# Effect of Wheat Genotype, Leaf Position, Growth Stage, Fungal Isolate, and Wet Period on Tan Spot Lesions

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**ABSTRACT**

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Resistance was related to wheat genotype, with 11 of 59 Chinese wheats more resistant to *Pyrenophora tritici-repentis* than resistant genotype BH1146, as expressed by lesion length rating. Resistance decreased with aging of the leaf. These findings were generally consistent for three highly aggressive fungal isolates. Increasing postinoculation foliage wet period related to increasing lesion size, with resistant genotypes acting similarly but further separating for degree of resistance.

Additional keywords: CIMMYT, *Drechslera tritici-repentis, Helminthosporium tritici-repentis, Pyrenophora trichostoma*, yellow leaf spot

*Pyrenophora tritici-repentis* (Died.) Drechs. causes tan spot or yellow leaf spot, characterized by small dark to larger oval tan to brown lesions, often with narrow to broad yellow borders, that enlarge most along the length of the leaf of wheat (*Triticum aestivum* L., *T. durum* Desf.) and other Gramineae (21,22,34,35,48,50). The disease is being recognized as economically damaging to wheat in many areas around the world (3,6,11,13,17,29,33,34,39,40,43,44,48-50). Fungal isolates vary in the severity of the damage they cause (7,13,16,17, 20,22,23,32) and cause comparable damage on wheat when obtained from wheat or bromegrass (*Bromus inermis* Leyss.) (22). The reaction of wheat cultivars to tan spot differs from one part of the world to another, suggesting regional strain differences in the fungus (13,14,17).

In some studies, resistance (ability to restrict damage, as measured by amount of leaf destroyed, or lesion size or length, or lesion ratings) in wheat appears to be polygenic and of intermediate to high heritability (10,36), and aggressiveness (degree of ability to cause damage) in the fungus appears to be polygenic (7-9,22,30-32,42). A single recessive gene for resistance has been reported in the winter wheat cultivar Carifen 12 (27). Lesion size on specific genotypes has been related to a toxic fungal protein of apparent 13,500 $M_r$ (2,23,25,45,46). An extensive chlorosis reaction to certain isolates of the fungus, detected in 5% of 695 wheat accessions, was related to a division of fungal isolates into three pathotypes: 1) inducing both tan necrosis and extensive chlorosis, 2) inducing tan necrosis only, and 3) inducing extensive chlorosis only (23-25). Susceptibility to tan necrosis and sensitivity to the fungal toxic protein were related to a single dominant gene (23,25). A fungal phytotoxic element unrelated to lesion size and of less than 10,000 $M_r$ also has been reported (4).

Resistance in wheat and oats (*Avena sativa* L.) occurs first as papillae formation (effective in oats only), then as a molecular-level restriction of lesion growth and of mycelial growth around mesophyll cells within (26) and beyond the lesion (23). Wheat genotypes differ in their ability to restrict growth of *P. tritici-repentis* and tan spot lesions. This restriction is overcome in differing degrees in each genotype as the period of postinoculation foliage wetness lengthens and/or the temperature rises (18,22,26,28,30). In one unconfirmed report, duration of postinoculation foliage wetness did not affect this resistance (25). Wetting wheat foliage before inoculation with the fungus had no effect on tan spot (14).

Epidemics of tan spot at different growth stages of wheat cause differing losses, with the highest losses recorded for inoculations at boot and flowering stages (40,43). Tan spot is often progressively more severe on lower leaves (8,38). The incubation period (inoculation to first symptoms) decreases with rising temperature (30).

Tan spot resistance for a given wheat genotype is similar for both seedling and adult greenhouse-grown plants and adult field-grown plants (8,13,23). Resistance has been found in species of *Aegilops* (1) and in the durum × *Aegilops* derivative Largo and Largo derivatives (L. R. Joppa, R. M. Hosford, Jr., and J. G. Jordahl, *unpublished*).

The rate of lesion development has been reduced by increasing nitrogen fertilizer rate, by increasing the proportion of nitrogen taken up as ammonium (19), and by application of KCl or $CaCl_2$ (5). Lower or higher lesion numbers also may be related to resistance in some (22,28,30) but not all (18,19,26,29) wheats and may differ with each genotype × temperature interaction (30).

Significant differences in lesion lengths among wheat genotypes have been detected (8,18,20,22,26,30) and related among seedlings and adult plants in the greenhouse to severity of tan spot on adult plants in the field (8,9,23,24,41). Lesion width also has been greater in susceptible than in resistant wheat (28). Recently, significant differences in lesion length also have been detected among oat genotypes attacked by *P. avenae* Ito & Kuribayashi (12) and among barley (*Hordeum vulgare* L.) genotypes attacked by *P. teres* (Died.) Drechs. (37). With *P. teres*, the resistance differences also were related to lesion numbers, incubation period, and lesion size (37). Differences have been detected among single ascospore isolates of *P. tritici-repentis* for lesion length and infection efficiency (20).

Our objectives were to examine lesion length as related to wheat genotype, leaf position, plant growth stage, fungal isolate, and wet period and to determine resistance to aggressive isolates of *P. tritici-repentis* from areas of the Great Plains of North America among Chinese spring wheats that had little leaf spotting in China.

## MATERIALS AND METHODS

**Wheat genotypes and fungal isolates.** Fifty-nine spring wheat genotypes, found

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to have relatively little leaf spotting in China by Sanjaya Rajaram, spring wheat breeder from Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), and recorded by CIMMYT as harineros Y86-87, were tested for resistance to North American isolates of *P. tritici-repentis*. Hard red spring wheat line ND495 was included as a susceptible check, and spring wheat cultivar BH1146 from Brazil was included in trials 1–22 as a resistant selection (8,18,28,32). All tested fungal isolates had been stored in liquid nitrogen for over 4 yr, and all were highly aggressive on ND495 and of lower aggressiveness on BH1146 in 30-hr post-inoculation wet periods (8,9,32). In a previous study (32), the descending order of aggressiveness on other wheats of the four virulent fungal isolates used were: 1) single-ascospore isolate PTI2 from wheat straw from Winner, South Dakota, in 1973; 2) single-ascospore isolate 78-62 from wheat straw from Bozeman, Montana, in 1978; 3) single-ascospore isolate PYD7 from wheat straw at Dickinson, North Dakota, in 1969; and 4) single-conidium isolate PTL1 from a spotted wheat leaf at Fargo, North Dakota, in 1978.

**Wheat culture.** Seeds of each wheat genotype were planted in Fison's sunshine blend No. 1 (Canadian sphagnum peat moss, perlite, vermiculite, dolomitic limestone, pH adjusted with wetting agent; Fison Horticulture, Vancouver, BC) at a rate of five seeds per 15-cm-diameter clay pot or two seeds per 4-cm-diameter, 17-cm-deep, 164-$cm^3$ Super Cell Cone-Tainer (Ray Leach Nursery, Canby, OR).

Each pot received 13 ml of Osmocote 14-14-14 controlled-release fertilizer (Sierra Chemical Co., Milpitas, CA). The plants in the Cone-Tainers were fertilized with 13 ml of Peters professional water-soluble fertilizer, geranium special 15-15-15, and 7 ml of Peters soil test fertilizer, soluble trace element mix (Peters Fertilizer Products, W. R. Grace and Co., Fogelsville, PA) per 3.8 L of warm water. Plants growing to the seedling four- and five-leaf stage were given two applications, and plants growing to later stages received three applications. Plants usually required 5 wk to grow to the five-leaf stage and 7 wk to grow to the flag leaf stage. When at the three-leaf stage, plants were thinned to three per pot and one per Cone-Tainer. To prevent lodging and to maintain separation of the wheat genotypes, plants in each pot were supported by a 1-m bamboo pole with a wire hoop mounted to a clothespin. The plant in the Cone-Tainer was supported by a 0.6-m bamboo pole with a plastic tie. The plants were grown in a glasshouse at 21 ± 7 C. Supplementary light was provided by Sylvania F40 CW fluorescent tubes to produce 14-hr day lengths. Plants were sprayed with resmethrin from a directed-spray insecticide aerosol generator as needed to control aphids and with acephate soluble powder (Orthene 75S) to control thrips.

**Inoculum.** Each isolate was grown on potato-dextrose agar (PDA) (liquid from 200 g of potato autoclaved in 500 ml of distilled water mixed with 20 g of dextrose and 20 g of agar in an additional 500 ml of distilled water) in four 15 × 150 mm glass petri plates. While on the PDA, the isolates were continuously illuminated by Sylvania F40 BLB and CW fluorescent tubes 35 cm above the plates with the temperature at 21 ± 3 C for 7–10 days. Each isolate grew to within 1 cm of the periphery of the plate and/or produced a dark zone of mycelium surrounded by a lighter gray middle zone merging to a nearly white outer zone on the PDA. A 1-cm round plug of mycelium and PDA taken from the light gray middle zone was transferred onto the center of a modified V-8 agar (150 ml of V-8 juice, 1.5 g of $CaCO_3$ and 20 g of agar in 850 ml of distilled water) in each of 20–36 15 × 150 glass petri plates. Because the fungus appeared to grow better on thick agar, each plate contained 30 ml of V-8 agar. The edges of each plate were sealed with Parafilm M, and the plates were transferred to a Percival 13LLVL temperature-controlled chamber (Percival Mfg. Co., Boone, IA) maintained at a temperature of 19 ± 1 C. Each group of plates on the four shelves in the chamber were illuminated by a General Electric F20 CW and a General Electric F20 BLB fluorescent tube mounted 15 cm above the plates and four Sylvania F40 CW tubes mounted on the sides of the chamber. A 14-hr light and 10-hr dark photoperiod was maintained in the chamber. The light cycle induced conidiophore formation and the dark cycle, conidia production. The cultures were grown in the chamber for 10 ± 3 days. Each plate was then flooded with 10 ml of sterile distilled water. The conidia and conidiophores were knocked down with a flat spatula, then in later runs with a glass slide. The inoculum was washed into a blender with sterile distilled water to produce a final volume of 450 ml and blended for 5 min. In trials 23–25, isolates PTI2, PTL1, 78-62, and PYD7 were each grown on nine plates of V-8 agar, for a total of 36 plates per trial. The conidia and conidiophore inoculum from the 36 plates was mixed together and adjusted to concentration. For the experiments utilizing the Cone-Tainers (trials 4–7 and 19–22), 25 plates of a single isolate were utilized per run. In all other trials, the fungal isolate was grown on 20 plates of V-8 agar. Conidia and conidiophores were counted with a hemacytometer, and the suspension was adjusted to 20,000–25,000 conidia and conidiophores per milliliter in trials 1–3 and 8–12; to 25,000 in trials 4–7, 13–15, 16–18, 19–22, 26–28, and 29-31; and to a 24,100–78,500 mixture from equal numbers of culture plates of each isolate in trials 23–25. Both intact and fragmented conidiophores were present in the resulting inoculum. Mycelial fragments (infective) in the inoculum were not recorded but presumably were relatively constant among trials. Usually a final volume of 500–600 ml of conidia and conidiophore suspension was obtained. Tween 20, two drops (0.04 ml) per 100 ml of suspension solution, was added as a wetting agent. A water check treatment was prepared with distilled water plus two drops of Tween 20 per 100 ml.

Among the 31 trials, the plants were inoculated when most genotypes were around the seedling four- and five-leaf growth stages, at flag leaf, and at dough (47); some genotypes deviated from these growth stages (GS) because of differences in growth rate. The inoculum suspension was sprayed in 125-ml amounts on wheat plants with a DeVilbiss No. 26 atomizer at 1.38 × $10^5$ Pa within 1 hr after preparation. An average 2.3 ml of inoculum per plant was delivered, which corresponded to the beginning of runoff of the inoculum from the plant leaves. The sprayed plants were already randomized in the inoculation chamber by container (pot or Cone-Tainer). Check plants were sprayed separately, then placed by container in their randomized locations in the chamber.

**Experimental design.** All but two of the trials (4 and 5) utilized a water-injected mist system. For trials 4 and 5, plants were incubated in an artificially lighted, moist chamber at 100% relative humidity and 21 ± 2 C. The dew on the wetted inoculated leaves was maintained for 30 hr by placing warm water in the bottom of the chamber and spraying cool water on the canvas of the walls in the chamber. For the remaining trials, a combination water and compressed air mist chamber was used as both the inoculation and the subsequent incubation chamber, with the plants misted at 23 ± 3 C for specific postinoculation wet periods. The mist chamber was located on a glasshouse bench (365 cm long, 90 cm wide, and 123 cm high) and covered with clear flexible plastic supported by a metal frame. Thick (2.4 cm), high-density Styrofoam insulation was placed on the top and sides of the chamber facing the south and west to help maintain an even temperature during the summer and days with bright sunshine. Within the chamber, four nozzles mounted horizontally 103 cm above the bench provided a fine mist. The nozzles were fed by the use of a time clock to deliver 1 min of Fargo city water every 5 min.

All 61 genotypes were inoculated in trials 1–28 (Tables 1–3), except that BH1146 was not in trials 23–25 and 26–28; trials 29–31 contained only seven genotypes (Table 4). In trials 1–3, 23–25,

and 29–31, three randomized pots of a genotype were used, each pot containing three plants. The experimental design was complete randomization with three replications (pots) and three subsamples (plants). In trials 4–7 and 19–22, six randomized Cone-Tainers, each containing one plant, were used for each genotype. Four plants were inoculated with PTI2 and two were inoculated with water. The experimental design was complete randomization with four replications (plants in Cone-Tainers). In trials 8–12, 13–15, 16–18, and 26–28, one randomized pot containing three plants of a genotype was used per block. The experimental design consisted of three subsamples (plants) per replication and was a randomized complete block with five replications for trials 8–12 and three replications for trials 13–18 and 26–28.

After the postinoculation wet period, plants were placed on a glasshouse bench and allowed to dry. In all trials, plants were rated 8 days after inoculation for the longest lesion on the top three leaves (one lesion per leaf), according to the following scale: 0 = no spots, 1 = spots ≤0.5 mm, 2 = 0.6–1.0 mm, 3 = 1.1–2.0 mm, 4 = 2.1–3.0 mm, 5 = >3 mm and coalescing. Generally, lesions were well separated, and long lesions were distinguished from coalesced lesions by the tiny dark sites of infection in the centers of individual lesions. To avoid any effects from water on the tips of the leaves for a prolonged period, the top one-fifth of each leaf was not rated.

Each trial was analyzed individually, and the experimental errors from each trial were pooled for an estimate of experimental error in the combined analysis. An SAS GLM procedure was used (15). Subgroups of trials testing individual factors were analyzed, and LSD ($P = 0.05$) values were determined for several comparisons within the study.

To compare genotypes and leaf positions to averaged rating length, the results of trials 1–25 were combined (Table 1). For growth stages, trials 1–3 (four-leaf), 4–7 (five-leaf), 8–12 (flag leaf), and 26–28 (dough) were compared (Table 2). Because lesions that begin at dough stage probably do not affect yield, trials 26–28 were not included in Table 1 for ranking genotypes for resistance. For isolates, trials 8–12 (PTI2), 13–15 (PYD7), and 16–18 (PTL1) were compared (Table 3). For initial wet period, trials 4–7 (PTI2, 30-hr mist, five-leaf stage) and 19–22 (PTI2, 48-hr mist, five-leaf stage) were compared, but to reduce the amount of data presented, only the means for each leaf location of the 61 genotypes were presented and compared. For comparison of higher resistance in longer wet periods, seven genotypes were studied in 48-, 72-, and 96-hr postinoculation wet periods in trials 29–31 (Table 4). For observing the simultaneous effect of four isolates (PTI2, PYD7, PTL1, and 78-62, 30-hr mist, four-leaf stage), trials 23–25 were conducted and the means of the 61 genotypes were compared with those of one isolate (PTI2, 30-hr mist, four-leaf stage, trials 1–3). To reduce data presented, only the means of the 61 genotypes were compared.

## RESULTS AND DISCUSSION

The data in the tables are restricted to representative genotypes and leaf locations that illustrate the points we wish to make. Computations are based on the entire set of 61 genotypes. We hope to publish the data on the entire 61 genotypes and top three leaves in the *Annual Wheat Newsletter*.

**Genotype.** Significant, consistent differences occurred among genotypes in all the trials, but variations in magnitudes of lesion size among all but trials 26–28 resulted in significant interactions involving trials, such as trials × genotype. It may be that conducting trials 26–28 in a short period in the fall when the temperature was less variable resulted in less variation among trials.

By 8 days after inoculation, the lesions produced by the fungal isolates used in this study had gone from yellow to tan necrosis on ND495 (susceptible check) but had not yet undergone that change on the other wheats. Some wheats may have had extensive enough chlorosis to fit the chlorotic reaction type (23). According to the rating scale on top leaves, lesion length was significantly different among the wheat genotypes, with 11 Chinese wheats significantly more resistant than BH1146 (Table 1). Ten moderately resistant to susceptible genotypes are included in Table 1 for comparison. Other genotypes with resistance greater than that of BH1146 recently have been reported in a Canadian study (23,24). In general, across leaf position (Tables 1–4), plant growth stage (Table 2), fungal isolates (Table 3), and wet period (Table 4), genotype resistance, expressed as a disease rating based on lesion length, was consistent and most evident on the top leaf.

**Leaf position.** Lesion length rating increased with lower leaf position, until the highest rating of five was reached (Tables 1–4). This agreed with earlier reports (8,38) and emphasized the desirability of making comparisons on the same leaf level. Excluding the apex one-fifth of each leaf from the rating may have occasionally resulted in lower lesion ratings. Sometimes ratings of four or five were evident on the apex one-fifth, one-third, or one-half of the leaf and a rating of three on the rest of the leaf. This should be studied further, and lesion position on the leaf should be related to resistance rating.

**Growth stage.** With one of our most aggressive isolates, PTI2 (8,9,32), at a given wet period, 30 hr (Table 2), mean lesion length rating on the top leaf of the 61 genotypes was 3.2 at flag leaf stage, 3.4 at four-leaf stage, 3.6 at five-leaf stage, and 4.6 at dough. On the lower leaves for these same growth stages, the trends were similar—4.1, 4.1, 4.4, and

**Table 1.** Genotype and leaf position effects on averaged rating length of tan spot lesions[z]

| CIMMYT no. | Genotype | Top leaf | Top-1 | Top-2 |
|---|---|---|---|---|
| 148 | 665 | 1.8 a | 2.7 a | 3.6 a |
| 142 | P83-5112 | 2.3 ab | 3.0 ab | 3.6 a |
| 115 | Shanghai7-18B-OY | 2.4 bc | 3.3 bc | 3.9 ab |
| 114 | Shanghai7-17B-OY | 2.6 b–d | 3.6 c–f | 4.2 bc |
| 116 | Shanghai7-19B-OY | 2.6 b–d | 3.5 c–e | 4.3 b–d |
| 117 | Shanghai7-31B-OY | 2.6 b–d | 3.5 c–e | 4.2 bc |
| 119 | Shanghai8-3B-OY | 2.6 b–d | 3.4 b–d | 4.2 bc |
| 131 | Wuhan2-43B-OY | 2.6 b–d | 3.3 bc | 4.2 bc |
| 149 | 1683-8 | 2.6 b–d | 3.4 b–d | 4.1 bc |
| 118 | Shanghai7-40B-OY | 2.7 b–d | 3.6 c–f | 4.2 bc |
| 120 | Shanghai8-7B-OY | 2.7 b–d | 3.5 c–e | 4.3 b–d |
| 141 | Qian Feng #2 | 2.7 b–e | 3.8 c–h | 4.4 c–e |
| 138 | YM#6-36B-OY | 2.8 b–f | 3.6 c–g | 4.4 c–e |
| 140 | Chuan Mai #18 | 2.8 c–g | 3.3 bc | 4.3 b–d |
| 139 | YM#6-40B-OY | 2.9 c–h | 3.8 c–h | 4.6 c–f |
| 147 | 793-3402 | 2.9 c–h | 3.8 c–h | 4.1 a–c |
| 101 | Ning 8331 | 3.0 d–i | 3.8 c–h | 4.5 c–f |
| 2 | BH1146 (Brazil) | 3.3 e–m | 4.2 h–l | 4.7 d–f |
| 96 | Nanjing 82049 | 3.5 i–n | 4.3 h–l | 4.7 d–f |
| 97 | Ning NO. 8180 | 4.3 q | 4.7 lm | 4.9 ef |
| 1 | ND495 (susceptible check) | 4.8 r | 4.9 m | 5.0 f |
| Mean (61 genotypes) | | 3.4 | 4.1 | 4.6 |

[z]Trials 1–25 (BH1146 in 1–22 only). Rating scale: 0 = no spots, 1 = spots ≤0.5 mm, 2 = 0.6–1.0 mm, 3 = 1.1–2.0 mm, 4 = 2.1–3.0 mm, 5 = >3.0 mm and coalescing. Isolates PT12, PYD7, PTL1, and 78-62 used, with 30- or 48-hr mist. Wheat growth stages were 14–59 (four-leaf to heading) at inoculation. In vertical columns, means followed by the same letter are not significantly ($P = 0.05$) different by SAS GLM/PDIFF. The LSD ($P = 0.05$) for comparison of lesion length rating within a genotype for top leaf vs. top-1, top leaf vs. top-2, and top-1 vs. top-2 is 0.4. For the mean of all 61 genotypes, the LSD is 0.1; only the 16 most resistant, four intermediate to susceptible, and the susceptible check ND495 are shown.

**Table 2.** Growth stage (GS) related to averaged rating length of tan spot lesions on the top and top-1 leaves of 21 wheat accessions[y]

| CIMMYT no. | Four-leaf (GS 14) (trials 1-3) | | Five-leaf (GS 15) (trials 4-7) | | Flag (GS39-59) (trials 8-12) | | Dough (GS 59-85) (trials 26-28) | |
|---|---|---|---|---|---|---|---|---|
| | Top | Top-1 | Top | Top-1 | Top | Top-1 | Top | Top-1 |
| 148 | 2.3 a–e | 3.7 c–i | 2.7 a–d | 3.8 a–c | 1.5 a | 2.5 a | 3.9 b–d | 4.4 bc |
| 142 | 1.6 a | 2.1 ab | 2.7 a–d | 3.6 a | 2.7 b–g | 3.5 b–f | 2.7 a | 3.3 a |
| 115 | 3.0 b–k | 3.8 c–j | 2.6 a–c | 3.8 a–c | 1.9 a–c | 2.3 a | 4.8 ef | 5.0 d |
| 114 | 3.0 b–l | 4.3 e–n | 2.4 ab | 1.6 ab | 2.4 a–f | 3.7 b–h | 4.3 b–f | 4.7 b–d |
| 116 | 2.9 b–j | 4.1 d–n | 3.0 a–f | 4.0 a–f | 1.9 ab | 3.1 a–c | 3.9 b–d | 4.8 b–d |
| 117 | 3.4 d–p | 4.5 f–n | 2.7 a–d | 3.9 a–d | 2.2 a–d | 3.3 a–e | 4.7 d–f | 4.7 b–d |
| 119 | 2.7 b–g | 3.9 c–k | 3.2 a–g | 4.3 c–k | 2.4 a–e | 2.9 ab | 4.3 b–f | 5.0 d |
| 131 | 2.2 a–c | 3.0 bc | 3.0 a–f | 3.6 ab | 2.1 a–c | 2.9 ab | 4.0 b–e | 4.9 cd |
| 149 | 2.7 b–g | 4.0 c–m | 3.5 d–i | 4.1 a–i | 3.0 b–j | 4.1 c–l | 3.5 b | 4.8 b–d |
| 118 | 2.7 b–g | 3.7 c–h | 3.3 a–h | 4.3 c–k | 2.1 a–c | 2.4 a | 4.4 c–f | 5.0 d |
| 120 | 2.3 a–d | 3.0 bc | 3.2 a–g | 4.3 c–k | 2.2 a–d | 3.2 a–d | 4.9 f | 4.9 cd |
| 141 | 2.8 b–h | 4.4 e–n | 3.5 d–i | 4.4 d–k | 3.0 c–j | 3.7 b–h | 4.7 d–f | 4.8 b–d |
| 138 | 2.8 b–h | 3.6 c–g | 2.4 a | 4.1 a–i | 2.7 b–h | 3.5 b–f | 5.0 f | 5.0 d |
| 140 | 2.1 ab | 1.9 a | 3.4 c–i | 4.1 a–h | 3.9 h–n | 4.5 f–l | 3.7 bc | 4.3 b |
| 139 | 2.9 b–i | 3.9 c–l | 3.3 b–h | 4.5 d–k | 2.3 a–d | 3.3 a–e | 4.9 f | 5.0 d |
| 147 | 2.5 a–f | 3.1 b–q | 3.2 a–g | 3.8 a–c | 3.6 g–l | 4.7 h–l | ··· | ··· |
| 101 | 3.1 b–m | 4.7 i–n | 2.9 a–e | 4.5 d–k | 2.4 a–e | 3.5 b–f | 5.0 f | 5.0 d |
| 2 | 2.6 b–f | 3.8 c–j | 3.7 e–j | 4.6 e–k | 3.6 g–l | 4.5 f–l | ··· | ··· |
| 96 | 4.0 h–q | 4.8 j–n | 3.6 d–j | 4.6 d–k | 3.4 e–l | 4.3 e–l | 4.9 f | 5.0 d |
| 97 | 4.2 k–q | 4.8 j–n | 3.7 e–j | ··· | 4.5 l–n | 4.4 f–l | 4.6 d–f | 4.8 b–d |
| 1 | 4.8 q | 5.0 n | 4.4 i–k | 4.6 d–k | 5.0 n | 5.0 i–l | 5.0 f | 5.0 d |
| Mean[z] | 3.4 | 4.1 | 3.6 | 4.4 | 3.2 | 4.1 | 4.6 | 4.9 |

[y] Rating scale: 0 = no spots, 1 = spots ≤0.5 mm, 2 = 0.6–1.0 mm, 3 = 1.1–2.0 mm, 4 = 2.1–3.0 mm, 5 = >3.0 mm and coalescing. Isolates PTI2 used, with 30-hr mist. In vertical columns, means followed by the same letter are not significantly ($P = 0.05$) different by SAS GLM/PDIFF. The LSD ($P = 0.05$) for comparison of lesion length rating with a genotype for top leaf vs. top-1 is 0.4. For comparison of the means of the 61 genotypes at a given leaf level, the LSD is 0.6; only the 21 genotypes in Table 1 are shown. Most genotypes in trials 26–28 were in GS 85 of plant development when inoculated.

[z] Mean of 61 genotypes.

**Table 3.** Effect of three isolates of *Pyrenophora tritici-repentis* and leaf position (top and top-1) on averaged rating length of tan spot lesions[y]

| CIMMYT no. | PTI2 (trials 8-12) | | PYD7 (trials 13-15) | | PTL1 (trials 16-18) | |
|---|---|---|---|---|---|---|
| | Top leaf | Top-1 | Top | Top-1 | Top | Top-1 |
| 148 | 1.5 a | 2.5 a | 0.6 ab | 2.0 ab | 1.7 a | 1.8 a |
| 142 | 2.7 b–g | 3.5 b–f | 1.8 b–g | 2.7 a–g | 3.0 a–g | 3.6 d–h |
| 115 | 1.9 a–c | 2.3 a | 0.3 a | 1.7 a | 2.0 ab | 2.9 a–e |
| 114 | 2.4 a–f | 3.7 b–h | 1.2 a–e | 2.1 a–c | 2.0 ab | 2.9 a–e |
| 116 | 1.9 ab | 3.1 a–c | 0.7 a–c | 1.9 ab | 2.7 a–d | 3.6 d–h |
| 117 | 2.2 a–d | 3.3 a–e | 1.1 a–d | 2.7 a–g | 2.7 a–d | 2.4 a–d |
| 119 | 2.4 a–e | 2.9 ab | 1.0 a–d | 2.3 a–d | 2.9 a–f | 3.0 a–f |
| 131 | 2.1 a–c | 2.9 ab | 1.0 a–d | 2.2 a–d | 3.2 b–h | 4.3 f–j |
| 149 | 3.0 b–j | 4.1 c–l | 2.1 c–h | 3.2 b–h | 2.0 ab | 2.2 a–c |
| 118 | 2.1 a–c | 2.4 a | 2.1 c–h | 2.3 a–d | 3.0 a–g | 3.8 e–j |
| 120 | 2.2 a–d | 3.2 a–d | 1.7 a–g | 2.2 a–d | 2.7 a–d | 3.6 d–h |
| 141 | 3.0 c–j | 3.7 b–h | 2.2 d–i | 3.6 d–i | 2.7 a–e | 3.4 c–h |
| 138 | 2.7 b–h | 3.5 b–f | 1.6 a–f | 1.7 a | 3.4 c–h | 4.2 f–j |
| 140 | 3.9 h–n | 4.5 f–l | 3.0 g–n | 4.1 h–j | 2.8 a–e | 2.2 a–c |
| 139 | 2.3 a–d | 3.3 a–e | 2.9 f–m | 2.4 a–e | 2.8 a–e | 3.8 e–j |
| 147 | 3.6 g–l | 4.7 h–l | 2.9 f–m | 4.4 h–j | 3.0 a–g | 3.9 e–j |
| 101 | 2.4 a–e | 3.5 b–f | 2.6 e–k | 2.2 a–d | 3.3 b–h | 3.0 a–f |
| 2 | 3.6 g–l | 4.5 f–l | 2.8 f–m | 3.9 f–j | 3.6 c–i | 4.4 g–j |
| 96 | 3.4 e–l | 4.3 e–l | 2.6 e–k | 3.6 d–i | 4.1 e–j | 4.6 h–j |
| 97 | 4.5 l–n | 4.4 f–l | 4.9 p–q | 4.8 ij | 4.6 h–j | 5.0 j |
| 1 | 5.0 n | 5.0 i–l | 5.0 q | 5.0 j | 5.0 j | 5.0 j |
| Mean[z] | 3.2 | 4.1 | 2.9 | 3.6 | 3.4 | 3.9 |

[y] Rating scale: 0 = no spots, 1 = spots ≤0.5 mm, 2 = 0.6–1.0 mm, 3 = 1.1–2.0 mm, 4 = 2.1–3.0 mm, 5 = >3.0 mm and coalescing. Plants inoculated when most of the genotypes were at flag leaf growth stage (GS 39), but some had grown up to GS 59 (emergence of inflorescence completed); 30-hr mist used. In vertical columns, means followed by the same letter are not significantly ($P = 0.05$) different by SAS GLM/PDIFF. The LSD ($P = 0.05$) for comparison of lesion length rating within a genotype for top leaf vs. top-1 is 0.4. For comparison of the means of the 61 genotypes at top leaf and at top-1, the LSD is 0.3; only the 21 genotypes in Table 1 are shown.

[z] Mean of 61 genotypes.

4.9 for top-1 and 4.7, 4.7, 4.8, and 5.0 for top-2. Only the increase at dough was significant at top leaf and between dough and flag or four-leaf at top-1, LSD ($P = 0.05$) = 0.6. It appeared that as long as the plant was actively growing vegetatively, susceptibility as measured by lesion rating did not vary greatly with growth stage but was least on the flag leaf as it fully extended. This was in general agreement with earlier findings (8,14,16,23) but did not significantly support the earlier concept that: "Severity of spotting was lowest and separation of genotypes for level of resistance was best on adult-plant flag leaves in the greenhouse." (8). Infection at boot to flowering has been reported to cause the highest yield loss (40,43). Apparently, this results from reducing yield-producing plant functions at a critical stage, and not from increase in susceptibility at this stage in plant growth. However, as the wheat leaves put food reserves into the developing seeds—and particularly by dough, when the seeds should have most of their dry weight—susceptibility of the top leaf increases greatly (Table 2).

Because of differences in growth rates, the actual growth stages at inoculation in trials 26–28 of the 11 genotypes with lowest average disease ratings in Table 1 were for CIMMYT 148 early milk to dough, 142 late milk, 115 mid-dough, 114 late milk, 116 early dough, 117 early heading, 119 early dough, 131 milk, 149

**Table 4.** Effect of genotype and length of wet period on averaged rating length of tan spot lesions on flag, flag-1, and flag-2 leaves[y]

| CIMMYT no. | Flag | Flag-1 | Flag-2 |
|---|---|---|---|
| 148 | 1.4 a | 2.1 a | 2.9 a |
| 149 | 1.5 a | 2.2 a | 3.0 a |
| 142 | 2.1 b | 2.7 b | 3.3 a |
| 140 | 2.2 b | 2.7 b | 3.3 a |
| 147 | 2.3 bc | 3.0 bc | 4.1 b |
| 138 | 2.5 c | 3.3 c | 4.3 b |
| 1[z] | 5.0 | 5.0 | 5.0 |
| **Wet period (hr)** | | | |
| 48 | 1.8 a | 2.3 a | 3.1 a |
| 72 | 2.1 b | 2.8 b | 3.6 a |
| 96 | 2.3 c | 3.1 c | 3.9 a |

[y] Rating scale: 0 = no spots, 1 = spots ≤0.5 mm, 2 = 0.6–1.0 mm, 3 = 1.1–2.0 mm, 4 = 2.1–3.0 mm, 5 = >3.0 mm and coalescing. Data are from trials 29–31 with isolate PTI2, and plants were inoculated at boot to milk growth stages (GS 45–75). Genotype × wet period, trial × wet period, and trial × genotype interactions are not significant, except for trial × genotype for flag-1 and for trial × genotype and trial × wet period for flag-2. In vertical columns means followed by the same letter are not significantly ($P = 0.05$) different by SAS GLM/PDIFF. The LSD ($P = 0.05$) for flag leaf vs. flag-1, flag leaf vs. flag-2, and flag-1 vs. flag-2 was 0.5 for genotype and 0.4 for wet period.

[z] Not included in the statistical analysis.

early milk, 118 early dough, and 120 late milk. Thus, lesion length rating related to both differing genotype resistance and a somewhat varying (uncontrolled) growth stage. CIMMYT 142, which rated 2.7 on the top leaf for late milk; 116, which rated 3.9 for early dough; and 149, which rated 3.5 for early milk, all might have rated higher at dough. All the ratings at dough (predominant in trials 26–28) on other genotypes were higher (Table 2).

**Isolate.** With comparison of aggressive isolates PTI2, PYD7, and PTL1, the mean lesion length of 61 genotypes for the top leaf were, respectively, 3.2, 2.9, and 3.4 (Table 3); for top-1, the means were 4.1, 3.6, and 3.9 (Table 3) and for top-2, 4.7, 4.3, and 4.5. Some of these values were significantly different, LSD ($P = 0.05$) = 0.3 for top leaf and top-1 and 0.2 for top-2, but only slightly. PYD7 appeared to be less aggressive (shorter lesions) than PTI2 or PTL1 on most genotypes. Also, compared with observations in earlier years, it appeared to have reduced aggressiveness (32). The genotype × isolate interaction of the combined trials 8–12, 13–15, and 16–18 (Table 3) was significant at all three leaf levels. The relative ranking of isolates with genotype was the same, with a few exceptions. With some individual wheat genotypes, PYD7 had higher lesion length ratings than PTI2 and significantly higher ratings than PTL1 (e.g., for CIMMYT 94, PTI2, PDY7, and PTL1 top leaf rated 3.7, 4.3, and 3.2, respectively). Inoculating with a mixture of four virulent isolates (PTI2, PYD7, PTL1, 78-62, trials 23–25) did not appreciably change the mean lesion lengths of the 60 genotypes tested (BH1146 excluded) compared with an individual isolate (PTI2, trials 1–3). For trials 23–25, length ratings for top, top-1, and top-2 were 3.5, 4.3, and 4.5, respectively, and for trials 1–3, 3.4, 4.2, 4.7, respectively.

**Wet period.** As the postinoculation wet period increased, using isolate PTI2, from 30 hr (trials 4–7) to 48 hr (trials 19–22) at a given growth stage (five-leaf), the mean rated lesion length of the 61 genotypes on the top leaf significantly increased from 3.6 to 4.1. On the top-1 leaf, it increased but not significantly (4.4 to 4.5), and on the top-2 leaf, it remained the same (4.8), LSD ($P = 0.05$) = 0.4. Although the general trend among genotypes was for increasing lesion length rating with increasing wet period and for decreasing difference as leaf position was lower and as genotype susceptibility increased, a few individual genotype averages showed no increase or showed decreases related to these factors. Further studies with 48- to 96-hr wet periods (trials 29–31) showed only increased lesion length rating with increasing wet period and lower leaf position and decreasing genotype differences with the lowest leaf position (Table 4).

Our findings support the concept that resistance, expressed as a rating of lesion length, is determined by wheat genotype. It also is affected by leaf position, with the higher leaf more resistant. Resistance is statistically similar between seedling and adult plant, with the developing flag leaf most resistant, but not significantly. By the dough stage, resistance is significantly reduced. This suggests that resistance decreases with aging of the leaf. Highly aggressive fungal isolates cause similar lesions on many wheat genotypes and dissimilar lesions on a few. Increasing the postinoculation wet period results in increasing lesion length rating (increasing lesion size), with resistant genotypes acting similarly but further separating for degree of resistance. Eleven of the Chinese wheats are significantly more resistant (shorter lesions on the top leaf) than an earlier resistant genotype, BH1146. Good separation for lesion length (resistance) occurs with infection of the apex leaf before the dough stage of plant development, with the best separation occurring when the flag leaf has just fully extended. A 30-hr postinoculation wet period appears sufficient to separate most genotypes for resistance, and 96 hr should separate the most resistant.


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