Resistant Winter Wheats Compared at Differing Growth Stages and Leaf Positions for Tan Spot Severity

D. J. COX, Assistant Professor, Department of Agronomy, and R. M. HOSFORD, JR., Professor, Department of Plant Pathology, North Dakota State University, Fargo 58105

ABSTRACT

Cox, D. J., and Hosford, R. M., Jr. 1987. Resistant winter wheats compared at differing growth stages and leaf positions for tan spot severity. Plant Disease 71:883-886.

Resistance to tan spot, caused by *Pyrenophora tritici-repentis*, was ranked among 14 winter wheat genotypes previously determined to have some resistance. Resistance was compared among greenhouse-grown adult plants, vernalized seedlings, and unvernalized seedlings using ratings of lesion size recorded from each of the top three leaves. Regardless of growth stage, the oldest leaf was most severely spotted and the youngest leaf was the least severely spotted. Severity of spotting was lowest and separation of genotypes for level of resistance was best on adult-plant flag leaves in the greenhouse. The correlation of tan spot severity for greenhouse-grown plants with adult field plants was significant for adult greenhouse plants ($r = 0.86$) and high for vernalized seedlings ($r = 0.64$) and unvernalized seedlings ($r = 0.79$).

Additional key words: *Drechslera tritici-repentis, Helminthosporium tritici-repentis, Pyrenophora trichostoma,* yellow leaf spot

Tan spot, caused by *Pyrenophora tritici-repentis* (Died.) Drechs. (syn. *P. trichostoma* (Fr.) Fckl.), anamorph *Drechslera tritici-repentis* (Died.) Shoem. (syn. *Helminthosporium tritici-repentis* Died.), causes chronic, economic wheat yield losses in several areas of the world (6,7). Races or groupings for virulence exist in the population of *P. tritici-repentis* (3,4,14) and the reaction of wheat cultivars to tan spot differs from one part of the world to another (3,4,14). *P. tritici-repentis* also causes major damage to prairie grasses (8,15). More than 26 species of Gramineae are attacked by the fungus (9). Resistance in wheat and oat hosts occurs first as papilla formation (ineffective in wheat), then as restriction of lesion growth and of mycelium growth within the lesion (10,11). Wheat genotypes differ in their ability to restrict growth of *P. tritici-repentis* as the period of postinoculation foliar wetness lengthens (5,6) and/or the temperature rises (10,13). Lesion numbers may differ on some genotypes (13). Resistance in wheat has been reported to be polygenic (1,16,17), monogenic (12), and of intermediate to high heritability (1,16,17). Tan spot severity usually increases with lower leaf position on the plant (19). Incubation period (inoculation to first symptoms) decreases with rising temperature (13). Tan spot resistance in a given wheat genotype is similar for both seedling and adult greenhouse-grown plants and adult field-grown plants (2–5,7,19) and unrelated to glaucousness (20). Resistance in wheat is only relative. Prolonging the postinoculation period of foliar wetness and increasing the inoculum load can eventually result in all leaves being severely spotted (6).

Our objectives were 1) to determine relative resistance of various hard red winter wheat genotypes to virulent strains of *P. tritici-repentis* from the

Published with the approval of the director of the North Dakota Agricultural Experiment Station as Journal Series Article 1564.

Accepted for publication 15 May 1987 (submitted for electronic processing).

Great Plains, 2) to determine the effect of wheat growth stages and leaf position on tan spot, and 3) to compare greenhouse and field ratings for tan spot resistance.

MATERIALS AND METHODS

Inoculation procedure. To give a broader spectrum of virulence to the testing of winter wheats for resistance than that obtainable with a single isolate, isolates Pti2 and PyD7 of *P. tritici-repentis* from the two most virulent races or groups detected in the central and northern Great Plains were used (14). Inoculum was produced by a modification of the technique described by Odvody et al (18). The fungus was grown on potato-dextrose agar (PDA) in petri plates for 10 days at 20 ± 1 C under one Norelco F40 WW and one Sylvania F40 BLB fluorescent tube suspended 30 cm above the petri plates. From each plate, 35 1-cm-diameter disks of gray mycelium and PDA were transferred to a petri plate of V-8 agar. A total of 24 V-8 agar plates were incubated for 24 hr in light at 18 ± 1 C followed by 24 hr in darkness at 18 ± 1 C in a Percival 13LLVL temperature-controlled chamber (Percival Mfg. Co., Boone, IA). The light was provided by one Norelco F20 WW and one Sylvania F20 BLB fluorescent tube suspended 28 cm above the petri plates. The 840 disks of PDA on the V-8 agar, now covered with mycelium, conidiophores, and conidia, were blended with distilled water for 3 min and adjusted to 1,000 ml of suspension. Over the years, blended disks of conidia, conidiophores, and mycelium have consistently produced severe spotting while measured numbers of conidia alone have been more erratic. The blended disks cause spotting comparable to that caused by 2,500 conidia per milliliter (6). Ten drops of Tween 20 (0.2 ml) were added, and the suspension was sprayed on 200 wheat plants with a DeVilbiss 26 atomizer at 0.55 kg cm^{-2} (DeVilbiss Co., Somerset, PA). Each plant received the mycelium, conidiophores, and conidia from 4.2 blended disks in 5 ml of water. The inoculated plants and four pots of water-sprayed check plants per trial were misted with tap water for 30 hr in a sunlit chamber at 23 ± 3 C. Most wheats become severely spotted by this fungus after 30 hr of misting (6). Plants were removed from the chamber and maintained in the greenhouse at 23 ± 3 C for 7–8 days and then rated for resistance to tan spot on the basis of lesion size (10) on a given leaf (Tables 1–3). Plants with ratings of 1–2 (small amount of associated leaf damage) were considered relatively resistant, plants with ratings of 3 (intermediate-sized lesions and more leaf damage) were considered moderately resistant, and plants with ratings higher than 3 (large lesions) were considered susceptible.

Experimental design. Of the 15 wheats (*Triticum aestivum* L.) used in this study, six had been reported as resistant (12,16,17,19), eight were resistant in previously unreported tests, and one was consistently reported as susceptible (5,6,16,17) (Table 1). Seed was planted at appropriate times to ensure that plants of each line reached the following growth stages at the time of inoculation: 1) four- to five-leaf stage, unvernalized; 2) four- to five-leaf stage, vernalized; and 3) flag leaf, vernalized. The three treatments are denoted as unvernalized seedlings (US), vernalized seedlings (VS), and adults (A). Four plants per treatment of each genotype were grown in autoclaved, aerated, and aged field soil in clay pots measuring 10 cm (for seedlings) and 15 cm (for adult plants). After emergence, plants assigned to VS and A treatments were vernalized for 40 days at 4.5 ± 2 C.

The experiment was repeated four times. Planting dates were coordinated with the intent of synchronizing the date of flag-leaf expansion (A) with the four-leaf stage (US and VS). However, flag-leaf expansion of plants assigned to the A treatment was synchronized with the four-leaf stage of VS plants for only two of the trials. Therefore, data were collected from four trials for the US treatment, from three trials for the A treatment, and from two trials for the VS treatment. Analyses of variance were computed for each treatment, leaf position, and trial and combined over all main factors. Replicates were nested within trials, four replicates per trial, and all interactions involving replicates within trials were pooled to calculate the error mean square. Least-square means were generated, and mean separation was performed according to Duncan's multiple range test and Fisher's least significant difference.

Observations of tan spot severity were made in the field at five locations in North Dakota during the years 1983–1986, for a total of 17 location-years. Tan spot severity was rated on Roughrider, Agassiz, Norstar, Seward, ND8061, and ND495 using a modified form of the 0–9 assessment scale of Luc Couture (6) (Table 3). Ratings of 1–6 were considered relatively resistant when the susceptible selection ND495 was rated from 7 to 9. Larez (10) and Larez et al (11) determined that the significant factor in this modified Luc Couture rating scale was lesion size. Therefore, we developed the scale used in the greenhouse on the basis of lesion size related to leaf location. Simple correlations were computed for the association between greenhouse and field ratings.

Table 1. Tan spot ratings[x] for 14 hard red winter wheats and ND495 hard red spring wheat[y] rated as adults (A), vernalized seedlings (VS), and unvernalized seedlings (US) in the greenhouse

Cultivar or line	Top leaf A	Top leaf VS	Top leaf US	Second leaf A	Second leaf VS	Second leaf US	Third leaf A	Third leaf VS	Third leaf US
Red Chief	0.8	2.1	1.9	1.1	2.6	3.1	2.3	3.6	4.3
Carifen 12	1.2	2.1	1.9	1.9	3.1	3.2	2.8	4.3	4.6
Siouxland	1.6	3.5	2.8	2.7	4.9	4.3	4.2	5.0	5.0
Bighorn	1.8	2.3	2.9	2.2	4.0	4.4	3.9	4.8	4.9
Agassiz	2.0	4.1	3.4	3.2	5.0	4.9	4.3	5.0	5.0
ND7716	2.0	3.1	2.9	3.0	4.5	4.2	4.3	5.0	4.8
Danne	2.0	2.9	2.5	3.7	4.5	4.4	3.9	5.0	4.8
Eklund	2.1	2.3	2.3	2.3	3.0	3.6	4.2	4.6	4.9
Roughrider	2.1	2.4	3.2	2.8	4.0	4.5	4.8	4.6	4.8
ND8061	2.2	2.9	2.6	2.7	4.6	4.3	4.0	5.0	4.9
Seward	2.3	3.9	3.6	2.9	4.9	4.7	4.4	5.0	5.0
Thunderbird	2.3	4.0	3.4	3.6	4.9	4.8	4.6	5.0	4.9
Norstar	2.6	3.1	2.6	3.6	4.9	4.5	4.2	5.0	5.0
Sundance	2.8	2.5	2.6	3.8	4.4	4.2	4.4	5.0	4.8
ND495	3.5	4.5	3.9	4.3	5.0	5.0	4.5	5.0	5.0
LSD (0.05)	0.7	0.7	0.9	0.8	0.7	0.6	0.8	0.5	0.3
$\bar{X}$[z]	2.1 c	3.1 b	2.8 c	2.9 b	4.3 a	4.3 b	4.1 a	4.8 a	4.8 a

Column group heading: Disease rating by leaf position and growth stage.

[x]Scale: 0 = no spotting, 1 = lesions ≤0.5 mm long, 2 = lesions ≤1 mm, 3 = lesions ≤2 mm, 4 = lesions ≤3 mm, and 5 = lesions >3 mm and coalescing.

[y]From previous reports: Eklund resistant to tan spot (16,17), Sundance CI 15327 resistant (16,17), Red Chief CI 12109 resistant (19), Carifen 12 resistant (12), Danne CI 13876 resistant (F. J. Gough, *personal communication*), ND7716 resistant (16,17), and ND495 susceptible (5,6,16,17). Resistance not previously published: Roughrider (CI 17439), Agassiz (PI 478771), Norstar (CI 17735), Siouxland (PI 483469), Bighorn, Thunderbird, Seward (PI 508289), and ND8061.

[z]Values across the row, for each respective growth stage, followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test, e.g., adult plants (2.1 c, 2.9 b, and 4.1 a). Also, for each respective leaf position, the growth stage means were not detected as being significantly different from each other, e.g., top leaf (2.1, 3.1, and 2.8).

RESULTS AND DISCUSSION

Analyses of variance indicated that cultivar effect was highly significant. Separation of cultivar means was observed for all growth stage-leaf position combinations (Table 1). The range among cultivar means for tan spot severity was greatest for the top leaf (flag leaf) of adult plants and least for lower leaves of unvernalized seedlings. Among the 14 winter wheats tested in the greenhouse, Red Chief was ranked most resistant and Sundance least (Table 1). In all trials, the water-inoculated check plants were not spotted. Rankings of cultivars were similar for unvernalized and vernalized seedlings tested in the greenhouse. Data computed for the top leaf indicated that relative ranking of cultivars based on the adult-plant greenhouse assay was sufficiently different from unvernalized seedlings (r = 0.54) and vernalized seedlings (r = 0.66) to warrant further study.

Leaf position had a significant influence on tan spot severity, which was greatest on the lowest leaf and decreased on each successively higher leaf. For example, the mean tan spot ratings for the top, second, and third leaves of the adult treatment were 2.1, 2.9, and 4.1, respectively, each significantly different from the other (Table 1). This was similar to the increased susceptibility of wheat to tan spot with leaf aging reported by Raymond et al (19). The difficulty in differentiating cultivars based on visual classification increased with each successively lower leaf because of both higher tan spot ratings and decreased differences between maximum and minimum values (Table 1).

The magnitude of mean tan spot rating for the adult and vernalized seedling treatments varied significantly among trials (Table 2). Similarly, differences were exhibited in magnitude of lesion size among growth stages for trials 1 and 2 (Table 2). These differences may be in part due to untested environmental factors such as photoperiod and incident radiation. However, the small range in values for coefficient of variation from individual analyses suggests that the procedure and the relative amount of experimental error were fairly consistent for the entire experiment.

Even though growth stage effect was significant when analyses of variance were computed for individual trials 1 and 2, the combined analysis (over all trials) indicated that growth stage effect was not significant. Significance was not detected for the combined analysis because the appropriate mean square for testing growth stage, the trial × growth stage interaction, was highly significant. For example, for trial 3, mean lesion size on the flag leaf was unusually large compared with flag leaf lesion size for trials 1 and 2 (Table 2), whereas for unvernalized seedlings, mean lesion size was similar for all four trials. However, this difference in mean tan spot rating among trials and growth stages had little effect on relative ranking of genotypes.

Disease severity ratings from both greenhouse and field evaluations are given in Table 3 for six wheats in which multiple location-year field data were obtained. The correlations between mean field severity and greenhouse rating, based on top leaf and mean of all three leaves, was only significant (P = 0.05) for the association between field- and greenhouse-grown adult plants. These significant correlations (r = 0.86, top leaf only, r = 0.87, mean of three leaves), were of similar magnitude, suggesting that tan spot evaluation based on ratings of the flag leaf would be the easiest and most efficient system for ranking resistance. The correlation between field severity and unvernalized seedling rating (r >0.75) was high but not statistically significant, largely because of the limited number (six) of genotypes being compared. Because of this high correlation, greenhouse screening of unvernalized winter wheat seedlings may be an effective method of evaluating diverse germ plasm (susceptible to resistant) before obtaining adult-plant reactions and ranking more resistant genotypes. However, detecting differences among resistant cultivars using unvernalized seedlings (top leaf was the fourth or fifth emerging leaf) may require greater testing precision, because the range of tan spot severity scores among seedlings is less than for adult plants.

A mean tan spot severity rating of ≤3 for the top three leaves of adult greenhouse plants was obtained for Red Chief, Carifen 12, Siouxland, Bighorn, Eklund, and ND8061 (1.4, 2.0, 2.8, 2.6, 2.9, and 3.0, respectively). The respective means of five of the six genotypes tested in the field were comparatively greater, indicating less resistance (Table 3). Only ND8061 was common to both groups, adult greenhouse plants with a mean rating ≤3 and field-tested. Carifen 12 previously had been reported as possessing resistance conditioned by a single recessive gene (12). Nagle et al (16,17) had reported the tan spot reaction of Eklund and Sundance to be similar and that of ND7716 as intermediate between these two wheats and ND495. The results of this study indicate that Sundance is more susceptible to tan spot than either Eklund or ND7716 (Table 1). Raymond et al (19) found greenhouse severity scores of seedlings of TAM-105 (susceptible) and Red Chief (resistant) to accurately reflect the yield loss sustained by these two cultivars in the field in response to tan spot infection.

The results of this study suggest that the reactions on flag leaves in the greenhouse most closely reflect those obtained on adult plants in the field and can be used to rank resistant wheats. However, if screening large numbers of early-generation materials of greatly varying reactions is an objective of a plant breeding program, then the use of

Table 2. Trial means for tan spot rating[a] of the top leaf of 15 wheats rated at three growth stages

Growth stage	Trial 1	2	3	4	LSD (0.05)
Adult (flag leaf)	1.2	1.7	3.3	···	0.3
Vernalized seedling	···	···	3.3	2.8	0.3
Unvernalized seedling	3.0	2.9	2.8	2.6	NS
LSD (0.05)	1.2	0.5	NS	NS	

[a] Scale: 0 = no spoting, 1 = lesions ≤0.5 mm long, 2 = lesions ≤1 mm, 3 = lesions ≤2 mm, 4 = lesions ≤3 mm, and 5 = lesions >3 mm and coalescing.

Table 3. Field and greenhouse ratings of six wheats to tan spot

		Greenhouse rating[b]					
		Top leaf			Mean of top 3 leaves		
Cultivar or line	Field rating[a]	A	VS	US	A	VS	US
Norstar	4.4	2.6	3.1	2.6	3.4	4.3	4.0
ND8061	4.7	2.2	2.9	2.6	3.0	4.2	3.9
Agassiz	4.9	2.0	4.1	3.4	3.2	4.7	4.4
Roughrider	5.2	2.1	2.4	3.2	3.3	3.7	4.2
Seward	5.5	2.3	3.9	3.6	3.2	4.6	4.4
ND495	8.2	3.5	4.5	3.9	4.1	4.8	4.6
LSD (0.05)	0.6	0.7	0.6	0.8	0.5	0.3	0.4
Correlation with field reaction[c]		0.86*	0.64	0.79	0.87*	0.49	0.77

[a] Tan spot severity at Zadok's (21) growth stages 75–77 (medium milk to late milk). Resistant: 0 = no leaves spotted; 1 = 1% lower leaf area spotted; 2 = 5% lower spotted; 3 = 10% lower spotted; 4 = 25–50% lower and 5% mid spotted; 5 = 50% lower and 25% mid spotted; and 6 = 50% lower, 25% mid, and 5% upper spotted. Susceptible: 7 = 50% lower, 50% mid, and 10% upper spotted; 8 = 50–100% lower, 50% mid, and 25% upper spotted; and 9 ≥50–100% lower, 50–100% mid, and 50–100% upper leaves spotted. Mean of 17 location-years.

[b] Resistant: 0 = no spotting, 1 = lesions ≤0.5 mm long, 2 = lesions ≤1 mm, and 3 = lesions ≤2 mm. Susceptible: 4 = lesions ≤3 mm and 5 = lesions >3 mm and coalescing. A = adult plant with flag leaf fully exposed, VS = vernalized seedlings, and US = unvernalized seedling.

[c] * = Significantly different from zero at P = 0.05.

unvernalized seedlings in the greenhouse to eliminate susceptible genotypes is justified by greater efficiency in terms of space and time. The simple rating scale developed, based on lesion size related to leaf location, appears to separate and rank winter wheats for resistance to tan spot.

ACKNOWLEDGMENTS

We wish to thank H. A. Lamey, B. D. Nelson, M. S. McMullen, and R. G. Cantrell for critically reviewing and Phyllis Hellem for typing the manuscript.

LITERATURE CITED

1. Cantrell, R. G., Elias, E., and Hosford, R. M., Jr. 1985. The inheritance of resistance to tan spot in durum wheat. Agron. Abstr. 77:50.
2. Elias, E., Cantrell, R. G., and Hosford, R. M., Jr. 1985. A field inoculation procedure for determining resistance to tan spot of wheat caused by *Pyrenophora tritici-repentis*. Agron. Abstr. 77:52.
3. Gilchrist, L. S. 1982. *Helminthosporium tritici-repentis* (=*Pyrenophora trichostoma*), como agente causal del tizon del trigo, prevalente en el estado de Michacan, Mexico. M.S. thesis. Colegio de Postgraduados, Chapingo, Mexico. 112 pp.
4. Gilchirst, L. S., Fuentes, S., and Isla de Bauer, M. de L. de la. 1984. Determinación de fuentes de resistencia contra *Helminthosporium tritici-repentis* bajo condiciones de campo e invernadero. Agrociencia 56:95-105.
5. Hosford, R. M., Jr. 1971. A form of *Pyrenophora trichostoma* pathogenic to wheat and other grasses. Phytopathology 61:28-32.
6. Hosford, R. M., Jr. 1982. Tan Spot. Pages 1-24 in: Tan Spot of Wheat and Related Diseases Workshop. R. M. Hosford, Jr., ed. North Dakota State University, Fargo. 116 pp.
7. Hosford, R. M., Jr., and Bush, R. H. 1974. Losses in wheat caused by *Pyrenophora trichostoma* and *Leptosphaeria avenaria* f. sp. *triticea*. Phytopathology 64:184-187.
8. Howard, R. J., and Morrall, R. A. A. 1975. The epidemiology of leaf spot disease in a native prairie. I. The progression of disease with time. Can. J. Bot. 53:1040-1050.
9. Krupinsky, J. M. 1982. Observations on the host range of isolates of *Pyrenophora trichostoma*. Can. J. Plant Pathol. 4:42-46.
10. Larez, C. R. 1985. Effect of environmental and biological factors on the infection process by *Pyrenophora tritici-repentis*. Ph.D. thesis. North Dakota State University, Fargo. 94 pp.
11. Larez, C. R., Hosford, R. M., Jr., and Freeman, T. P. 1986. Infection of wheat and oats by *Pyrenophora tritici-repentis* and initial characterization of resistance. Phytopathology 76:931-938.
12. Lee, T. S., and Gough, F. J. 1984. Inheritance of Septoria leaf blotch (*S. tritici*) and Pyrenophora tan spot (*P. tritici-repentis*) resistance in *Triticum aestivum* cv. Carifen 12. Plant Dis. 68:848-851.
13. Luz, W. C. 1986. Development of the wheat leaf spot syndrome as influenced by temperature, interactions among fungal pathogens, and triadimenol seed treatment. Ph.D. thesis. Cornell University, Ithaca, NY. 118 pp.
14. Luz, W. C., and Hosford, R. M., Jr. 1980. Twelve *Pyrenophora trichostoma* races for virulence to wheat in the Central Plains of North America. Phytopathology 70:1193-1196.
15. Morrall, R. A. A., and Howard, R. J. 1975. The epidemiology of leaf spot disease in a native prairie. II. Airborne spore populations of *Pyrenophora tritici-repentis*. Can. J. Bot. 53:2345-2353.
16. Nagle, B. J. 1981. Inheritance of resistance to *Pyrenophora trichostoma*. Ph.D. thesis. North Dakota State University, Fargo. 69 pp.
17. Nagle, B. J., Frohberg, R. C., and Hosford, R. M., Jr. 1982. Inheritance of resistance to tan spot of wheat. Pages 40-45 in: Tan Spot of Wheat and Related Diseases Workshop. R. M. Hosford, Jr., ed. North Dakota State University, Fargo. 116 pp.
18. Odvody, G. N., and Boosalis, M. G. 1978. A rapid technique to study sporulation requirements of *Pyrenophora trichostoma*. Phytopathol. News 12:212-213.
19. Raymond, P. J., Bockus, W. W., and Norman, B. L. 1985. Tan spot of winter wheat: Procedures to determine host response. Phytopathology 75:686-690.
20. Roberts, D. E. 1984. Effect of glaucousness on tan spot infection of spring wheat. M.S. thesis. North Dakota State University, Fargo. 70 pp.
21. Tottman, D. R., Makepeace, R. J., and Broad, H. 1979. An explanation of the decimal code for the growth stages of cereals, with illustrations. Ann. Appl. Biol. 93:221-234.