidia	OIP[w]	Conidia[x] (%)	Conidia	OIP[w]	Conidia[x] (%)
1	913 bc[y]	6 de	99.3	1676 a	1	99.9
2	852 b-e	14 c-e	98.7	1690 a	2	99.9
3	857 b-e	7 de	99.4	1379 ab	4	99.7
4	650 b-g	9 de	98.7	750 de	2	99.8
5	781 b-f	6 de	99.2	1272 a-c	3	99.8
6	747 b-f	3 de	99.8	1530 ab	1	99.9
7	902 b-d	9 de	99.3	1540 ab	4	99.7
8	572 e-g	5 de	99.4	1209 bc	1	99.9
9	963 b	28 bc	97.4	506 d-g	6	98.3
10	500 f-h	26 bc	92.5	405 e-g	2	99.2
11	1320 a	46 a	96.7	690 d-f	2	99.8
12	570 e-g	13 c-e	97.6	632 d-g	4	99.3
13	572 e-g	32 ab	95.7	410 e-g	3	99.4
14	476 f-h	6 de	99.1	417 e-g	4	98.5
15	580 d-g	15 c-e	97.7	682 d-f	4	99.3
16	582 d-g	9 de	98.9	845 cd	6	99.4
17[z]	0 j	0 e	-	206 g	3	99.3
18	25 j	1 de	92.3	364 e-g	4	99.3
19	215 h-j	6 de	99.1	313 fg	5	95.6
20	383 g-i	9 de	99.0	506 d-g	7	98.1
21	92 ij	0 e	100.0	431 d-g	8	97.5
22	508 f-h	4 de	99.2	600 d-g	2	99.5
23	131 ij	1 de	99.8	624 d-g	3	99.7
24	605 c-g	17 b-d	98.1	361 e-g	2	99.5

[v]Isolates 1-8, 9-16, and 17-24 represent single ascospore isolates from three asci from Altus, Guymon, and Braman, Oklahoma respectively.

[w]Other infective propagules, i.e., conidiophores and hyphal fragments.

[x]Data were transformed to arcsin square root values for analysis; actual percentages are shown.

[y]Means within columns followed by the same letter are not significantly different according to the Waller-Duncan Bayesian *k*-ratio *t*-test with a specified *k*-ratio of 100. Means in columns without letters are not significantly different.

[z]Omitted from the analysis of percent conidia in Test 1.

Table III. Analysis of variance for the comparison of several techniques used to harvest inoculum of *Pyrenophora tritici-repentis* from the surface of clarified V-8 juice medium

Source	df	Mean square values × 10^8 Conidia (no./ml)	OIP[v] (no./ml)	Conidia[w] (%/ml)
Test	1	1313.33 **[x]	276.36 *	0.1335 **
Rep(Test)	6	85.94 NS	28.93 NS	0.0099 *
Method[y]	2	2905.43 **	2549.13 **	1.4919 **
Isolate[z]	5	254.34 NS	35.86 NS	0.0104 *
Method × Isolate	10	5.78 NS	10.40 NS	0.0034 NS
Error	119	77.95	17.03	0.0045

[v]Other infective propagules, i.e., conidiophores and hyphal fragments.
[w]Values were transformed by the arcsin square root method for the analysis of variance.
[x]NS=not significant, *=significant at $P \leq 0.05$, and **=significant at $P \leq 0.01$.
[y]Inoculum was removed from cultures by scraping with a rubber spatula or a glass microscope slide or by washing with a directed stream of water from a plastic wash bottle.
[z]Isolates were collected from wheat fields near Altus, Braman, Clinton, Goltry, Guymon, and Ponca City, Oklahoma.

Table IV. Quantification of inoculum composition using three harvest methods evaluated over two tests utilizing six isolates of *Pyrenophora tritici-repentis* grown in clarified V-8 juice medium in a factorial design

	Inoculum composition per milliliter[w]		
Method[w]	Conidia	OIP[x]	Conidia[y] (%)
Directed washing	4033 a	68 c	98.2 a
Rubber spatula	2900 b	1419 a	67.7 b
Microscope slide	2542 c	1217 b	67.7 b

[w]Means within columns followed by the same letter are not significantly different following the Waller-Duncan Bayesian *k*-ratio *t*-test with a specified *k*-ratio of 100 to compute the minimum significant difference.

[x]Inocula were removed by scraping colonies with a flat rubber spatula; scraping with a glass microscope slide; or washing colonies with a directed stream of water from a plastic wash bottle.

[y]OIP = other infective propagules (conidiophores and hyphal fragments).

[z]Percent conidia were analyzed after transforming the data to arcsin square root values. The actual percentages are presented.

Table V. Quantification of mean inoculum yield and conidia density of *Pyrenophora tritici-repentis* per petri dish of clarified V-8 juice medium comparing two inoculum production techniques in tests with one or two photoperiods[v]

Test[v] Method	Harvested inoculum composition[w] × 10^2 Conidia		OIP[x]		Conidia[y] (no./mm^2)	
One-photoperiod test						
Conidial lawn	3395	a[z]	93	a	56.8	a
Conidial ring	955	b	81	a	16.1	b
Two-photoperiod test						
Conidial lawn	2977	a	211	a	50.1	a
Conidial rings	1582	b	206	a	26.6	b

[v]Each test was conducted twice using isolates 1, 11, and 22, (Table II) in a factorial design with four replications.

[w]Inoculum was removed by washing colonies with a directed stream of water from a plastic wash bottle.

[x]Other infective propagules, i.e., conidiophores and hyphal fragments.

[y]Spore density per unit surface area of medium was derived by dividing the yield of conidia by the surface area of medium in a 100 × 15 mm petri dish.

[z]Mean propagule yields per petri dish within columns of each test followed by the same letter are not significantly different according to the least significant difference test ($P = 0.05$).

CHAPTER III

INOCULUM DENSITY AND INFECTION EFFICIENCY OF CONIDIA AND CONIDIOPHORES OF ISOLATES OF *PYRENOPHORA TRITICI-REPENTIS*

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ABSTRACT

Evans, C. K., Hunger, R. M., and Siegerist, W. C. 1995. Inoculum density and infection efficiency of conidia and conidiophores of isolates of *Pyrenophora tritici-repentis*. Plant Dis. 79:(Submitted January 4, 1995).

Glass slides and wheat leaves were inoculated with conidia and conidiophores of a *P. tritici-repentis* isolates to compare the density (no./cm^2) of propagules on non-host 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 'TAM 105' (susceptible) and 'Red Chief' (resistant) wheat cultivars to compare disease severity resulting from the different forms of propagule. Conidia caused 26 times more lesions than did conidiophores, and differences among the isolates for disease severity 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 disease severity as a function of the density of propagules per unit of inoculated leaf area. 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 estimates 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 (7-9,17,19). Studies with *P. tritici-repentis* have utilized different inoculum concentrations and/or focused on seasonal airborne spore populations relating inoculum density to the progression of tan spot (12,18,26,32). Other studies of tan spot on wheat focused on the effects of temperature (11,25), post-inoculation leaf wetness duration (10,11), leaf position or age (2,10,28), and the role of toxins in tan spot reactions (1,22-24,33). However, 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 (21,23,24), bent glass rods (11), the edge of a glass slide (10), and blended agar discs with conidia, conidiophores and hyphae (33) to prepare inoculum of *P. tritici-repentis* for subsequent inoculations onto

wheat plants. All of these techniques for inoculum production 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 (31) 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 (5) examined the effects of propagule type, inoculum dosage, and spatial interaction among lesions on infection phenotype, infections per unit area, and percent disease severity. 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 fully expanded leaf at the time of inoculation to effectively differentiate between resistant and susceptible wheat genotypes. Riaz *et. al.* (28) described a procedure to quantify production of *P. tritici-repentis* conidia on wheat leaves. They revealed 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"; to do this would require an understanding of 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 on defined areas of wheat leaves and glass slides, compare the severity of tan spot resulting from inoculation with conidiophores and conidia at equal inoculum density on wheat leaves, 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), Oklahoma (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 hr 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) Tween 20 per 100 ml. Conidiophores were then dislodged by 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 cheese-cloth. Concentrations of conidiophores or conidia were determined in a nematode counting dish and suspensions were adjusted to the desired concentration(s) (13). In all tests, the viability of conidia and

conidiophores from each *P. tritici-repentis* isolate was assessed by inoculating each propagule onto water agar in petri dishes (100 × 15 mm). Following inoculation, petri dishes were maintained at 21 C in the dark. After 12 hr, 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 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 (DeVilbiss Pulmo-Aide Model 5601D) 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, 2555 Kerper Boulevard, Dubuque, Iowa 52001) on an adjustable stand to allow height adjustment. An inoculation stage 10 cm long by 7 cm wide was fabricated from a one 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 three 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,

Ohio). Timed intervals for all applications were set for 1.5 seconds. The glass cylinder was swabbed between inoculations with a paper towel. The cylinder was sterilized with ethanol between inoculations of each isolate.

Suspensions of conidia and conidiophores prepared from the 91RBB6 isolate were used in inoculum density assessments. The concentrations of conidia used in the first density assessment experiment were 1,968, 4,592, 6,128, and 10,688 conidia/ml and in the next density assessment experiment were 2,048, 4,512, 7,528, 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 three cm^2 area on each of five glass slides (3 $\times$ 1 cm, L $\times$ W) and defined areas on the adaxial surface of five leaves of the wheat cultivar 'TAM-105'. Propagules inoculated onto the surfaces of glass slides and wheat leaves were counted using a stereomicroscope. Propagule density (no./cm^2) was derived by dividing the number of propagules observed by the area that was inoculated. Inoculated leaf areas were 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 as a function of conidia or conidiophore inoculum concentration, on adaxial wheat leaf surfaces and defined areas of glass microscope slides was determined. Statistics were computed using the regression (REG) procedure of the SAS statistical program (29).

Estimates of slope parameters and *y*-intercepts of the linear models were compared using the general linear models (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 disease severity of each propagule at an equal density; in the third experiment they were utilized to estimate the infection efficiencies of the three *P. tritici-repentis* isolates utilizing their conidia and conidiophores separately.

COMPARISON OF DISEASE SEVERITY DUE TO INFECTIONS CAUSED BY CONIDIA AND CONIDIOPHORES. The second experiment determined the disease severity on two wheat cultivars from inoculations of 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 propagules of the pathogen causing tan spot (conidia vs. conidiophores). 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 (110 mm) and fertilized with 1.6 g/pot of 14-14-14 Osmocote® fertilizer. Wheat plants were grown in a controlled environment growth chamber under a 12 hr lighted period (610 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 20 C and 12 hr dark period at 15 C. Relative humidity in the growth chamber was maintained at 30% and wheat plants were

inoculated after five or six weeks growth. The middle three cm of the uppermost fully expanded leaf (top leaf-1) of four plants in each pot (replication) was inoculated with each form of propagule of each isolate on the two wheat cultivars. Using the regression functions (Figs. 1 and 2) for the respective propagules, inoculum concentrations of conidia were adjusted to 10,000/ml and conidiophores were adjusted to 15,000/ml to standardize the propagules densities when inoculated on wheat leaf surfaces. Leaves of the control plant leaves were inoculated in the same fashion with distilled water and Tween 20. Following inoculation, plants were allowed to air dry and then placed in a mist chamber providing >95% relative humidity for 12 hr in light (46 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 22-24 C followed by 12 hr in darkness at 18-20 C. After the 24 hr post-inoculation wet period, misting was stopped and plants were maintained in the mist chamber. The number of lesions in the length of inoculated wheat leaf were counted four to six days following the post-inoculation wet period. After counting the lesions, the inoculated leaf area was excised and measured as described earlier. Estimates of the density of propagules that were deposited onto the wheat leaves were based on the inoculated leaf areas. Inoculum concentrations for the respective propagules were entered into their corresponding regression functions (Figs. 1 and 2) to estimate the propagules 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 (top leaf-1 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 (disease

severity). Statistical analyses were conducted on the combined data of the repeated tests following the SAS analysis of variance procedure (ANOVA) (29). Main effect means were separated using Fishers least significant difference test (*L.S.D.)* computed at $P \leq 0.05$.

INFECTION EFFICIENCY OF CONIDIA AND CONIDIOPHORES. The third set of experiments were designed to estimate infection efficiencies of the three *P. tritici-isolates* 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 (disease severity) 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 that was utilized. Four concentrations of conidial suspensions containing 4,000, 5,500, 7,500, and 10,000 conidia/ml, were utilized to inoculate leaves. A total of 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 disease severity (no. 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 the *P. tritici-repentis* isolates conidia (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 that was utilized. Wheat leaves of TAM 105 and Red Chief were inoculated with suspensions of conidiophores containing 10,000, 20,000, and 40,000 conidiophores/ml. A total of six experiments were conducted. Infection efficiency of conidiophores of the *P. tritici-repentis* isolates was determined from the regression of disease severity as a function of the density of conidiophores on wheat leaf surfaces averaged over the two wheat cultivars as described previously.

Residual analyses were conducted for disease severity data. The disease severity values were weighted for both propagules using the weight option described in the GLM procedure of the SAS program (29) 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.

RESULTS

The three *P. tritici-repentis* isolates utilized in these and other studies (3), had a typical dark green appearance on potato dextrose agar. Conidiophores and conidia were produced prolifically on clarified V8 juice agar. Inoculation of conidia from these isolates onto leaves of TAM 105 and Red Chief resulted in lesions typical of tan spot, which have a small black necrotic spot surrounded by a tan necrosis with associated chlorotic halos.

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(s). Germination of conidia on water agar was greater than 98%, and germination of conidiophores on water agar was greater than 97%.

INOCULUM DENSITY ASSESSMENT. The first experiment demonstrated that inoculating propagules of *P. tritici-repentis* onto artificial surfaces such as 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, respectively). 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, respectively). 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. The comparison of the slope parameters indicated the density of conidia inoculated on glass was four times greater than the density of conidia inoculated on adaxial surfaces of wheat leaves (P=0.0001). 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). 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 propagules density on adaxial surfaces of wheat leaves as indicated by significant differences between the slopes and intercepts of the regression functions.

COMPARISON OF DISEASE SEVERITY 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 (Table VI). 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 to the deposition of conidiophores per wheat leaf surface (Table VII). There was a significant difference between wheat cultivars, isolates, and propagules when each was averaged over the levels of the other two main effects for disease severity. Mean separations of the two wheat cultivars for disease severity confirmed that Red Chief was more resistant to tan spot having fewer lesions/cm^2 on inoculated leaf portions than TAM 105. Mean separations of the three *P. tritici-repentis* isolates for disease severity revealed that isolate 91ALA1 was more virulent than the other isolates as evidenced by the greater number of lesions/cm^2 produced by 91ALA1 on inoculated wheat leaf portions. Mean separations of the two propagules for disease severity was dramatic. Conidia were 26 times more effective at causing infection than conidiophores as shown by the large difference in the number of lesions/cm^2 each produced when inoculated onto portions of wheat leaves. This extreme difference in disease severity at a nearly equivalent 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 disease severity. 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 level of the isolates. The estimated number of conidia deposited per leaf portion of Red Chief was 29.04 (*se*=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 disease severity 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). Thus, averaged over the level of isolates, conidia were much more infective than conidiophores on the two wheat cultivars.

The significant isolate by propagule interaction for disease severity revealed that each isolates' 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 conidial infections 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). Thus, averaged over the level of 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 disease severity due to infection of the three *P. tritici-repentis* isolates. Disease severity of the three isolates increased in a linear fashion with increasing density of conidia (Fig. 3). The *y*-intercepts of the regression functions of disease severity 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 disease severity 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 44-70% of the variation of disease severity. Disease severity due to infection from conidiophores was much lower than infections caused by conidia (Fig. 4). The *y*-intercepts of the regression equations of disease severity 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 disease severity due to infection by conidiophores of 91RBB6 was significantly different from zero (P=0.0001). The slopes of the regression equations of disease severity 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 disease severity due to conidiophore

infections of 91ALA1 and 91GYA3 respectively, revealed there was no difference between the models estimating their infection efficiencies. The slope of the regression equation of disease severity due to infection by conidiophores of 91RBB6 was significantly different from zero (P=0.0001). The comparison of the slope and y-intercepts of the regression of disease severity due to infection by conidiophores revealed the 91RBB6 isolate was significantly different from the 91ALA1 (P=0.001 and P=0.001, respectively) and the 91GYA3 (P=0.003 and P=0.009, respectively) isolates. However, residual analysis (4) demonstrated that the variance of disease severity was proportional to the increase in deposition of conidiophores of the 91RBB6 isolate. Thus there seems to be either no relationship between conidiophore density and disease severity (Fig. 3A and 3B), or the relationship is extremely variable (Fig. 3C).

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 oriented where the adaxial and abaxial surfaces of some portion of their length is oriented 90 degrees to the direction of the inoculum being applied. In this manner portions of wheat leaves have little or no potential of being inoculated. Schein (30) and others (5,6,20,27) 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 from readily available lab supplies so that the adaxial surface of a three 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 (5).

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 (14). 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, however, direct counts of propagule density on wheat leaves was not reported. We found our estimates of infection efficiency would be three to four times too low had we based them on the propagule density we observed on glass slides. We did not quantify differences of propagule density that may occur among wheat cultivars; this remains to be examined. The extent propagules overlap (eg. 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 epidemiological studies of *P. tritici-repentis* on wheat should be based on estimates of the pathogens propagule density on wheat leaf surfaces.

The results of disease severity from infection by conidia and conidiophores on wheat confirm and strengthen those of Francl and Jordahl (5,15). They reported that conidia were 25 times more infectious than conidiophores (15) 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 (5). We determined that disease severity on wheat from infection by conidia of *P. tritici-repentis* was 26 times greater than infection by conidiophores (Table VII). In addition, we found that the 91ALA1 isolate was the most virulent and caused greater disease severity than 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 (16) 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 results were similar in that our methods of inoculum production and inoculation enable us to quantitatively identify isolates with high levels of virulence and infection efficiency to screen for improved resistance to tan spot of wheat.

The focus of the third study was to specifically demonstrate differences among the *P. tritici-repentis* isolates, utilizing their conidiophores and conidia separately, to assess their infection efficiency. In part, this was appropriate due to a lack of cultivar by isolate interaction for disease severity in the second test. The regression of disease

severity 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 disease severity as a function of conidiophore density were extremely variable. Hence, making accurate estimates of the infection efficiency of *P. tritici-repentis* isolates, using conidiophores, is highly questionable. These experiments demonstrate the importance of excluding conidiophores from P. *tritici-repentis* inoculum when assessing the virulence 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 an isolate(s) inoculum composition as demonstrated by the variability of infection efficiency among the isolates utilizing their conidiophores to infect wheat leaves (Fig. 4A-C).

The 91ALA1 isolate exhibited the highest level of virulence of the three that were studied as determined by the slope of its regression function from infection with conidia. Francl and Jordahl (5) 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 disease severity ratings (%) as a function of inoculum concentration. The slope coefficients of the isolates we studied were much higher but were derived from disease severity as a function of estimated propagule density on wheat leaf surfaces. Previous investigators (10,16) reported that Hunger and Brown (13) 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. Although our estimates of infection efficiency likely are affected by other parameters such as temperature and duration of leaf wetness, additional studies incorporating these parameters are needed in order to develop predictive models for field based epidemiological studies of tan spot.

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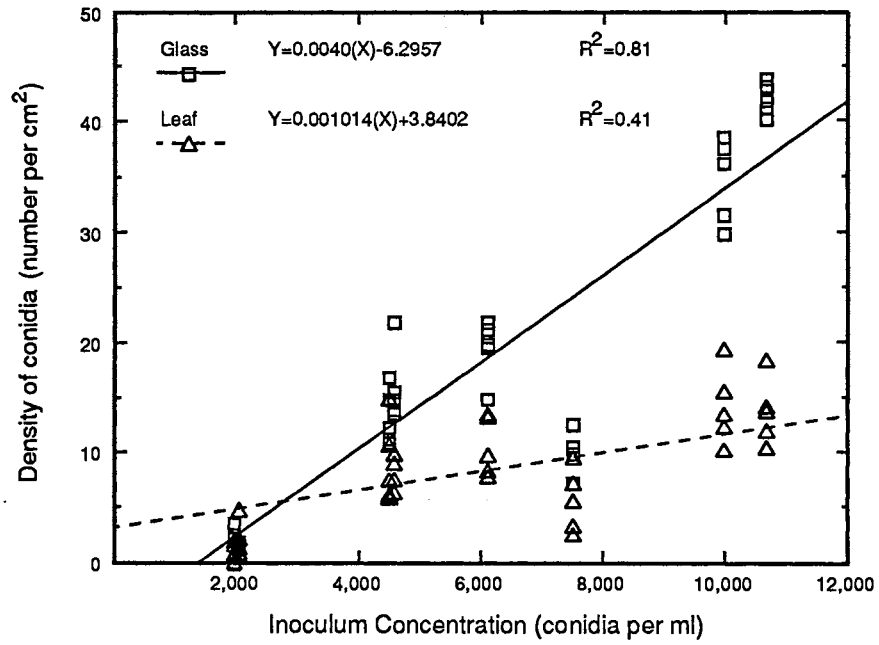

Fig. 1. Density of conidia on the surface of defined areas of glass (□) slides and adaxial surfaces of 'TAM 105' wheat leaves (Δ) as a function of conidial inoculum concentration.

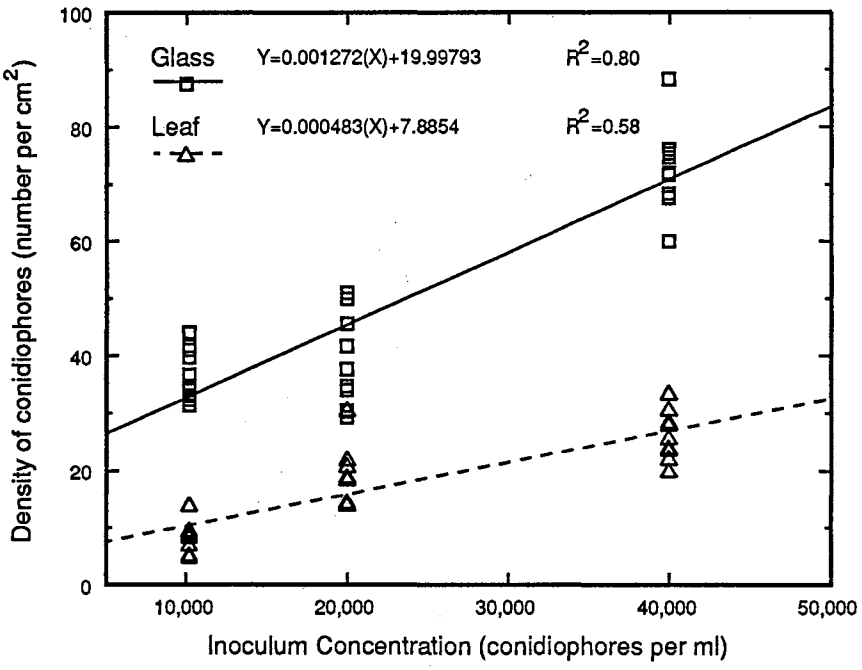

Fig. 2. Density of conidiophores on the surface of defined areas of glass (□) slides and defined adaxial surfaces of 'TAM 105' wheat leaves (Δ) as a function of conidiophore inoculum concentration.

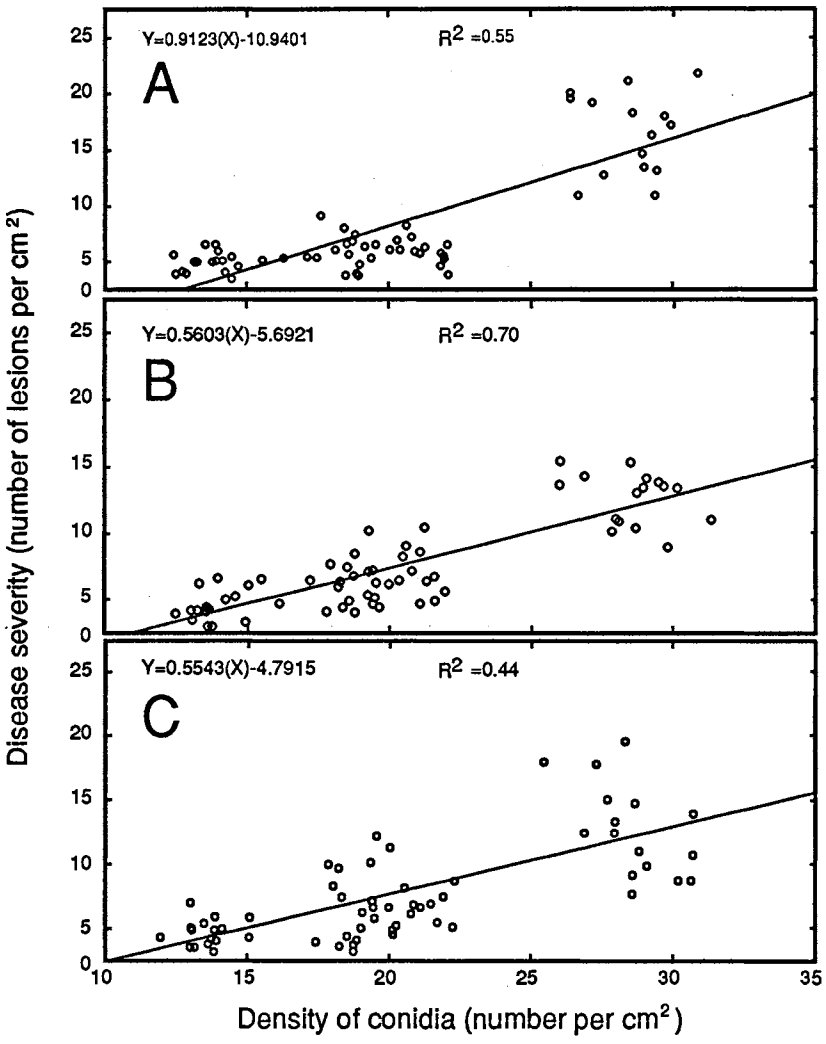

Fig. 3. Relationship between disease severity and estimated density of conidia on adaxial surfaces of 'TAM 105' and 'Red Chief' wheat leaves with three *P. tritici-repentis* isolates. Figures A-C represent the linear equations for the isolates 91ALA1 (A), 91GYA3 (B), and 91RBB6 (C), respectively.

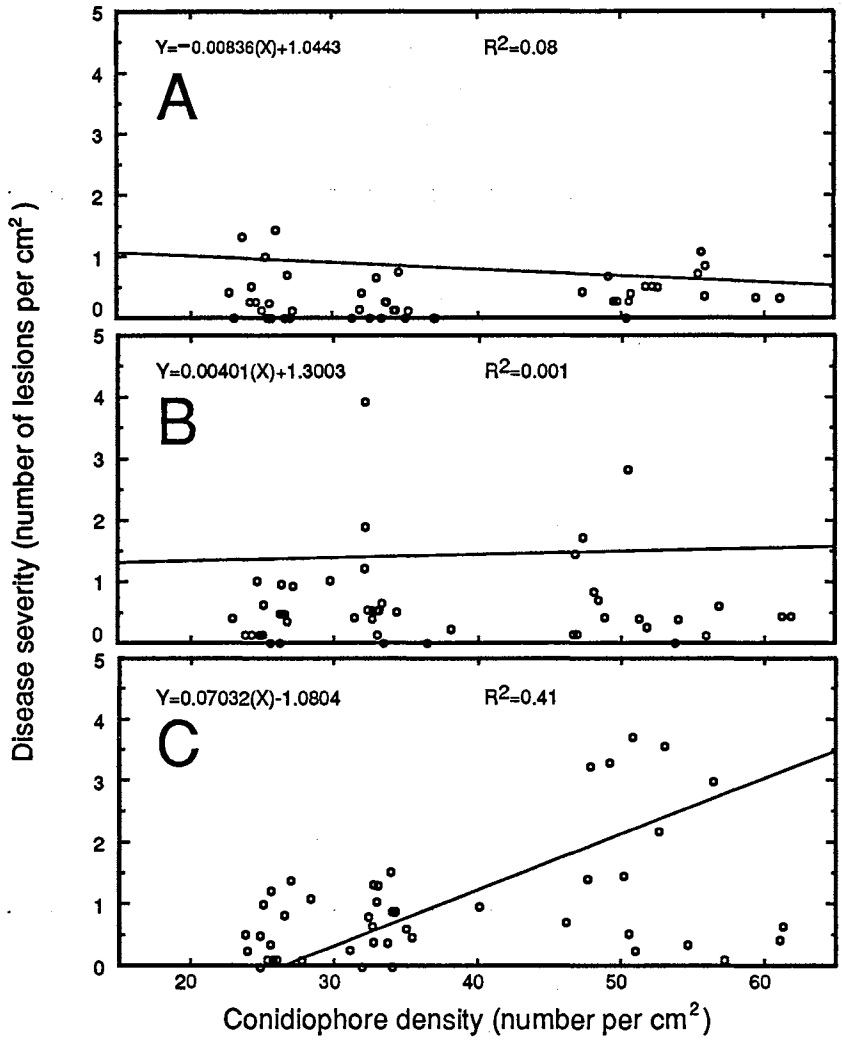

Fig. 4. Relationship between disease severity and estimated density of conidiophores on adaxial surfaces of 'TAM 105' and 'Red Chief' wheat leaves with three *P. tritici-repentis* isolates. Figures A-C represent the linear equations for the isolates 91ALA1 (A), 91GYA3 (B), and 91RBB6 (C), respectively.

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

		Mean square values		
Source	df	Inoculated Leaf Area (cm^2)	Estimated[u] Propagule Deposition (no./leaf)	Disease[v] Severity (no. lesions/cm^2)
		($\times 10^{-3}$)		($\times 10^{-2}$)
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**	3108.33**
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 was derived using the inoculum concentrations with the regression functions (Fig. 1) corresponding to each propagules deposition onto adaxial wheat leaf surfaces. The resulting value was multiplied by the measured leaf area to arrive at an estimated propagule deposition value per inoculated leaf section.

[v]Disease severity was assessed by counting the number of visible lesions on a three 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, Oklahoma, respectively.

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

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

Main effect[y] Levels	Inoculated Leaf Area (cm^2)	Estimated[w] Propagule Deposition (no./leaf)	Disease[x] Severity (no. lesions/cm^2)
Cultivar			
Red Chief	2.02[z]	29.42	4.95 b
TAM-105	2.03	29.68	7.31 a
L.S.D.	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
L.S.D.	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
L.S.D.	0.03	0.57	0.68

[w]Propagule deposition was derived using the inoculum concentrations with the regression functions (Fig. 1) corresponding to each propagules deposition onto adaxial wheat leaf surfaces. The resulting value was multiplied by the measured leaf area to arrive at an estimated propagule deposition value per inoculated leaf section.

[x]Disease severity was assessed by counting the number of visible lesions on a three cm length of inoculated area in the middle of a wheat leaf.

[y]The three main effects consisted of two wheat cultivars, three *P. tritici-repentis* isolates, and two propagules of the same pathogen.

[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 test (P=0.05).

CHAPTER IV

EVALUATION OF WHEAT GENOTYPES FOR RESISTANCE TO TAN SPOT UNDER GREENHOUSE AND FIELD CONDITIONS

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ABSTRACT

Evans, C. K., Hunger, R. M., and Siegerist, W. C. 1995. Evaluation of wheat genotypes for resistance to tan spot under greenhouse and field conditions. Plant Dis. 79:(In preparation).

Eight winter wheat genotypes selected from the 1992 Southern Regional Performance Nursery were evaluated for tan spot resistance at two locations in Oklahoma based on their reaction to *P. tritici-repentis* infection in previous greenhouse screening tests. A mist irrigation system was constructed in the field to increase the duration of free moisture in plots that were inoculated with an aqueous suspension containing conidia from three *P. tritici-repentis* isolates using a gas powered backpack sprayer. Tan spot infection was promoted by misting plots for 30 minutes at varying intervals following inoculation. Tan spot infection was uniform at both locations. Visual assessments were conducted at seven-day intervals following infection and differences among the eight wheat genotypes resistance to tan spot were determined using the area under the disease progress curve (AUDPC). The mean grain yield of tan spot-inoculated split-plots was reduced an average of 15 to 17% (P=0.05) compared to the yield of the

disease-free split-plots. Rank correlation coefficients of greenhouse measurements of tan spot lesion lengths and field assessments of the AUDPC on the eight wheat genotypes demonstrated a significant level of concordance ($P \leq 0.05$) between the two assessment methods of tan spot resistance. Thus, greenhouse assessments of tan spot reactions on wheat seedlings provided a good indication of field reaction to tan spot. Additionally, the technique of inoculation and mist irrigation could be utilized further to screen promising lines of wheat under field environments.

Pyrenophora tritici-repentis (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.) Shoemaker) is a fungal foliar pathogen of many gramineous species (20-25) and is also a foliar and seed pathogen of wheat (*Triticum* spp.) causing tan spot on leaves and stems and pink smudge of grain (12,16,34,40). Tan spot is capable of causing significant yield and quality losses of spring and winter wheat (*Triticum aestivum* L.) (17,31,35) and occurs in major wheat producing regions world wide (41). Investigations of environmental conditions favorable for tan spot infection on wheat have shown that a post-inoculation wet period of 6-48 hr is required which is influenced by temperature as well as host genotype (18,19). Additional investigations have found that tan spot infections are usually more severe on older (lower) leaves compared to younger (higher) leaves of wheat plants (8,18). In another study (32), it was demonstrated that secondary inoculum was produced on leaves of susceptible wheat cultivars at a faster rate and in larger quantities than on leaves of resistant cultivars. Older leaves of a tan spot-resistant wheat cultivar also produced more secondary inoculum compared to younger leaves of the same cultivar. Other studies have demonstrated that dramatic yield losses result when infections occur during the

boot to heading stages resulting in severe flag leaf damage (8,31,41). Toxins of *P. tritici-repentis* have been identified (3,6,39) and evidence of a variable tan spot reaction, based on the specific combination of isolate and host genotype, has been proposed (27-30). Furthermore, differences in virulence among isolates have been demonstrated in several studies (9,18,23,24).

Reduced tillage or low-till systems utilized by some wheat producers have been shown to maintain higher quantities of wheat residue on the soil surface, thus increasing the potential for inoculum of *P. tritici-repentis* to build up (1,4,44). The combinations of cultural practices, host genotype, weather conditions for infection, and isolate variability, present breeders with a multitude of factors to consider when breeding for tan spot resistance. Plant pathologists have developed resistance screening procedures that involve inoculating wheat seedlings with propagules of *P. tritici-repentis* and initiating tan spot infection in the greenhouse (11,30). Several methods of inoculating wheat plots in the field have also been developed (1,30). These methods involve inoculation with oat kernels or wheat straw infested with the fungus. The inoculum can be distributed within or between wheat plots and after a period in which pseudoperithecia mature, ascospores are finally ejected to initiate tan spot infections on wheat leaves. Other methods utilized inoculum consisting of conidia, conidiophores, and mycellium which are applied directly onto wheat plants (9,30). However, no method of initiating tan spot infection in a large scale field screening test at a specific point in time has been reported. Few reports have been published comparing the performance of wheat genotypes in greenhouse tests compared to field based assessments for their reaction to tan spot (5,30). Therefore, the objectives of this study

were to develop a field screening technique to evaluate tan spot reaction of wheat genotypes in the field initiated on a large scale over several gentoypes; assess disease progress on several wheat genotypes that were selected based on previous grenhouse tests, and to compare their field and greenhouse reactions (11).

MATERIALS AND METHODS

Ten wheat genotypes were selected from the 1992 Southern Regional Performance Nursery to test for resistance to tan spot under field conditions based on their reactions to tan spot in previous greenhouse screening tests (12). The wheat cultivar 'TAM 105' was included as a tan spot-susceptible check and 'Red Chief' was included as the tan spot-resistant check cultivar. Based on the analyses of mean tan spot lesion length, wheat genotypes 'KS84170E-8-3' and 'TX88V5440' had resistance similar to Red Chief. Mean lesion length on the genotypes 'KS87H325-2', 'OK88W833', 'TX88V4636', and 'XH1319', indicated they were moderately susceptible to tan spot; whereas, mean lesion length on leaves of wheat genotypes 'CO870449' and 'N87V106' indicated that they were equally or more susceptible than TAM 105 (Table VIII).

PLOT DESIGN AND INOCULATION. One test was conducted at the Agronomy Research Station near Perkins, a second test was conducted at the Plant Pathology Research Farm in Stillwater, Oklahoma. The soil type at Perkins was a Teller loam whereas the soil type at the Stillwater test location was an Easpur loam. Soil tests were conducted at the two locations and fertilizer was applied for a yield goal of 2016 kg/ha. The test site at Perkins was fertilized with ammonium nitrate to provide 52.6 kg/ha of additional N seven days prior to planting. The test site at Stillwater was fertilized in a similar fashion to provide 38.1 kg/ha of additional N seven days prior to planting.

Plots consisted of 2 rows on 22.8 cm centers that were 1.52 m in length. Plots were separated from each other by a blank row (i.e. a 22.8 cm skip). Thus, the area of each plot represented 1.04×10^{-4} ha. Each row was planted with 3.5 g of wheat seed. The test at Stillwater was seeded September 28 and the test at Perkins was seeded October 1, 1992. The experimental design was a split-plot with four randomized blocks. Blocks were separated by a 1.52 m alley width and the entire test was bordered on all sides with the wheat cultivar 'Cimmaron'. Main plots at each location consisted of the ten wheat genotypes. The split-plot treatments consisted of one half of each main plot sprayed with propiconazol (Tilt®) as a disease-free control; the second half of each plot was inoculated with conidia of *P. tritici-repentis* prepared as described earlier (10). Disease-free split-plots were sprayed by placing a fiberglass sheet at the midpoint of each plot to prevent applications of the fungicide from drifting onto the tan spot-inoculated split-plot of each main-plot. Plots were sprayed with a hand sprayer at a rate equivalent to 187 L/ha using a solution containing 6.28 g of active ingredient per liter. Split-plots at Stillwater were first sprayed with propiconazol on March 26, 1993 whereas split-plots at Perkins were first sprayed with propiconazol on April 2. Thereafter, disease-free split-plots at each location were sprayed three times at 14 day intervals to insure the control of tan spot and other foliar wheat diseases.

Conidia were produced and harvested from three separate *P. tritici-repentis* isolates that were utilized in previous studies (10,11). Inoculum concentrations were adjusted to 3000 conidia/ml. The propagules in the inoculum suspension consisted of 93% conidia and the remaining 7% of propagules in inoculum suspension consisted of conidiophores and hyphal fragments. Hence, the total concentration of *P. tritici-*

repentis propagules in suspension was 3225/ml.

Inoculum was applied with a gas powered back-pack sprayer/fogger (Model 11, Vandermollen Corp., Livingston, NJ 07039) which was calibrated to deliver the inoculum suspension at 9600 L/ha. Each split-plot received ca. 0.5 L of inoculum suspension. Plots were inoculated at Stillwater on March 29, 1993. Plots at Perkins were inoculated on April 9, 1993. The growth stage (42) of the ten wheat genotypes at each location at the time of their inoculation ranged from Zadoks' 31 to 32 scale. Petri dishes (100 $\times$ 15 mm) containing water agar, were placed in the middle of each plot prior to inoculation. After inoculation, the water agar plates were collected, incubated for 12 hr in the dark, and conidia were assessed for percent germination by randomly counting 100 conidia per dish using a stereomicroscope. Following inoculation, each location received overhead mist treatment to provide periods of free moisture on wheat leaves which is required for tan spot infection (18,19). The mist irrigation system was supported by steel fence-posts driven into the ground every 1.82 m along the midpoint of each block. The mist system was constructed with schedule-40 PVC plastic pipe (19 mm inside diameter) suspended 1.52 m above the ground from the fence posts. A 10 m span of the misting system was suspended over each block. Mist was produced at 2.76×10^4 Pa through mist producing nozzles (Flora-Mist model 300-B, A. H. Hummert Seed Co. 2746 Chauteau Avenue, St Louis, MO 63103) spaced every 0.66 m along the suspended PVC pipes. Following inoculation at the Stillwater at 1500 hr, plots received a 20 min mist period at 1700, 2000, and 2100 hr. A rainfall event occurred at approximately 2330 hr in which 15 mm of rainfall was recorded over an eight hr period. Sky conditions remained cloudy for the entire following day. Thus

continuous free moisture was present on wheat leaves for approximately 30 hr at Stillwater.

Mist was applied at Perkins after inoculation for 30 min intervals at 1030, 1400, and 1800 hr and at 0030, 0700, 0830, and 1000 hr on the following dayl. Following the initial application of mist, subsequent infections and progression of the tan spot at each location was dependent on natural dew periods and rainfall events.

Twenty-two days ater inoculation, leaves showing tan spot symptoms were taken from each split-plot at both locations to confirm infection. Each leaf was surface disinfested by immersion in a 1% sodium hypochlorite solution for 30 sec, rinsed in deionized water, dried on a paper towel, and placed on moistened filter paper in plastic petri dishes (15 × 100 mm). Dishes were then incubated for 2-3 days at 21 C with an alternating 12 hr light and dark period. After two or three days, leaves were examined with a stereomicroscope for the presence of conidiophores and conidia of *P. tritici-repentis*.

DISEASE ASSESSMENT. Tan spot was visually assessed on ca. seven day intervals beginning on April 20 using the Horsfall and Barratt rating scale (13-15). Visual assessments of the amount of necrotic leaf area caused by tan spot, were conducted on the uppermost, fully developed, four leaves of four randomly selected tillers per inoculated split-plot. The extent of foliar damage caused by other foliar fungal diseases was minimal at both locations and did not affect tan spot ratings. Final ratings were taken on May 13 and on May 19 at Stillwater. The area under the disease progress curve (AUDPC) (36) was calculated for each plot and data were subjected to analyses of variance by location following the ANOVA procedure of the

SAS statistical program (33). The analyses of variance were performed as a randomized complete block (i.e. no disease was present in fungicide-sprayed split-plots). Mean separations were performed following Fishers' least significant difference test (P=0.05) for AUDPC data and are presented in Table VIII with lesion length means from previous greenhouse experiments (11). Spearmans' rank correlation coefficients (38) were calculated using the genotypes ranks of tan spot lesion length determined in the greenhouse to compare their ranks using the AUDPC data from the field (Table XI).

YIELD ASSESSMENT. Plots were harvested at maturity by cutting 304.8 mm from the center of each row. Grain yields and kernel weights were determined after hand threshing. Kernel weight was determined by weighing 100 seed from each split-plot. Analyses of variance of plot yield (g/plot) and kernel weight (mg/kernel) were performed as a split-plot following the ANOVA procedure of SAS, means are presented in Tables IX and X. An assessment of the performance each wheat genotype infected with tan spot relative to the split-plot sprayed with propiconazole was performed by dividing the grain yield and kernel weight data from tan spot-inoculated split-plots with the corresponding disease-free split-plot data. The percentage values were transformed to arcsine-square root values and analyses of variance were performed on the transformed data following the ANOVA procedure of SAS to determine the performance and rank of each genotype infected with tan spot as a percentage of their performance when no foliar diseases were present; the actual percentage values are presented in Tables IX and X. Spearmans' rank correlation coefficients were calculated to compare the wheat genotypes rankings of lesion length

measurements in the greenhouse , their field AUDPC assessments, and their percent of disease-free control plot yield and kernel weight ranks.

RESULTS AND DISCUSSION

Germination of *P. tritici-repentis* conidia on the surface of water agar was never less than 95%. Samples of wheat leaves from inoculated split-plots were predominantly infected with *P. tritici-repentis*. Tan spot infection was uniform over all inoculated split-plots at both test locations. Throughout the entire test at both locations, minor levels (<5% severity) (2) of leaf rust (*Puccinia recondita*) appeared late in the season. Applications of propiconazol provided excellent control of foliar diseases and no tan spot was cultured from leaves sampled from sprayed split-plots.

For AUDPC data, there was a significant difference between the locations for the extent of tan spot damage that was observed and there was a significant genotype by location interaction; thus, mean AUDPC data were presented separately for each location. The mean AUDPC averaged over the ten genotypes, was significantly greater for Stillwater and was twice that compared to the mean AUDPC for Perkins. There were significant differences (P=0.05) among the 10 wheat genotypes for AUDPC (Table VIII). Previous greenhouse tests indicated that wheat genotypes TX88V5440 and KS84170E-8-3 had lesion lengths that were comparable to the resistant cultivar Red Chief (Table VIII). However, field assessments of the AUDPC values Red Chief was significantly lower than the other genotypes at Perkins and Stillwater (Table VIII), thus confirming other assessments of tan spot resistance of Red Chief (5,11,30). Field assessments of AUDPC for the genotypes TX88V5440 and KS84170E-8-3 were higher than expected and were similar to the those of genotypes

XH1319 and KS87H325-2. Initially we regarded TX88V5440 and KS84170E-8-3 as moderately resistant to tan spot. Hence, differences among the wheat genotypes, as indicated by their AUDPC values, were less clearly defined under field conditions compared to greenhouse assessments. However, the level of concordance between the ranking of the 10 wheat genotypes in the greenhouse and their AUDPC exhibited at separate field locations , were significant (Table XI). Thus, greenhouse assessments may not accurately predict a wheat genotypes reaction in the field. However, it appears that greenhouse tests can provide an approximate indication of a particular wheat genotypes performance relative to others that are being tested, especially when resistant and susceptible check cultivars are included. The rankings of the 10 wheat genotypes for grain yield and kernel weight were not significantly correlated with their greenhouse rankings of tan spot lesion lengths. Although the influence of tan spot on grain yield and kernel weight of the 10 wheat genotypes was significant, differences of performance among the genotypes for grain yield and kernel weight were likely more influenced by environmental factors and the genotypes genetic adaptation within the two field environments they were tested (37). Averaged over the 10 wheat genotypes, mean yield of tan spot-inoculated split-plots was 15% to 17% less than the plots in which foliar diseases were controlled. Mean kernel weight of the 10 genotypes were reduced by 7% at the Perkins location and were reduced 13% at the Stillwater location.

One of the most important aspects of these experiments was the apparent success in initiating tan spot infection in the field utilizing laboratory cultured conidia of three *P. tritici-repentis* isolates. We feel this was due to the mist system that we employed to produce a simulated dew period at the Perkins and Stillwater test locations. These tests

were also unique in that we were able to initiate tan spot infection at a specific point in time in the growing season rather than relying on weather conditions to mature pseudoperithecia of *P. tritici-repentis* on oat kernels or wheat straw, thus eliminating the reliance on extreme climatic variability that often occurs during Oklahoma's wheat growing seasons. The mist irrigation system we constructed was simple to assemble and parts were readily available from plumbing supply and greenhouse supply businesses. This mist irrigation system could be enlarged to initiate tan spot infections on a much larger scale for breeding and pathological studies of tan spot, and possibly other foliar wheat pathogens. In the event a dry year occurs during field testing, the mist irrigation system could be further employed to produce simulated dew periods from time to time, thus decreasing the reliance on climatic conditions to maintain the development of tan spot disease throughout the growing season.

In comparison to the oat kernel inoculation technique (30), our inoculation and infection technique does not rely on prevailing weather to produce initial inoculum. This provided us with the ability to initiate tan spot infection at a chosen point in time. Additionally, the misting system eliminated the need for plastic tenting which has been utilized in field based testing of tan spot on spring wheat (7), which is impractical in Oklahomas' windy conditions. Our test was limited in that only one form of post-inoculation treatment was used to initiate tan spot infection. However, a preliminary study by Brûlé-Babel and Lamari (7) compared tenting versus mist irrigation as tan spot post-inoculation treatments to determine which was more effective. Although they did not present any statistical comparisons, they concluded that mist irrigation was the most practical way of providing a post-inoculation environment suitable for

screening large scale wheat breeding nurseries. The design of this study did not address the occurrence of the cryptic effect (43) which likely masked the degree of partial resistance to tan spot that was present in the genotypes we tested. However, the design of this study was not necessarily intended to discriminate among the genotypes for small differences of partial resistance to tan spot; rather, it was intended primarily to demonstrate an effective technique of initiating large scale tan spot infection in field tests as well as compare the relative performance of wheat genotypes reaction to tan spot in greenhouse and field tests. We found our techniques of inoculum production and plot inoculation, coupled with the mist irrigation system, facilitated the large scale field infection of tan spot which would be useful to screen promising wheat lines under field conditions for resistance to *P. tritici-repentis*.

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Table VIII[w]. Greenhouse ratings of tan spot lesion lengths and field assessments of the area under the disease progress curve for eight selected genotypes from the 1992 Southern Regional Performance Nursery

Wheat Genotypes	Greenhouse ratings of lesion length (mm) and rank						AUDPC[x] from field ratings and rank					
	Experiment 1			Experiment 2			Perkins location[y]			Stillwater location[y]		
Red Chief[z]	0.65	a	2	1.49	a	1	106	a	1	346	a	1
TX88V5440	0.44	a	1	1.92	ab	2	320	b	4	811	cd	5
KS84170E-8-3	1.07	a	3	2.12	ab	3	315	b	2	615	b	2
XH1319	2.44	bc	5	2.62	bc	4	389	bc	5	721	bc	3
OK88W833	2.91	bc	7	2.80	bc	5	456	c	7	827	cde	6
KS87H325-2	2.11	b	4	3.42	cd	7	310	b	3	729	bcd	4
TAM 105[z]	3.34	cd	8	3.75	cd	9	459	c	8	845	cde	7
TX88V4636	2.69	bc	6	3.15	cd	6	424	c	6	897	de	9
N87V106	4.77	e	10	3.68	cd	8	627	d	10	997	e	10
CO870449	4.22	de	9	4.06	d	10	460	c	9	868	cde	8

[w]Means within columns followed by the same letter are not significantly different (P=0.05) following Fishers' least significant difference (*L.S.D.*) test.

[x]AUDPC was calculated following Shaner and Finneys' (36) descriptions.

[y]Data are from tan spot-inoculated split-plots.

[z]Red Chief and TAM 105 were included as resistant and susceptible check cultivars, respectively.

Table IX. Grain yield of tan spot inoculated and disease control plots of eight selected genotypes from the 1992 Southern Regional Performance Nursery over two locations in 1993

	Grain yield (g/plot)							
	Perkins location[x]				Stillwater loction[x]			
Wheat Genotypes	Tan spot	Disease Control	Percent of Control[y]	Rank	Tan spot	Disease Control	Percent of Control[y]	Rank
Red Chief[z]	52.4	64.6	87.5	3	52.2	63.3	86.8	6
TX88V5440	72.6	84.5	88.9	2	86.2 a	110.4 b	83.5	7
KS84170E-8-3	76.4	97.5	80.5	6	80.7	98.9	82.6	8
XH1319	62.7	78.9	80.2	7	90.1	98.2	93.1	2
OK88W833	80.2	100.2	82.6	5	98.6 a	118.0 b	87.5	5
KS87H325-2	69.5	70.6	95.8	1	83.3	70.7	99.3[w]	1
TAM 105[z]	68.5	93.7	80.2	8	60.2 a	99.7 b	59.6	10
TX88V4636	59.5 a	91.1 b	73.3	10	76.0	85.2	88.5	4
N87V106	62.5	83.9	76.5	9	70.3	79.8	92.0	3
CO870449	52.1	64.5	84.3	4	69.1 a	97.7 b	76.6	9

[w]Values that were ≥100 percent were substituted with a value of (100-1/4n), where n is the number of units upon which the percentage data was based.

[x]Means within rows of each location followed by different letters are significantly different (P=0.05) using the standard error (sqrt($2E_b/r$)) for the pair comparison of two subplot means at the same mainplot treatment to compute the least significant difference. Means within rows of each location without letters are not significantly different from each other.

[y]Means of percent control of the wheat genotypes within columns of each location were not significantly different (NS).

[z]Red Chief and TAM 105 were included as a tan spot resistant and susceptible cultivars, respectively.

Table X. Kernel weight of tan spot inoculated and disease control plots of eight selected genotypes from the 1992 Southern Regional Performance Nursery over two locations in 1993

	Kernel weight (mg/kernel)							
	Perkins location[x]				Stillwater loction[x]			
Wheat Genotypes	Tan spot	Disease Control	Percent of Control[y]	Rank	Tan spot	Disease Control	Percent of Control[y]	Rank
Red Chief[z]	30.8	31.3	97.9	5	28.5 a	32.3 b	90.3	3
TX88V5440	27.5	27.3	99.3[w]	1	25.5 a	28.8 b	90.1	5
KS84170E-8-3	33.0 a	35.5 b	93.8	6	29.3 a	33.5 b	90.2	4
XH1319	31.3	32.0	97.9	4	31.8 a	35.0 b	91.3	2
OK88W833	34.5	34.8	98.8	2	31.8 a	36.0 b	88.4	6
KS87H325-2	27.8	28.0	98.0	3	29.3	30.3	95.5	1
TAM 105[z]	23.3 a	30.0 b	78.8	10	21.0 a	28.5 b	73.7	10
TX88V4636	27.3 a	32.0 b	85.3	9	25.5 a	31.3 b	82.0	8
N87V106	27.0 a	30.3 b	89.5	7	25.5 a	30.3 b	87.3	7
CO870449	25.5 a	29.5 b	87.0	8	25.5 a	33.3 b	77.5	9

[w]Values that were ≥100 percent were substituted with a value of (100-1/4n), where n is the number of units upon which the percentage data was based.

[x]Means within rows of each location followed by different letters are significantly different (P=0.05) using the standard error (sqrt($2E_b/r$)) to compute the least significant difference for the pair comparison of two subplot means at the same mainplot treatment. Means within rows of each location without letters are not significantly different from each other.

[y]Means of percent control of the wheat genotypes within columns of each location were not significantly different (NS, P=0.05).

[z]Red Chief was included as a tan spot-resistant cultivar, TAM 105 was included as a tan spot-susceptible cultivar.

Table XI. Rank correlations of eight selected wheat genotypes from the 1992 Southern Regional Performance Nursery in two greenhouse ratings of tan spot lesion lengths and two tests measuring the area under the disease progress curve from tan spot inoculation at Zadoks growth stage 31 at two field locations[y]

	Greenhouse experiments		Ratings of AUDPC	
	1	2	Perkins	Stillwater
Greenhouse exp. 1	--	0.87**[z]	0.93**	0.79**
Greenhouse exp. 2		--	0.81**	0.75*
AUDPC at Perkins			--	0.89**

[y]The ranks of Red Chief and TAM 105 were included in calculating the Spearmans' rank correlation coefficients.

[z]Correlation coefficients followed by * or ** are significant at the $P=0.05$ and $P=0.01$ level of probability, respectively.

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APPENDIXES

APPENDIX A

REACTION OF THE NORTHERN REGIONAL PERFORMANCE NURSERY TO TAN SPOT, 1993

Evans, C. K., Hunger, R. M., and Siegerist, W. C. 1994. Reaction of the Northern Regional Performance nursery to tan spot, 1993. Biol. & Cult. Tests for Control of Plant Dis. Vol 9:123.

The reaction of the entries in the 1993 Northern Regional Performance Nursery (NRPN) to tan spot of wheat caused by Pyrenophora tritici-repentis (PTR) was determined in two tests conducted in the greenhouse. Three genotypes were included to serve as resistant and susceptible checks. These were 'Red Chief' (resistant), 'Agrotricum' (OK906, resistant), and 'TAM 105' (susceptble). Ten seeds of each entry were planted as a clump in soil contained in wooden flats. Flats were planted in a randomized complete block design with four replications. The inoculum consisted of equal amounts of conidia produced from three single ascospore isolates of PTR. These isolates were obtained from naturally infested straw collected in 1991 from different wheat producing regions of Oklahoma. Each isolate produces abundant quantities of conidia *in vitro* and causes the typical lesions associated with the tan spot disease. In both tests, seedlings were inoculated when the first leaf was fully expanded. Seedlings were inoculated with a conidial suspension (1000 conidia ml^{-1} + 1 drop of Tween 20 per 100 ml) suing a DeVilbiss sprayer (model # 5601 D) until incipient runoff. Following inoculation, plants were allowed to dry for one hr and then placed in a mist

chamber that provided near 100% relative humidity. After 24 hr in the mist chamber plants were placed on greenhouse benches. The length of the largest lesion that occurred in the middle 50% of the first leaf was determined after eight days using a dial caliper with an accuracy range of $\pm$ 0.05 mm. One measurement was made on four separate leaves from each clump of plants. Measurements were made from the border of the visible edge of the chlorotic or necrotic lesions longest dimension which generally was oriented parallel with the leaf axis. Statistical analyses were conducted on the mean of the four measurements make per entry.

Data from both tests of the 1993 NRPN were combined and the analysis revealed there were no significant differences between tests. Significant differences among genotypes were observed (P=0.01), and a significant test by genotype interaction was observed. An *L.S.D.*(P=0.05) was used for mean separations. Mean lesion lengths from the two tests are presented separately as well as over tests (Table XII). Mean lesion lengths from the combined analysis ranged from 1.26 to 3.71 mm. Red Chief (resistant) and TAM 105 (susceptible) check cultivars provided good comparisons to assess the seedling reactions of the NRPN entries to tan spot. The genotype MT8719 demonstrated seedling stage resistance comparable to the level of resistance of the check cultivar Red Chief. The genotypes MT8713, WI88-083, ND89142, W-235, and NE89526 expressed levels of resistance comparable to the tan spot resistant check Agrotricum.

Table XII. Mean lesion lengths from tan spot infection on wheat genotypes in the 1993 Northern Regional Performance Nursery

Rank[1]	Entry No.	Sel. No.	Mean lesion lengths (mm) Test 1[2]	Test 2[2]	Mean[3]
1	Chk	Red Chief	0.08	1.71	1.26
2	32	MT8719	1.20	1.35	1.27
3	31	MT8713	2.33	1.71	2.02
4	30	WI88-083	2.33	1.99	2.16
5	Chk	Agrotricum	2.58	2.08	2.33
6	14	ND891422	2.70	2.02	2.36
7	2	Roughrider	2.65	2.13	2.39
8	35	W-235	2.79	2.36	2.57
9	3	Abilene	2.96	2.20	2.58
10	18	NE89526	3.26	1.90	2.58
11	33	ID0426	2.70	2.50	2.60
12	29	XNH1712	2.87	2.35	2.61
13	25	XNH1643	2.96	2.30	2.63
14	7	SD89102	2.62	2.67	2.64
15	28	XNH1687	2.84	2.50	2.67
16	34	W-198	2.79	2.55	2.67
17	10	SD89333	2.81	2.60	2.70
18	15	ND8889	2.78	2.65	2.71
19	1	Kharkof	2.74	2.73	2.74
20	4	SD88201	2.94	2.63	2.78
21	26	XNH1648	3.44	2.43	2.94
22	27	XNH1650	3.39	2.55	2.97
23	Chk	TAM-105	3.07	2.88	2.97
24	12	ND8933	3.39	2.58	2.98
25	19	NE89657	3.30	2.77	3.03
26	21	NE90625	3.52	2.60	3.06
27	11	ND8930	3.06	3.10	3.08
28	23	NE88595	3.53	2.76	3.14
29	5	SD87143	3.25	3.06	3.15
30	22	NE88526	3.73	2.62	3.17
31	8	SD88231	3.42	2.96	3.19
32	17	NE89522	3.43	3.00	3.21
33	20	NE87615	3.60	2.90	3.25
34	16	ND90109	3.47	3.22	3.35
35	9	SD89119	4.11	2.64	3.37
36	24	NE88588	3.39	3.37	3.38
37	13	ND8955	4.13	2.85	3.49
38	6	SD89204	4.38	3.03	3.71
Mean			3.03	2.53	2.78
L.S.D.			0.62	0.38	0.36
C.V.			14.69	10.69	13.25

[1]Rankings are from the combined analysis of the two tests.
[2]Mean lesion lengths were computed over four replications.
[3]Mean lesion length values from the combined analysis.

APPENDIX B

REACTION OF THE SOUTHERN REGIONAL PERFORMANCE NURSERY TO TAN SPOT, 1993

Evans, C. K., Hunger, R. M., and Siegerist, W. C. 1994. Reaction of the Southern Regional Performance nursery to tan spot, 1993. Biol. & Cult. Tests for Control of Plant Dis. Vol 9:124.

The reaction of the entries in the 1993 Southern Regional Performance Nursery (SRPN) to tan spot of wheat caused by *Pyrenophora tritici-repentis* (PTR) was determined in two tests conducted in the greenhouse. Three genotypes were included to serve as resistant and susceptible checks. These were 'Red Chief' (resistant), 'Agrotricum' (OK906, resistant), and 'TAM-105' (susceptible). Ten seeds of each entry were planted as a clump in soil contained in wooden flats. Flats were planted in a randomized complete block design with four replications. The inoculum consisted of equal amounts of conidia produced from three single ascospore isolates of PTR. These isolates were obtained from naturally infested straw collected in 1991 from different wheat producing regions of Oklahoma. Each isolate produces abundant quantities of conidia *in vitro* and causes the typical lesions associated with the tan spot disease. In both tests, seedlings were inoculated when the first leaf was fully expanded. Seedlings were inoculated when the first leaf was fully expanded. Seedlings were inoculated with a conidial suspension (1000 conidia ml^{-1} + 1 drop Tween-20 per 100 ml) using a DeVilbiss sprayer (model # 5601 D) until incipient runoff. Following inoculation,

plants were allowed to dry for one hr and then placed in a mist chamber that provided near 100% relative humidity. After 24 hr in the mist chamber, plants wee placed on greenhouse benches. The length of the largest lesion that occurred in the middle 50% of the first leaf was determined after eight days using a dial caliper with an accuracy range of $\pm$ 0.05 mm. One measurement was made on four separate leaves from each clump of plants. Measurements were made from the border of the visible edge of the chlorotic or necrotic lesions longest dimension which generally was oriented parallel with the leaf axis. Statistical analyses were conducted on the mean of the four measurements made per entry.

Data from both tests of the 1993 SRPN were combined and the analysis revealed there were no significant differences between tests. Significant differences among genotypes were observed (P=0.01), and a significant test by genotype interaction was observed. An *L.S.D.*(P=0.05) was used for mean separations. Mean lesion lengths from the two tests are presented separately as well as over tests (Table XIII). Mean lesion lengths from the combined analysis ranged from 1.80 to 3.80 mm. Red Chief (resistant) and TAM-105 (susceptible) check cultivars provided good comparisons to assess the seedling reactions of the SRPN entries to tan spot. The genotypes KS831374-142, KS92P059E, T70, and XH1520 demonstrated levels of resistance in the seedling stage equal to the resistant check cultivar Red Chief.

Table XIII. Mean lesion lengths from tan spot infection on wheat genotypes in the 1993 Southern Regional Performance Nursery

Rank[1]	Entry No.	Sel. No.	Mean lesion lengths (mm) Test 1[2]	Test 2[2]	Mean[3]
1	22	KS83174-142	1.90	1.70	1.80
2	Chk	Red Chief	1.93	1.91	1.92
3	24	KS92P059E	1.74	2.50	2.12
4	Chk	Agrotricum	1.97	2.69	2.33
5	43	T70	2.44	2.37	2.40
6	36	XH1520	2.63	2.20	2.41
7	4	OK88767-11	2.61	2.27	2.44
8	10	TX90D9277	2.17	2.85	2.51
9	38	XH1620	2.04	3.04	2.54
10	42	T4731	2.74	2.60	2.67
11	7	OK88767-24	2.84	2.51	2.67
12	16	TX91V3308	2.69	2.72	2.70
13	14	TX90V7911	3.12	2.50	2.81
14	34	XH1455	2.33	3.35	2.84
15	12	TX91V4931	2.67	3.05	2.86
16	39	W87-017-44	2.85	2.93	2.89
17	40	WI89055	3.25	2.68	2.96
18	45	TH905	2.86	3.16	3.01
19	26	KS92P0363-134	2.67	3.38	3.03
20	41	T13	3.16	2.97	3.06
21	9	TX88A6533	3.39	2.74	3.06
22	1	Kharkof	2.64	3.53	3.08
23	21	CO880240	3.11	3.06	3.09
24	35	XH1485	3.21	2.98	3.10
25	18	CO880054	3.15	3.06	3.11
26	27	KS92P0425-155	3.39	2.85	3.12
27	Chk	TAM-105	3.54	2.75	3.15
28	5	OK88767-02	3.51	2.80	3.15
29	19	CO880169	3.41	2.94	3.17
30	6	OK88767-15	3.18	3.18	3.18
31	20	CO880210	3.55	2.88	3.22
32	25	KS92P0263-137	3.15	3.31	3.23
33	13	TX90V8410	3.10	3.42	3.26
34	31	NE90479	3.37	3.15	3.26
35	8	TX88A6480	2.99	3.59	3.29
36	15	TX91V5739	3.59	3.07	3.30
37	32	NE90524	3.43	3.32	3.37
38	17	TX89A7141	3.69	3.18	3.44
39	3	TAM-107	3.82	3.14	3.48
40	30	NE88584	3.93	3.03	3.48
41	11	TX89A7137	3.62	3.34	3.48
42	37	XH1529	3.38	3.70	3.54
43	23	KS84063-9-7	4.15	2.94	3.55
44	28	KS89H48-1	3.93	3.20	3.56
45	33	NE90574	4.14	3.09	3.62
46	44	T64	3.99	3.31	3.65
47	29	N87V106	3.90	3.52	3.71
48	2	Scout 66	4.13	3.47	3.80
Mean			3.10	2.96	3.03
L.S.D.			0.65	0.80	0.51
C.V.			14.89	19.35	17.16

[1]Rankings are from the combined analysis of the two tests.
[2]Mean lesion lengths were computed over four replications.
[3]Mean lesion length values from the combined analysis.

APPENDIX C

REACTION OF THE SOUTHERN REGIONAL PERFORMANCE NURSERY TO TAN SPOT, 1992

Evans, C. K., Hunger, R. M., and Siegerist, W. C. 1993. Reaction of the Southern Regional Performance nursery to tan spot, 1992. Biol. & Cult. Tests for Control of Plant Dis. Vol 8:92.

The reaction of entries in the 1992 Southern Regional Performance Nursery (SRPN) to tan spot of wheat was determined in the greenhouse. Ten seeds of each entry were planted in hills in pasteurized soil (soil, sand, & peatmoss in a 1:1:1 ratio) contained in wooden flats. Flats were planted in a randomized complete block design (one rep per flat) with two replication in the first test and four replication in the second test. Three genotypes were included as resistant ('Red Chief' and 'Agrotricum') and susceptible ('TAM-105') checks. Inoculum consisted of equal amounts of conidia produced from three single ascospore isolates of *Pyrenophora tritici-repentis* (PTR). These isolates were obtained from naturally infested straw collected in 1991 from different wheat producing regions of Oklahoma. Inoculum (1000 conidia ml^{-1} + 1 drop Tween 20 per 100 ml) was prepared by mixing equal amounts of conidia from the PTR isolates. The first leaves (first test) or third leaves (second test) were inoculated when fully expanded using a DeVilbiss sprayer (model # 5601 D) until incipient runoff. Plants were then allowed to dry for one hr and then placed in a mist chamber that provided near 100% relative humidity. After 24 hr in the mist chamber plants

were transferred to greenhouse benches. The length of the largest lesion that occurred in the middle 50% of the first leaf (first test) or third leaf (second test) was determined after eight days using a dial caliper with an accuracy range of $\pm$ 0.05 mm. One measurement was made on four separate leaves from each clump of plants. Measurements were made from the border of the visible edge of the chlorotic or necrotic lesions longest dimension which generally was oriented parallel with the leaf axis. Statistical analyses were conducted on the mean of the four measurements make per entry.

Data from both tests of the 1992 SRPN were combined and the analysis revealed there were no significant differences between tests or among replication within tests. Significant differences among genotypes were observed (P=0.01) , and no significant test by genotype interaction was observed. An *L.S.D.* (P=0.05) was used for mean separations. Mean lesion lengths from the two tests are presented separately as well as over tests. Mean lesion lengths from the combined analysis ranged from 1.2 to 4.1 mm. The resistant (Red Chief) and susceptible (TAM-105) check cultivars provided good comparisons to assess the seedling reactions of the SRPN entries to tan spot. Four genotypes (entries 10, 15, 23, & 24) had levels of resistance in the seedling stage nearly equal to that of the resistant cultivar Red Chief which had a mean lesion length of 1.2 mm. Three of these entries (10, 15, & 23) have 'Karl' in their pedigree.

Table XIV. Mean lesion lengths from tan spot infection on wheat genotypes in the 1992 Southern Regional Performance Nursery

Rank[1]	Entry No.	Sel. No.	Mean lesion lengths (mm) Test 1[2]	Test 2[3]	Mean[4]
1	Chk	Red Chief	0.65	1.49	1.21
2	10	TX88V5440	0.44	1.92	1.43
3	23	KS8313374-142	0.68	1.98	1.54
4	15	TX88V5433	1.19	1.76	1.57
5	24	KS84170E-8-3	1.07	2.12	1.77
6	42	T67	2.30	2.46	2.41
7	31	NE88427	1.75	2.75	2.42
8	22	KSSB-369-7	1.47	2.94	2.45
9	Chk	Agrotricum	1.92	2.74	2.46
10	34	XH1319	2.44	2.62	2.56
11	32	NE88584	2.69	2.54	2.59
12	19	CO860094	3.08	2.53	2.71
13	44	TH901	2.39	2.90	2.73
14	43	T21-3	2.40	2.92	2.75
15	38	W87-018	2.93	2.67	2.76
16	3	TAM-107	2.64	2.84	2.77
17	45	TH902	2.85	2.82	2.83
18	4	OK88W833	2.91	2.80	2.84
19	2	Scout 66	2.66	2.93	2.84
20	6	OK89399	2.96	2.83	2.87
21	40	WI88-028	3.05	2.80	2.89
22	25	HBC302E	3.09	2.85	2.93
23	39	WI88-181	2.98	2.94	2.95
24	37	XH1497	2.83	3.02	2.96
25	30	NE88595	3.81	2.56	2.98
26	26	KS87H325-2	2.11	3.42	2.99
27	8	TX88V4636	2.69	3.15	3.00
28	41	T13	2.67	3.23	3.05
29	35	XH1436	2.98	3.12	3.06
30	16	TX88A6480	3.20	3.00	3.07
31	13	TX88V4524	2.81	3.27	3.12
32	36	XH1437	3.16	3.11	3.12
33	7	OK89421	2.98	3.24	3.15
34	18	CO860086	2.86	3.31	3.17
35	5	OK89499	3.38	3.07	3.17
36	14	TX89V4138	2.68	3.45	3.20
37	9	TX84V1418HF	3.54	3.03	3.20
38	20	CO860236	3.23	3.19	3.20
39	33	NE88588	2.88	3.53	3.32
40	17	TX88A6533	2.98	3.52	3.34
41	27	KS89H48-1	3.13	3.49	3.37
42	28	HS89H50-4	3.24	3.49	3.41
43	Chk	TAM-105	3.34	3.75	3.61
44	12	TX87V1613	3.82	3.58	3.66
45	1	Kharkof	3.71	3.69	3.70
46	11	TX88V4635	3.36	3.89	3.72
47	29	N87V106	4.77	3.68	4.04
48	21	CO870449	4.22	4.06	4.11
Mean			2.73	2.99	2.90
L.S.D.			1.06	0.90	0.71
C.V.			19.30	21.80	21.40

[1]Rankings are from the combined analysis of the two tests.
[2]Mean lesion length values were computed from two replications.
[3]Mean lesion lengths were computed from four replicaitons.
[4]Mean lesion length values from the combined analysis.

VITA

Conrad Kent Evans

Candidate for the Degree of

Doctor of Philosophy

Thesis: INFECTION EFFICIENCY OF PROPAGULES OF *PYRENOPHORA TRITICI-REPENTIS* ISOLATES AND EVALUATION OF WHEAT FOR REACTION TO TAN SPOT

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