Tan Spot of Winter Wheat: Procedures to Determine Host Response

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ABSTRACT

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Techniques are described to quantify winter wheat cultivar reaction to tan spot under greenhouse and field conditions. A greenhouse seedling assay, which includes an improved technique for in vitro sporulation of *Drechslera tritici-repentis*, produced results which were highly correlated ($r = 0.91$) with those of field trials. The assay involves applying a conidial suspension (2×10^3/ml) to four-leaf seedlings in flats, exposing the inoculated plants to a 48 hr dew period in a mist chamber, and rating disease severity 7–10 days later. Preparation and use of inoculum for field experiments and a disease severity rating scale are described. When these techniques were employed under field conditions, a cultivar showing resistance in the greenhouse sustained 7.2% yield loss from tan spot compared to 27.7% for a cultivar identified as susceptible in the greenhouse. Under field conditions, ascospores were the only infecting propagules observed on leaves until 1 May when conidia were first detected and by 13 May only conidia were observed.

Additional key words: *Pyrenophora tritici-repentis*.

Pyrenophora tritici-repentis (Died.) Drechs., and its anamorph *Drechslera tritici-repentis* (Died.) Shoem., causes a foliar disease on wheat and other gramineous plants (3,6,7). Commonly called tan spot, yellow leaf spot, or eyespot of wheat, this destructive disease has been noted throughout the world (4,5), and losses attributed to this pathosystem under severe epidemic conditions have ranged from 19.7 to 49.4% of the grain yield (17,18), presumably due to a reduction in grain size (14,17,18). First noted in Kansas in 1947, tan spot has increased in incidence and severity and caused an estimated average annual loss of 200×10^6 kg of grain over the 1979-1983 seasons (20). This increase in importance is probably attributable to continuous wheat cultivation, stubble retention on the soil surface, and the lack of high levels of resistance in cultivars currently grown in Kansas.

The epidemiology of tan spot has been investigated in Australia (15) and North Dakota (19). Pseudothecial initials develop on infested wheat stubble on or above the soil surface soon after harvest (2). The pseudothecia mature during the fall and winter and forcibly eject ascospores during wet periods the following spring (2,15). The ascospores function as primary inoculum and are followed by secondary spread within the crop canopy by conidia produced on senescing infected leaves and infested stubble (5,6,15). Production of conidia and infection efficiency both increase with frequent rains and prolonged wetting of the foliage (3,5,6,15).

Control measures for tan spot include crop rotation, burning or incorporating crop residue, spraying with fungicides, and planting resistant cultivars. Crop rotation and residue management both reduce refuse left on or above the soil surface and thus, the amount of primary inoculum. However, under a favorable environment for disease development, a small amount of residue can result in significant incidence and severity of tan spot (17). Furthermore, in locations where wheat is grown continuously, soil erosion and conservation of soil moisture and fuel are increasing in importance; it is becoming desirable to leave large amounts of residue on the soil surface (1). The relatively low grain yield potential under Kansas conditions and number of fungicide applications required for disease control often preclude their use. Thus, resistant cultivars would be a highly desirable form of control.

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The identification of resistant germplasm previously has been done by greenhouse screening methods for producing the disease and then scoring disease severity. Luz and Hosford (9) divided isolates of *P. tritici-repentis* into 12 races based upon virulence on a set of differentials. Although Sharp et al (18) used conidial and mycelial fragment suspensions to inoculate field plots and quantified the effect of tan spot on thousand-kernel weight of 30 spring wheat cultivars, the disease ratings were not consistently correlated with the reduction in thousand-kernel weight.

There were six objectives of our work: to develop an in vitro technique for obtaining profuse sporulation of *P. tritici-repentis*, to produce spore suspensions relatively free of mycelial fragments, to develop a greenhouse assay for plant resistance to tan spot that correlates with cultivar reaction in the field, to develop a technique for inoculating field plots with *P. tritici-repentis*, to devise a field disease rating system to identify resistance and quantify disease progress for tan spot, and to determine yield losses for a susceptible wheat cultivar relative to one identified as "resistant" in our greenhouse and field disease nurseries. We also observed some parameters of the epidemiology of tan spot in Kansas.

MATERIALS AND METHODS

Fungal isolates. Fourteen isolates of *P. tritici-repentis* were obtained from naturally-infected wheat at a number of locations in Kansas. Infected leaf segments (0.5 cm square) were placed in a mixture of 95% ethanol and 5% sodium hypochlorite (1:1, v/v) for 20 sec and separately rinsed twice for 30 sec in sterile distilled water. These segments were then placed on V-8 agar (150 ml of V-8 juice, 3.0 g of $CaCO_3$, 15 g of agar, in 850 ml of distilled water) and incubated at 21 ± 3 C under a 12-hr light/12-hr dark regime to induce sporulation from the tissue. After 2–3 days, a single conidium per isolate was transferred to V-8 agar slants.

In vitro sporulation. Suspensions of conidia were produced by using the overlapping of agar wedges (13). Sterile, molten one-fourth strength potato-dextrose agar (1/4 PDA) was poured into slanted 100-mm-diameter plastic petri dishes to form a 70 mm long agar wedge tapering from 8 mm to 0 mm thick. After the agar had solidified the dishes were laid flat and about 5–7 ml of molten V-8 agar added on the 0 mm thick side. A mycelial plug, taken from the advancing margin of a 6-day culture of *P. tritici-repentis* grown on PDA, was placed on the outer edge of the 1/4 PDA portion of the plate and incubated at 21 C under continuous light.

When the colony's advancing margin had progressed 1-2 cm onto the V-8 portion of the plate, the aerial mycelium on this portion was knocked down with a sterile, bent glass rod. This stimulated conidiophore formation and reduced mycelial fragments in the final spore suspension. The dish was then incubated at 16 ± 3 C at the beginning of a 12-hr light/12-hr dark cycle for 24 hr. The light cycle stimulated the production of conidiopores while the dark cycle induced completion of the sporulation process (11,12). The conidia were produced in a band near the advancing edge of the colony on the V-8 side of the plate and were harvested by flooding the plate with 5 ml of sterile distilled water and dislodging the conidia with a bent glass rod followed by transferring the suspension to a suitable container.

Resistance screening in the greenhouse. A mist chamber (8) consisting of a 1- × 1- × 4-m redwood frame covered on all sides with 0.025 mm polyethylene film was set up in the greenhouse. The chamber could be opened along one side by a hinged frame and mist was generated by two centrifugal atomizing humidifiers (Percival Manufacturing Co., Boone, IA). Distilled water was continuously provided to each humidifier via a self-contained float valve in the basin of the humidifier. The humidifiers were electrically controlled to operate for 1 min every 9 min, which maintained a continuous fog throughout the chamber.

Wheat cultivars to be screened for resistance were planted in galvanized flats filled with a steam-sterilized mixture of Chase silty clay loam, sand, and sphagnum moss (2:1:1, v/v). Each flat was planted with 12 rows, three rows per cultivar, arranged in a randomized block design with four replicates.

The hard red winter wheat cultivars planted were Newton (CI 17715), Red Chief (CI 12109), Tam 105 (CI 17826), and Sturdy (CI 13684). After being planted, they were placed in the greenhouse and watered and fertilized as needed. When the plants reached the four-leaf stage they were inoculated with a conidial suspension (2×10^3 conidia per milliliter) of six isolates of *P. tritici-repentis* in distilled water with 0.05% Triton B 1956 added as a surfactant. An atomizer (model 152; the DeVilbiss Co., Somerset, PA) at 0.7 kg/cm^2 pressure was used to uniformly spray the leaves with the suspension up to but just prior to the "drip" stage. After a 1-hr dry-down period to allow spores to adhere to the leaves, the inoculated flats were placed in the mist chamber for 48-hr. Flats were then removed from the chamber and maintained in the greenhouse for 7–10 days at 25 ± 3 C before being rated for severity of tan spot. In a separate experiment, 10 winter wheat cultivars (Red Chief, Auburn [CI 17898], Triumph 64 [CI 135679], Lancota [CI 17389], Arkan [PI 475771], Tam 105, Caprock [CI 14516], Homestead [CI 17264], Sturdy, and Parker76 [CI 13285]) were seeded in two replicate flats (25 seeds per cultivar per row) and grown in the greenhouse. When plants had reached the four-leaf stage they were inoculated as described above, placed in the mist chamber 48 hr, and subsequently rated for disease severity.

Disease severity rating system. A system to rate the severity of tan spot was developed to take into account lesion size, amount of leaf area affected, and leaf location. A 0–5 assessment scale was employed, in which 0 = no reaction, 1 = flecks or minute lesions (<1 mm square), 2 = lesions (>1 mm square) with distinct yellow halos covering <10% of the leaf area, 3 = lesions (>1 mm square) with distinct yellow halos covering 10–50% of the leaf area, 4 = numerous coalescing lesions, large or small with >50% of the leaf area affected, and 5 = total leaf collapsed, necrotic.

Since the upper leaves contribute most to grain filling relative to lower leaves, a weighted severity scale was devised taking into account leaf position. Leaf location in greenhouse tests was determined by counting the leaves from the soil line up. Leaf one represented the first leaf from the soil line; leaf two, the leaf above leaf one; leaf three, the leaf above leaf two; and leaf four, the leaf above leaf three. Thus, each plant that was rated received four scores corresponding to the rating for the four inoculated leaves. Ten randomly selected plants from each replicate of each cultivar were rated. The mean for each leaf position was calculated and multiplied by the leaf number. The four "weighted means" were then added and divided by four to obtain a weighted disease severity score for that cultivar. An analysis of variance was carried out on the results and mean separation was performed according to Duncan's multiple range test ($P = 0.05$).

Preparation of field inoculum. Inoculum for the field experiment consisted of autoclaved oat kernels infested with 14 separate isolates of *P. tritici-repentis*. Inoculum was prepared by placing 150 g of oat kernels overnight in 130 ml of distilled water in a 1-L canning jar. This was then capped with a perforated cotton plugged lid and autoclaved for 90 min. When the decoction had cooled, 5 ml of conidial suspension (2×10^3) from a single isolate was aseptically introduced and the jar was shaken to distribute the inoculum. The jars were shaken every 3 days during incubation for about 14 days at room temperature (23 ± 3 C) until the kernels were thoroughly colonized, and then the oats were spread out to dry.

Field experiments. Two winter wheat cultivars (Red Chief and Tam 105) were planted in five-row plots (1.2- × 7.6-m) on 10 September 1982 at the Rocky Ford Experimental Farm, Manhattan, KS, where the soil is a Chase silty clay loam (pH = 6.2). Plots were arranged in a randomized complete block design with three treatments and four replicates per cultivar. The treatments consisted of three different levels of inoculum produced from bulked inoculum from 14 isolates of *P. tritici-repentis*. All plots received 900 g of oat kernels either 0, 25 or 100% infested with *P. tritici-repentis*. Kernels were evenly spread over the surface of the soil in each plot during the first week of November when plants were at the four-leaf stage. Commencing on 9 May, the noninfested plots were sprayed at weekly intervals until maturity with Mancozeb (Manzate 200) fungicide at 2.3 kg/ha.

In a separate experiment, the 10 wheat cultivars that had been screened in the greenhouse for reaction to *P. tritici-repentis* were planted in the field (28 September 1981) in a randomized block design with flour replications. Plots consisted of single rows 1.0 m long with each row receiving 2.0 g of wheat seed. When plants had grown to the four-leaf stage (1 November) 900 g of oat-kernel inoculum of *P. tritici-repentis* per 9.3 m^2 of plot area was evenly spread over the surface of the soil.

Field disease severity rating. For the field experiment using large plots, a total of eight observations of disease severity were made approximately every 7–10 days on each plot starting at the end of the tillering stage and continuing through the late dough stage. Twenty random tillers were selected per plot and the top four leaves were scored for disease severity by using the 0–5 scale described above. The position of the leaf being rated was also noted when 4 = flag leaf, 3 = penultimate, 2 = two leaves down, and 1 = three leaves down. The mean for each leaf position was calculated and multiplied by the leaf number. A weighted mean disease severity score was obtained by adding the products and dividing by four. Mature plots were harvested with a plot combine and yields adjusted to 12% moisture. Percent yield loss was calculated for each cultivar-inoculum level treatment by comparison with the appropriate uninoculated control.

For the field experiment involving 10 wheat cultivars planted in smaller plots, three ratings were made 9 days apart commencing on 24 May. The weighted severity scale had not been developed at that time so an unweighted 0 to 4 scale was used in which: 0 = no tan-spot lesions evident on flag and penultimate leaves; 1 = flag and penultimate leaves showing lesions <1 mm^2 with no yellow halo; 2 = flag and penultimate leaves showing lesions with yellow halos but less than 25% of leaf blade affected; 3 = flag and penultimate leaves with lesions covering 25–50% of leaf area; and 4 = flag and penultimate leaves with lesions covering >50% of leaf area. Scores from four replications were used to calculate a mean severity score for each cultivar for each rating date and these were used to calculate an average score across the three rating dates.

Oat kernels were periodically collected from the soil surface and inspected microscopically for spore production by *P. tritici-repentis*. Small tan-spot lesions on leaves were also inspected to determine the type of infective propagule. Oat-kernel inoculum was observed under a dissecting microscope and leaves were collected, mounted in lactophenol-cotton blue and observed under a compound microscope.

RESULTS

The isolates used in this study sporulated profusely on the split agar wedge media. Up to four conidial harvests (one per day) were possible from a single sporulation plate if it was again run through the 12-hr light/12-hr dark regime after each harvest. The conidia were relatively free from hyphal fragments and were useful in greenhouse screening for resistance and for the production of field inoculum.

Significant differences in severity of tan spot were detected among cultivars (Table 1). In the greenhouse the number and size of lesions were all smaller on the cultivar Red Chief than on the other three cultivars. Furthermore, on a particular leaf, the disease severity was always the lowest on this cultivar (Table 1). Based on the means for a particular leaf position obtained from all cultivars, disease severity was highest on the lowest leaf and diminished significantly on each successively higher leaf (Table 1). Rankings of cultivars were similar whether unvernalized or vernalized seedlings were used in the greenhouse assay.

Disease severity scores from both greenhouse and field experiments for the 10 wheat cultivars tested are given in Table 2. In these experiments, Red Chief again had the lowest reaction of any cultivar. The correlation between greenhouse and average field reaction was 0.91.

Infestation of field plots with oat-kernel inoculum resulted in significant development of tan spot (Table 3). In early spring, most plots showed evidence of infection initiated by ascospores. The mean severity within each cultivar was related to the amount of inoculum present and the differences were significant. Differences in reaction to tan spot between the two cultivars were significant at each inoculum level and on each leaf rated. As in the greenhouse tests, disease severity was highest on the lowest leaf that was rated.

Observations on the disease progression throughout the growing season of the noninfested and 900 g-infested treatments were used to construct Fig. 1. The differences between the disease progress curves for the 900-g and 230-g infested treatments were not statistically significant within a cultivar after 16 May (boot stage) and thus the 230-g curves are not presented. Significant differences were seen between cultivars for both amount and progression of disease. From the heading through the late milk stages, disease progressed more rapidly on TAM 105 than on Red Chief. Lesion number and size were always smaller in Red Chief resulting in an overall lower disease severity over all observations except between the boot stage and heading 25% complete.

Lesions were not detected on leaves in the field until early spring (4 April 1983) at the pseudostem-erect growth stage (Table 3, Fig. 1). Disease rating for plants in the inoculated plots then declined until the boot stage. At this time conidia of *P. tritici-repentis* were first detected and the disease severity scores for inoculated plots again increased for the remainder of the season (Fig. 1). Disease severity scores obtained on uninoculated plots were due to incomplete disease control with the fungicide and leaf senescence from causes other than tan spot. Nevertheless, scores were significantly less than scores from infested plots except at the boot growth stage.

Yields and yield losses for the cultivar treatments are given in Table 4. The inoculation of the plots with infested kernels reduced the yield in both cultivars. At the high infestation rate, the reduction in yield (7.2%) for the cultivar Red Chief was not significant, but the reduction in yield of TAM 105 (27.7%) was significant. The different levels of infestation (i.e., 230 g and 900 g inoculum) did not produce significantly different yields or yield losses for either cultivar.

Observations on the propagule type. Pseudothecial initials were observed on the infested oat-kernels at the end of the incubation period in the laboratory, but they did not contain mature asci. Pseudothecia with mature asci and ascospores were first observed after the onset of leaf symptoms (April). The infections on leaves at that time were caused exclusively by ascospores. Conidia were not observed on infected leaves until the first week in May. By 13 May, the infective propagules were exclusively conidia and no ascospores were found after that date. Kernels collected 13 May bore numerous conidiophores and conidia, most of them associated with the setae on the beaks of pseudothecia. Profuse sporulation of *P. tritici-repentis* from older infected leaves was also observed at this time.

TABLE 1. Tan spot severity scores of winter wheat seedlings in the greenhouse[w]

Cultivar	Disease severity				Weighted mean[x]
	Top leaf	Second leaf	Third leaf	Fourth leaf	
TAM 105	1.9 a[y]	2.9 a	4.4 a	4.8 a	7.4 a
Sturdy	2.1 a	2.8 a	4.1 a	4.9 a	7.4 a
Newton	1.0 b	2.4 a	4.0 a	4.8 a	6.0 b
Red Chief	0.7 b	1.3 b	2.6 b	4.2 b	4.0 c
Mean	1.4 d[z]	2.3 c	3.8 b	4.7 a	

[w] Plants inoculated with $2–3 \times 10^3$ conidia per milliliter at the four-leaf stage followed by a 48-hr postinoculation wet period and rated after 7–10 days.
[x] Weighted disease severity = [(top leaf rating × 4) + (second leaf rating × 3) + (third leaf rating × 2) + (fourth leaf rating)]/4.
[y] Values within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Values across the row followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 2. Reaction of 10 wheat cultivars to tan spot in the greenhouse and field

Wheat cultivar	Greenhouse reaction[w]	Field reaction[x]
Red Chief	5.7[y]	1.4[z]
Auburn	7.0	1.9
Triumph 64	7.2	2.7
Lancota	8.1	2.6
Arkan	8.2	3.0
TAM 105	8.6	3.8
Caprock	8.6	3.4
Homestead	8.9	3.3
Sturdy	9.6	3.4
Parker 76	9.7	3.6
Correlation of greenhouse with field reactions = 0.91		

[w] Seedlings with four leaves were inoculated in the greenhouse and individual leaves were rated 10 days later using a 0 to 5 scale with 0 = no tan spot lesions visible and 5 = total leaf collapsed, necrotic. A weighted score is presented in which disease severity = [(fourth leaf rating × 4) + (third leaf rating × 3) + (second leaf rating × 2) + (first leaf rating)]/4.
[x] Cultivars grown in 1-m plots under field conditons and rated at three times for tan spot severity using a nonweighted scale of 0 to 4 with 0 = no tan spot lesions visible on flag and penultimate leaves and 4 = severe necrosis from coalescing lesions on flag and penultimate leaves.
[y] Means of four replications.
[z] Means of four replications and three rating dates.

TABLE 3. Tan spot severity score of winter wheat cultivars in the field rated at the pseudostem-erect growth stage

Cultivar	Inoculation treatment[w]	Disease severity and leaf position				Weighted mean[x]
		Flag leaf	Penultimate leaf	Third leaf	Fourth leaf	
Red Chief	None	0.0 c[y]	0.2 d	0.6 d	1.3 d	0.8 e
Red Chief	230	0.2 bc	1.0 c	1.7 c	2.6 bc	2.4 d
Red Chief	900	0.3 bc	1.8 b	2.6 b	3.2 b	3.8 c
TAM 105	None	0.0 c	0.3 d	0.8 d	1.9 cd	1.1 e
TAM 105	230	0.7 ab	2.7 a	3.9 a	4.7 a	5.8 b
TAM 105	900	1.1 a	3.2 a	4.5 a	4.8 a	7.0 a
Mean		0.4 c[z]	1.5 b	2.3 ab	3.1 a	

[w] Amount (grams) of oat kernels colonized by *Pyrenophora tritici-repentis* applied to 9.3 m^2 of plot area.
[x] Weighted disease severity = [(flag leaf rating × 4) + (penultimate leaf rating × 3) + (third leaf rating × 2) + (fourth leaf rating)]/4.
[y] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Row means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 4. Effect of different inoculum infestation levels of *Pyrenophora tritici-repentis* on winter wheat yield and percent yield loss[x]

Inoculum infestation (g/9.3m^2)	Cultivar	Yield[y]	Yield loss (%)
0	TAM 105	3,739 a[z]	-
	Red Chief	2,638 b	-
230	TAM 105	2,788 b	25.4 a
	Red Chief	2,442 b	7.4 b
900	TAM 105	2,704 b	27.7 a
	Red Chief	2,448 b	7.2 b

[x]Percent yield loss = yield of infested treatment subtracted from yield of control divided by yield of control × 100.
[y]Yield = grams per 9.3 m^2 plot. Values represent the mean of four replicates.
[z]Means within columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

DISCUSSION

Previous reports (9,11,12) have described methods to obtain conidia of *P. tritici-repentis* on agar media. The advantages of the procedure described here are: a reduction in the number of manipulations required, reduced amounts of mycelial and conidiophore fragments in the final spore suspensions, and the ability to produce up to four successive harvests of conidia from a single sporulation plate. The procedure may be applicable to other fungi in the genera *Dreschslera*, *Bipolaris*, and *Exserohilum*.

In the greenhouse experiment, leaf position significantly affected the severity score (Table 1); younger leaves of all cultivars had lower tan-spot ratings than older leaves even though all were inoculated at the same time. This suggests that leaf age contributes to tissue susceptibility and was confirmed by similar data obtained from the field (Table 3). Sequential leaf senescence has been noted by Madsen et al (10) to enhance leaf spot severity on *Poa pratensis* infected by *Drechslera sorokiniana* and is a general phenomenon in cereals.

The weighted disease severity score reflected differences in susceptibility of leaves of different ages. Although cultivar Red Chief is resistant to tan spot and its younger leaves remain relatively healthy compared to those of other cultivars. its older leaves were severely affected. Since the flag and penultimate leaves are most important for grain filling, the weighted score was used to quantify disease severity.

Cultivar disease severity scores in the greenhouse were highly correlated ($r = 0.91$) with scores in the field (Table 2). In both environments the cultivar Red Chief was resistant compared to TAM 105 which was susceptible. These cultivars sustained 7.2 and 27.7%, respectively, yield loss in the field. Thus, the greenhouse resistance screening may be an accurate indicator of performance (yield loss) in the field.

Percentage yield loss rather than yield should be the primary factor in identifying resistance to tan spot. The cultivars Red Chief and TAM 105 had statistically similar yields with moderately severe tan spot (Table 4). If yield under disease pressure were used as an indicator of resistance both cultivars would have been rated identically. However, Red Chief rated resistant (7.2% yield loss) and TAM 105 susceptible (27.7% yield loss) due to the relatively low yield potential of Red Chief in the absence of tan spot. The disease control strategy should be to combine the disease resistance gene(s) from Red Chief into a higher yielding cultivar such as TAM 105. Although a race/differential system has been identified for tan spot (9), Red Chief was resistant under field conditions to 14 different isolates collected across Kansas and it has not become severely diseased in 3 yr of testing with over three dozen other isolates.

The oat-kernel inoculum used to infest field plots with *P. tritici-repentis* simulated the natural survival and sporulation of the fungus from straw. Pseudothecia initials were produced on the oat kernels during the fall, and asci matured within them by early spring. The technique allows known amounts of inoculum to be applied to plots to give different amounts of primary infection (Table 3) and enables known fungal isolates to be applied to research plots. It should prove useful in field experiments involving fungicidal control and virulence differences between isolates as well as screening for resistance.

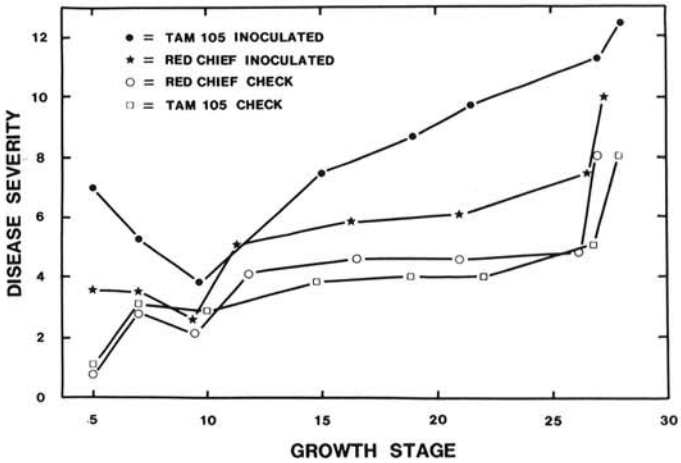

Fig. 1. Relationship between tan spot disease severity and growth stage for winter wheat cultivars TAM 105 (susceptible) and Red Chief (resistant). Growth stage numbers represent the following: 5 = pseudostem erect, 10 = boot stage, 15 = heading 95% complete, 20 = kernels one-quarter formed, 25 = milk stage, and 30 = ripe.

Disease severity scores for Red Chief were significantly lower than those for TAM 105 whether primary inoculum (at the pseudostem-erect stage) or secondary inoculum (at the heading stage) was producing the disease (Fig. 1). Neither propagule type nor plant age affected the resistance response of Red Chief. Between those growth stages (Fig. 1, stages 6-10), both Red Chief and TAM 105 appeared to "grow out" of the disease as reflected by declining severities. This may have been due to the relatively short distance (5.0 cm) that ascospores are ejected from pseudothecia (B. L. Norman, *unpublished*) or the lack of suitable conditions for formation of conidia and the initiation of infection until the boot stage.

In Kansas, only ascospores are important for disease progress from winter until May. During this period, the amount of infested refuse is directly correlated with disease severity ([21] and Table 3); however, it is not known how much the primary infections contribute to yield loss in winter wheat. In Australia, Rees and Platz (16) attributed one-quarter of the total loss in yield to severe disease from seedling to pseudo-erect stages. By mid-May (heading) through harvest only conidia are epidemiologically important. During this period severe tan spot is not necessarily associated with fields with large amounts of infested residue (17) and disease progress is similar in areas that have different amounts of primary infection. This is due to the airborne, repeating conidial stage (3,6).

If disease severity in the field is used to quantify tan spot damage, ratings should not be made between the last-leaf-visible stage and the 1/4 headed stage or after the late milk stage. At those times there was little difference between Red Chief and TAM 105 (Fig. 1) although Red Chief possesses significant resistance to tan spot. This agrees with the results of others (17) where the average level of tan spot on the flag and penultimate leaves of plants around the milk stage is used in a disease-loss relationship. Several readings more accurately indicate damage than does a single reading. If only one disease severity reading can be made, it should be taken between the end of the heading stage and the beginning of the early milk stage even though cultivar resistance differences are also evident at the pseudostem-erect growth stage.

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