Response of Durum Wheat Kernels and Leaves at Different Growth Stages to *Pyrenophora tritici-repentis*

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ABSTRACT

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Fourteen durum wheat genotypes were evaluated for the reaction of leaves at different growth stages and kernels to *Pyrenophora tritici-repentis*. Plants were artificially inoculated with *P. tritici-repentis* at the two-leaf stage and after emergence of the flag leaf, and were rated for tan spot reaction by percent leaf area with symptoms and lesion type or length. At two locations in southern Saskatchewan, the same genotypes were rated in the field for leaf spots at late milk to early dough in 1991 and 1992. There was no correlation among trials in percent leaf area with symptoms at the seedling stage. Lesion type was considered the best method to determine tan spot reaction at the seedling stage. Lesion length and percent leaf area with symptoms on the flag leaf were correlated among trials. Lesion length was correlated with percent area with symptoms on the flag leaf and was considered the best method for rating tan spot reaction in adult plants. Percent leaf area with symptoms at the seedling stage was not correlated with that on the flag leaf or with field leaf spot ratings. Most lesions on field plants were attributed to *P. tritici-repentis*. Field leaf spot ratings were correlated with both percent leaf area with symptoms and lesion length on the flag leaf of artificially inoculated plants. Percent incidence of red smudge in kernels of plants artificially inoculated with *P. tritici-repentis* at the milk to early dough stage was not correlated, or was negatively correlated, with tan spot reaction at the seedling or adult stages, and with field leaf spot ratings. In the durum wheat genotypes tested, different resistance mechanisms to *P. tritici-repentis* seemed to be operating in different organs of the plant, and resistance to tan spot observed at the adult stage was not expressed at the seedling stage.

Tan spot is caused by *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph *Drechslera tritici-repentis* (Died.) Shoemaker). Resistance to tan spot in wheat (*Triticum* spp.) has been reported to be under polygenic (8,17) or monogenic (11,15) control. The same fungus also causes red smudge symptoms on seed (9,22), which cause downgrading of wheat (3).

Some reports have indicated that the tan spot reactions of seedlings and of adult field-grown wheat plants were correlated (14,19), as were those of field-grown and greenhouse-grown adult plants (6). However, the reaction of adult wheat plants to tan spot did not always correspond to that of seedlings (6,13). Different reactions to *Leptosphaeria nodorum* E. Müller (anamorph *Septoria nodorum* (Berk.) Berk.) were also reported among different growth stages of spring durum (*Triticum turgidum* L. var. *durum*) and common wheat (*T. aestivum* L.), and winter wheat (1,12, 16,18). Conner (4) found no correlation in the reaction of hexaploid wheat to black point, spot blotch, and common root rot, caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur (anamorph *Bipolaris sorokiniana* (Sacc.) Shoemaker).

The present study was initiated to compare the tan spot reaction of adult durum wheat genotypes in the field to that of artificially inoculated plants at the seedling and adult stages. The association of tan spot reaction on leaves with red smudge developed in *P. tritici-repentis*-infected kernels was also examined.

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MATERIALS AND METHODS

Growth chamber study. Fourteen durum wheat genotypes (including registered cultivars; breeding lines developed at the Agriculture and Agri-Food Canada, Swift Current Research Station; and introductions) were tested under controlled conditions for reaction to *P. tritici-repentis* at the seedling (1 1/2- to 2-leaf) stage and after emergence of the flag leaf. These were eight semidwarf genotypes (Westbred 881, 9060E-01A, DT 369, 8678-DB3A, 8770-K3E, 8774-AF2A, 8663-BP2B, and 8464-DH2D), two genotypes of medium height (Sceptre and Medora), and four tall genotypes (8466-DP1C, 8563-BN4A, Plenty, and Wakooma). Westbred 881 is an introduction from the United States, and 9060E-01A is from ICARDA.

The testing of seedling and adult plants was repeated in three trials. For trials 1 and 2, seeds were planted in pots 7 × 7 × 18 cm using a 1:1 mixture of local Swinton loam soil (2) and peat moss. For trial 3, plants were grown in bedding plant containers (6 × 6 × 6 cm) using the same soil mixture. Plants were fertilized every 2 wk using 1,300 ppm of N, P, and K (20-20-20).

Seedlings at the 1 1/2- to 2-leaf stage were inoculated with a conidial suspension of *P. tritici-repentis* ($2\text{–}3 \times 10^3$ conidia) and incubated at 100% humidity for 24 hr (16 hr dark/8 hr light) at 20 C. The seedlings were then kept in a growth chamber (16 hr daylight, 22 C day/15 C night) for 6–7 days, at which time they were rated for lesion type (14), and the first leaf was rated for percent leaf area with symptoms. Adult plants at Zadoks' growth stage (GS) 45–58 (booting to emergence of inflorescence) (23) were inoculated and incubated in a similar manner, and the flag leaf of the main tiller of each plant was rated for lesion length and percent leaf area with symptoms at

7 days after inoculation. Percent leaf area with symptoms in adult plants was not determined in trial 3. Planting dates of the adult plant test were adjusted so that all genotypes reached the desired growth stage simultaneously. For each genotype, there were four replicates with four plants each. Plants were arranged in a randomized complete-block design.

Spikes of the same 14 genotypes were inoculated with *P. tritici-repentis* at GS 71–83 (milk to early dough), using the same conidial concentration, and incubated in the same way as above. Kernels from the spike of the main tiller were rated for red smudge incidence after harvest. This experiment was also repeated three times, using the same design as above. All three trials were planted in pots and fertilized as indicated above for trials 1 and 2.

An isolate of *P. tritici-repentis,* identified by Lamari and Bernier (14) as ASC1, was used for all artificial inoculations. This isolate was reported as the most common pathotype in western Canada. To compare this isolate with those present in Swift Current and Outlook, Saskatchewan, where the field trials were conducted, 15 isolates of *P. tritici-repentis* were collected at random from infected leaves of durum wheat in 1991 at both locations. Single-spore isolates of these and of ASC1 were then inoculated onto seedlings at the two-leaf stage of five genotypes: Katepwa (common wheat), Medora, Wakooma, Sceptre, and 4B1149 (durum wheats). Lesion type was rated 6–7 days after inoculation, as described above.

Field ratings. Two field trials were planted at Swift Current and Outlook in 1991 and 1992, as described by Fernandez et al (10). The 150–200 genotypes seeded in 1991 and 1992 included the 14 tested here. Irrigation water was applied by overhead sprinklers as required to maximize grain yield.

Plants were rated for leaf spots at Zadoks' GS 77–84 (late milk to early dough) at both locations, using Couture's (5) 1–9 rating system (1 = all leaves free of symptoms, 9 = all leaves with more than 50% of their area covered with leaf spots). Leaf spot ratings were an average of the scores of about 20 plants from the center rows of each plot. To identify the leaf spotting fungi, leaf pieces of about 1 cm^2 were selected randomly from lesions from each of 15–20 upper leaves taken at random from each plot at the time of rating. Leaf pieces were surface-disinfested, plated, and incubated as described by Fernandez et al (10). Percent isolation of the fungi based on the total leaf area colonized by each was calculated for each genotype.

Mean lesion length and arcsine-transformed percent incidence were subjected to an analysis of variance (ANOVA) or general linear model (GLM), and least significant differences (LSD) were calculated (20). Homogeneity of variances was tested using Bartlett's test. Simple correlations were also performed on the data from the field experiments and controlled-environment study.

RESULTS

Field studies. Leaf spot field ratings varied significantly ($P \leq 0.01$) among genotypes in both locations and years. Severity of leaf spots ranged from a score of 5 (0% of area covered with lesions in upper, 10–25% in middle, and 50% or more in lower leaves) to 8 (25–50% of area covered with lesions in upper, and 50% or more in middle and lower leaves) (5). The semidwarfs Westbred 881, 8678-DB3A, 8770-K3E, 9060E-01A, and DT 369 were the most susceptible; whereas the semidwarfs 8663-BP2B, 8464-DH2D, and 8774-AF2A were among the most resistant genotypes (Table 1).

Leaf spots in these 14 genotypes at both Swift Current and Outlook in 1991 and 1992 were attributed primarily to *P. tritici-repentis,* with a mean percent isolation of 84% in 1991 and 68% in 1992. However, the genotypes 8663-BP2B, 8464-DH2D, 9060E-01A, and Westbred 881 in 1992 had similar proportions of both *P. tritici-repentis* and *L. nodorum* at Swift Current, and a higher proportion of *L. nodorum* (70–77%) than of *P. tritici-repentis* (22–30%) at Outlook. In 1991, tan spot lesions observed on flag leaves of the 14 genotypes at GS 77–84 were mostly small (<2 mm) and intermediate (about 2–3 mm) in the most resistant genotypes, and mostly intermediate to large (>3 mm) in the most susceptible genotypes.

The 30 isolates of *P. tritici-repentis* collected from both locations in 1991 gave a similar reaction based on lesion type (14) at the two-leaf stage to that of the isolate used for the artificial inoculation study (below). These were a 2 (moderately resistant) on 4B1149, 3–4 (intermediate–moderately susceptible) on Sceptre, 4–5 (moderately susceptible–susceptible) on Wakooma and Medora, and 5 (susceptible) on Katepwa.

Growth chamber study. Based on lesion type on seedlings inoculated at the two-leaf stage, the 14 genotypes were all susceptible. They rated 4–5 (moderately susceptible–susceptible), except for 8563-BN4A, 8464-DH2D, and 8770-K3E, which rated 4 (moderately susceptible), and Sceptre, which rated 3–4 (intermediate–moderately susceptible). Because of coalescence of lesions on genotypes rated 4–5, lesion length was not determined. Six to seven days after inoculation, percent area of the first leaf with tan spot symptoms was 4–16% for trial 1, 4–14% for trial 2, and 8–16% for trial 3. In all three trials, there was a significant difference ($P \leq 0.01$) among genotypes. Whereas ratings based on lesion type on seedlings were consistent among tests, percent leaf area affected was not. The genotype × test interaction was significant ($P \leq 0.01$), and there was no correlation ($P > 0.05$) among trials. However, overall, 8464-DH2D, 8563-BN4A, and 8770-K3E had the lowest (average for all three trials of 7–8%) and Westbred 881 the highest (14%) percent leaf area affected.

In contrast, percent area of flag leaf with symptoms at 7 days after inoculation in trials 1 (3–7%) and 2 (4–9%) was correlated ($r = 0.56$, $P \leq 0.05$). Lesion length on the flag leaf in trials 1–3 (Table 2) was also correlated ($r = 0.65$, $P \leq 0.05$, to $r = 0.74$, $P \leq 0.01$). The genotype × test interaction was not significant ($P > 0.05$) for percent leaf area with symptoms but was significant ($P \leq 0.01$) for lesion length. Percent leaf area with symptoms was correlated with lesion length in both trials ($r = 0.78$–0.85, $P \leq 0.01$).

The genotypes differed ($P \leq 0.01$) for percent leaf area with symptoms and le-

Table 1. Severity of leaf spots in 14 durum wheat genotypes grown at two locations in Saskatchewan in 1991 and 1992

	Leaf spots[z]			
	1991		1992	
Genotype	Swift Current	Outlook	Swift Current	Outlook
Westbred 881	7	8	7.8	7.3
9060E-01A	7	8	7	7
8678-DB3A	7	8	7	7
8770-K3E	7	8	7	6.8
DT 369	6.3	8	7	6.8
Medora	6	7.8	7	6
Sceptre	6	7.3	6.5	6
Wakooma	5.3	7	6.5	5.8
Plenty	5.3	7	5.3	5.3
8774-AF2A	5.5	7.3	6.3	5.5
8464-DH2D	5.5	7	5.8	6
8466-DP1C	5	7	5	5.3
8563-BN4A	5.5	7	5.3	5.8
8663-BP2B	5.3	7	5	5.3
LSD (0.05)	0.5	0.5	0.5	0.5

[z] Leaf spots based on a scale of 0 (all leaves free of symptoms) to 9 (all leaves >50% covered with leaf spots) (5).

sion length on the flag leaf at 7 days after inoculation. Genotypes with the shortest lesions (average of <2 mm) had mostly small dark and tan lesions, whereas those with larger lesions (average of >2 mm) had mostly both small and large tan lesions (Table 2). It should be noted that all genotypes developed tan spot lesions when inoculated with *P. tritici-repentis;* therefore, none were regarded as possessing adequate resistance to this disease. However, the most susceptible genotypes also developed dark lesions, indicative of a resistant reaction (14). The presence of different types of lesions on the same flag leaf made categorization of genotypes based on lesion type difficult.

Differences among the more resistant and susceptible genotypes became more pronounced with time. By 15 days after inoculation, lesions on genotypes with the largest lesion size (>2 mm) had expanded and coalesced, and percent leaf area with symptoms was estimated to be more than 30% in most cases (*data not shown*). Although there was also expansion of lesions in the most resistant genotypes (lesions <2 mm), in general there was very little coalescence of lesions; and on average, percent leaf area with symptoms was estimated to be less than 20% (*data not shown*).

There was no correlation ($P > 0.05$) between percent leaf area with symptoms at the two-leaf stage and on the flag leaf. Leaf spot ratings taken in the field at Swift Current and Outlook in 1991 and 1992 were correlated with lesion length and percent leaf area with symptoms on the flag leaf (Table 3), but not with percent leaf area with symptoms at the seedling stage ($P > 0.05$).

Artificial inoculations of spikes of the 14 genotypes at GS 71–83 resulted in a significant difference ($P \leq 0.01$) among genotypes in the incidence of red smudge assessed visually after harvest. Variances of the three trials were homogeneous, and the genotype × test interaction was not significant at $P = 0.01$. Therefore, data from the three trials were combined (Table 4). The most susceptible genotypes included the registered cultivars Medora, Sceptre, Wakooma, and Plenty; whereas the introductions Westbred 881 and 9060E-01A were among the most resistant genotypes.

Incidence of red smudge (all three trials combined) and lesion length or percent leaf area with tan spot symptoms at the seedling or adult stage were not correlated ($P > 0.05$), except for trial 2, where lesion length on the flag leaf was negatively correlated with incidence of red smudge ($r = -0.63$, $P \leq 0.05$). Percent incidence of red smudge in artificially inoculated plants was also negatively correlated with field leaf spot ratings at Outlook in 1992 ($r = -0.60$, $P \leq 0.05$), but it was not correlated ($P > 0.05$) with leaf spot ratings at Swift Current in 1992 or at either location in 1991.

DISCUSSION

The correlation of percent leaf area with symptoms and lesion length on the flag leaf agrees with Diaz de Ackerman et al (7), who suggested that lesion length identifies genotypes with different levels of resistance. Therefore, lesion length on the flag leaf can be regarded as a good indicator of tan spot reaction and as the most practical way to assess genotypes for resistance to this disease. Lesion type, on the other hand, was considered less adequate for flag leaves because different types of lesions were commonly seen on the same leaf. However, lesion type (14) on seedlings was considered better than percent leaf area with symptoms for determination of seedling reaction. Percent area with symptoms on the first leaf of two-leaf seedlings was very variable among tests. Because of coalescence of lesions in the most susceptible genotypes, lesion length on seedlings could not be determined with accuracy.

The lack of correlation between the reactions of adult plants and seedlings, and the observation that the reaction of artificially inoculated flag leaves, but not seedling leaves, was correlated with field leaf spot ratings, agrees with Cox and Hosford (6). This study also showed that, in most cases, there was no correlation between susceptibility to red smudge and tan spot reactions in artificially inoculated adult plants or seedlings field leaf spot ratings.

These observations suggest that in durum wheat, there might be resistance to tan spot expressed in adult plants but not in seedlings, and that different resistance mechanisms to *P. tritici-repentis* appear to operate in different plant organs. This is similar to the conclusion reached by Arseniuk et al (1) in relation to resistance of spring and winter wheat to *L. nodorum* at different plant growth stages, and to findings by Schilder and Bergstrom (21) and Conner (4) in relation to resistance of hexaploid wheat to *P. tritici-repentis* and *C. sativus,* respectively. The lack of correlation between tan spot reaction at the two-leaf stage and after emergence of the flag leaf suggests that both seedlings and adult plants should be examined when testing

Table 2. Length of tan spot lesions on flag leaves of durum wheat artificially inoculated with *Pyrenophora tritici-repentis* in three trials

Genotype	Lesion length (mm)[z]		
	Trial 1	Trial 2	Trial 3
Westbred 881	3.1	2.6	2.3
9060E-01A	2.4	3.5	2.4
8678-DB3A	3.3	3.1	2.4
8770-K3E	2.7	2.8	2.0
DT 369	2.5	2.5	1.6
Medora	2.3	2.2	2.5
Sceptre	2.2	1.8	1.9
Wakooma	1.6	1.7	1.8
Plenty	1.6	1.8	1.5
8774-AF2A	1.8	2.1	1.6
8464-DH2D	2.0	1.7	1.1
8466-DP1C	1.8	2.0	1.7
8563-BN4A	1.7	2.1	1.8
8663-BP2B	1.5	1.9	1.2
LSD (0.05)	0.5	0.4	0.4

[z] Mean length of longest lesion on flag leaves of four replicates of four plants each.

Table 3. Correlation of field leaf spot ratings of 14 durum wheat genotypes at two locations and in 2 yr with percent leaf area affected and lesion length in flag leaves of growth room-grown plants artificially inoculated with *Pyrenophora tritici-repentis*

Growth room measurements	Field leaf spot ratings[x]			
	Swift Current		Outlook	
	1991	1992	1991	1992
Leaf area (%)[y]				
Trial 1	0.59*[z]	0.59*	0.62*	0.54*
Trial 2	0.80**	0.56*	0.82**	0.77**
Lesion length (mm)				
Trial 1	0.90**	0.80**	0.88**	0.90**
Trial 2	0.88**	0.65*	0.87**	0.83**
Trial 3	0.70**	0.71**	0.71**	0.61*

[x] Leaf spots rated based on Couture (5).
[y] Leaf area and length of tan spot lesions measured on flag leaves of plants artificially inoculated with *P. tritici-repentis.*
[z] * = Significant at $P \leq 0.05$, and ** = significant at $P \leq 0.01$.

Table 4. Incidence of red smudge in kernels of durum wheat plants artificially inoculated with *Pyrenophora tritici-repentis*

Genotype	Incidence (%)[z]
Medora	26.6 a
Sceptre	21.9 ab
8663-BP2B	21.2 ab
8466-DP1C	18.8 ab
Wakooma	17.9 b–d
8563-BN4A	16.4 bc
Plenty	16.3 b–d
8464-DH2D	15.7 b–d
8770-K3E	14.2 b–e
8774-AF2A	11.5 c–f
8678-DB3A	10.8 d–f
DT 369	9.9 d–f
9060E-01A	9.1 ef
Westbred 881	7.3 f

[z] Mean incidence of three trials. Values followed by the same letter are not significantly different ($P > 0.05$) according to an LSD test performed on arcsine-transformed values.

genotypes for resistance to this disease. Screening only seedlings for reaction to tan spot might not allow the detection of resistance at the adult stage.

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LITERATURE CITED

1. Arseniuk, E., Fried, P. M., Winzeler, H., and Czembor, H. J. 1991. Comparison of resistance of triticale, wheat and spelt to septoria nodorum blotch at the seedling and adult plant stages. Euphytica 55:43-48.
2. Ayers, K. W., Acton, D. F., and Ellis, J. G. 1985. The soils of the Swift Current Map Area 72J Saskatchewan. Univ. Saskatchewan, Saskatoon, SK, Ext. Publ. 481.
3. Canadian Grain Commission. 1991. Official Grain Grading Guide. Canadian Grain Commission.
4. Conner, R. L. 1990. Interrelationship of cultivar reactions to common root rot, black point, and spot blotch in spring wheat. Plant Dis. 74:224-227.
5. Couture, L. 1980. Assessment of severity of foliage diseases of cereals in cooperative evaluation tests. Can. Plant Dis. Surv. 60:8-10.
6. 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 Dis. 71:883-886.
7. Diaz de Ackerman, M., Hosford, R. M., Jr., Cox, D. J., and Hammond, J. J. 1988. Resistance in winter wheats to geographically differing isolates of *Pyrenophora tritici-repentis* and observations on pseudoperithecia. Plant Dis. 72:1028-1031.
8. Elias, E., Cantrell, R. G., and Hosford, R. M., Jr. 1989. Heritability of resistance to tan spot in durum wheat and its association with other agronomic traits. Crop Sci. 29:299-304.
9. Fernandez, M. R., Clarke, J. M., DePauw, R. M., Irvine, R. B., and Knox, R. E. Black point and red smudge in irrigated durum wheat in southern Saskatchewan in 1990-1992. Can. J. Plant Pathol. In press.
10. Fernandez, M. R., Clarke, J. M., DePauw, R. M., Irvine, R. B., and McLeod, J. G. 1993. Evaluation of durum wheat for resistance to tan spot and pink smudge. Pages 28-32 in: Proc. Intl. Tan Spot Workshop, 2nd. L. J. Francl, J. M. Krupinsky, and M. P. McMullen, eds. North Dakota State University, Fargo.
11. Frohberg, R. C. 1982. Breeding hard red spring wheat for resistance to tan spot. Page 48 in: Tan Spot of Wheat and Related Diseases Workshop. R. M. Hosford, Jr., ed. North Dakota State University, Fargo.
12. Gilbert, J., and Tekauz, A. 1993. Reaction of Canadian spring wheats to *Septoria nodorum* and the relationship between disease severity and yield components. Plant Dis. 77:398-402.
13. Hosford, R. M., Jr., Jordahl, J. G., and Hammond, J. J. 1990. Effect of wheat genotype, leaf position, growth stage, fungal isolate, and wet period on tan spot lesions. Plant Dis. 74:385-390.
14. Lamari, L., and Bernier, C. C. 1989. Evaluation of wheat lines and cultivars to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. Can. J. Plant Pathol. 11:49-56.
15. 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.
16. Mullaney, E. J., Scharen, A. L., and Bryan, M. D. 1983. Resistance to *Septoria nodorum* in a durum wheat cultivar as determined by stage of host development. Can. J. Bot. 61:2248-2250.
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.
18. Nelson, L. R., and Cowder, J. 1992. Effect of growth stage on components of partial resistance of wheat to *Septoria nodorum*. Cereal Res. Comm. 20:33-40.
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. SAS Institute. 1985. SAS User's Guide: Statistics. Version 5 ed. SAS Institute, Cary, NC.
21. Schilder, A. M. C., and Bergstrom, G. C. 1993. Infection of wheat seed by and seed transmission of *Pyrenophora tritici-repentis*. Pages 56-60 in: Proc. Intl. Tan Spot Workshop, 2nd. L. J. Francl, J. M. Krupinsky, M. P. McMullen, eds. North Dakota State University, Fargo.
22. Valder, P. G. 1954. Yellow leaf spot and pink grain in wheat. Agric. Gaz. N.S.W. 65:36-37.
23. Zadoks, J. C., Chang, T. T., and Konzak, C. F. 1974. A decimal code for the growth stages of cereals. Weed Res. 14:415-421.