Slow Rusting of Wheat with Stem Rust Detected in the Glasshouse

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ABSTRACT

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Race 15B2-TLM of *Puccinia graminis* f. sp. *tritici* produced significantly smaller colonies in seedlings of wheat accessions that rusted slowly, such as Thatcher, than in seedlings of accessions that rusted rapidly, such as Prelude. No significant differences in infectibility were noted in seedlings, but the rapidity with which uredia developed from flecks was significantly greater in seedlings of the fast-rusting accessions. Adult plants of slow-rusting wheats were significantly less receptive to the stem rust fungus than their fast-rusting counterparts.

Lack of simple procedures for screening and evaluation hampers selection for slow rusting, and in field tests the quantities of inoculum (5,12,14,17) and the severity of the disease (9) may mask slow rusting. In small plots, slow rusting may be underestimated when inoculum is blown from adjacent plots sown to cultivars that rust rapidly (12,17). Tests with adult plants in the glasshouse are tedious because space is limited and uniform inoculation of large plants is difficult (1,16). Detached-leaf methods are useful for preliminary tests (1,16), but detached leaves are more susceptible than intact ones (1).

Slow rusting may be detected in seedlings (2,3,6,8,10,15) but slow rusting in seedlings as a possible screening tool needed to be evaluated.

MATERIALS AND METHODS

Host. Wheat accessions that rust slowly or rapidly with stem rust in the field were used in this study. Those that rust rapidly were Prelude (CI 4323) and Thatcher × Prelude (line 243-8); those that rust slowly were Thatcher (CI 10003), Thatcher × Prelude (line 244-6), and Idaed 59 × Baart (line 124-2). In some tests Thatcher × Prelude (line 255-2) was used in the place of line 124-2. Accessions were sown in a 7.5-cm pot filled with a mixture of three parts of field soil, one part sand, and one part peat in two of six equidistantly spaced holes 0.4–0.6 cm from periphery. Seedling tests consisted of 48 pots with two seedlings of Thatcher and two seedlings of Prelude in each pot. These were arranged in three groups of 16 pots in which each of the remaining accessions was sown in pots of one group only, producing two seedlings in a pot. Thus, each seedling test consisted of 96 seedlings of Thatcher, 96 of Prelude, and 32 of each of the remaining accessions. Each pot contained six randomly distributed seedlings and was treated as a replicate.

In adult plant tests, seed of each accession was sown separately in a 10-cm pot. The emerging seedlings were thinned to one plant per pot. The plants were periodically watered and fertilized. In adult plant trials, the soil in each pot was drenched once in 3 wk with a 40-ppm suspension of ethirimol to control powdery mildew.

Inoculation and incubation. Race 15B2-TLM of *Puccinia graminis* Pers. f. sp. *tritici* was used for inoculation. Pots of 7-day-old seedlings were inoculated with 0.2 ml of a urediospore suspension (1 mg of spores in 3 ml of Soltrol 170 paraffinic oil) using Rowell and Olien's method (13). After inoculation, the pots were placed in a dew chamber for 12 hr of darkness at 21 C and then for 4 hr of illumination with 22,000 lux at 25 C. The dry plants were then maintained in a growth chamber programmed for 12 hr at 21 C and 22,000 lux and 12 hr of darkness at 18 C until flecks appeared; then the seedlings were moved to a glasshouse at 18–20 C.

Adult plants were inoculated at the flowering stage in a settling tower with a rotating platform carrying two plants of each of four accessions. The rotating plants were exposed for 5 min in a cloud of dry urediospores created by discharging 5 mg of urediospores, with the aid of a CO_2 gun, into the air above the eight plants. After inoculation, adult plants were treated the same as the inoculated seedlings.

Mycelium staining. Holz's (4) whole-leaf clearing and staining method was employed in studies of the mycelium in the leaf blade tissue. Segments 4–5 cm long, cut from the middle of leaf blades 144 hr after inoculation, were cleared for 24 hr or more in 20% KOH solution, rinsed in water, immersed for 3–5 min in glacial acetic acid, rinsed again in water, and finally stained in cotton blue solution for several hours. After excess stain was removed by gentle washing in water, segments were mounted in glycerine for microscopic examination. The mycelium was distinguishable by its blue-purple color. The mycelial colony around an infection site was approximately elliptical. Its area was calculated from its length and width using the formula: (length × width × π)/4 for elliptical area computation.

Because of missing data and unequal sample size in tests of rust development on seedlings (Table 1), an unweighted analysis of variance was used, applying a model in which the groups of pots were between variables and the wheat accessions and readings were within variables. Data in Table 2 were subjected to multivariate analysis of variance. The results were scaled to achieve equal variances.

RESULTS

Mycelium development. Colonies of *P. graminis tritici* were compared among 99 colonies in 37 segments from 28 Prelude seedlings, 75 colonies in 44 segments of 32 Thatcher seedlings, 55 colonies in 35 segments of 26 seedlings of line 243-8, 56 colonies in 35 segments of 27 seedlings of line 244-6, and 50 colonies in 24 segments of 20 seedlings of line 124-2. The average area of the colonies for fast-rusting Prelude (247 mm^2) and line 243-8 (240 mm^2) was significantly different ($P = 0.01$) from the slow-rusting Thatcher (68 mm^2), line 244-6 (77 mm^2), and line 124-2 (52 mm^2). The sizes of colonies in the fast and slow rusting wheats did not differ significantly. Adjacent colonies in Prelude and line 243-8 frequently coalesced, whereas such colonies were rare in Thatcher, line 244-6, and line 124-2. In the slow-rusting wheats many colonies were small and were seen only after they were stained. After 14–20 days, the uredia on all plants were of infection type 3-4.

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Table 1. Infections on one-leaf seedlings of wheat accessions after plants were inoculated with *Puccinia graminis* f. sp. *tritici* race 15B2-TLM

Accession	Mean visible infections per leaf[a]			Percent infections with erumpent uredia per leaf[a]		
	7.5 days	9.5 days	12.5 days	7.5 days	9.5 days	12.5 days
Prelude	14.0 y	14.5 y	14.5 y	80 y	86 y	94 y
Line 243-8	12.8 y	12.6 y	13.0 y	66 y	88 y	94 y
Line 244-6	13.6 y	13.9 y	14.5 y	23 z	51 z	82 z
Thatcher	11.2 y	12.5 y	12.5 y	23 z	53 z	77 z
Line 255-2	10.0 z	10.7 z	10.7 z	32 z	52 z	74 z

[a] Different letters assigned to means that are significantly different ($P = 0.01$). Data based on 92 leaves of Thatcher, 86 of Prelude, 30 of line 243-8, 32 of line 244-6, and 32 of line 255-2.

Rust development on seedlings. Flecks and erumpent uredia in the seedling leaves were counted 7.5, 9.5, and 12.5 days after inoculation. In one test, Thatcher, Prelude, line 244-6, line 243-8, and line 124-2 were used, and in a second test, line 124-2 was replaced by line 255-2.

Inoculated seedlings were examined daily. Flecks became noticeable 6 days after inoculation. In the first test (data not shown) the number of visible infections on Prelude and line 243-8 was higher than on Thatcher, line 124-2, and line 244-6. In the second test the total number of infections on the slow- and fast-rusting lines did not differ significantly except that they were significantly reduced on line 255-2 (Table 1). Uredia erupted significantly earlier in seedlings of Prelude and line 243-8 than in Thatcher, line 244-6, and line 255-2 (Table 1).

Receptivity to infection of adult plants. Results were recorded 16 and 23 days after inoculation in one test and 20 days after inoculation in the second test. The infectibility of an accession was determined by counting the uredia on equal-length segments of the flag and penultimate leaves, on equal lengths of the stem between the head and the flag leaf, and on equal lengths of the sheath of the flag leaf.

The numbers of uredia on each of the plant parts did not differ significantly on Prelude and line 243-8 (Table 2) but were significantly larger ($P = 0.05$) than on Thatcher and line 244-6. Also, the number of uredia was significantly higher on line 243-8 ($P = 0.01$) than on Thatcher and on line 243-8 and Prelude than on line 244-6. The numbers of uredia on Thatcher in all parts compared differed significantly from those on line 244-6 ($P = 0.05$).

Table 2. Uredia formed by *Puccinia graminis* f. sp. *tritici* race 15B2-TLM on segments of plant parts of wheat accessions

Accession	Average number of uredia per segment on:[a]			
	Flag blade	Penultimate leaf blade	Peduncle	Flag sheath
Line 243-8	20.2 x	22.2 x	27.2 x	26.5 x
Prelude	16.8 x	15.6 x	17.4 x	16.2 x
Thatcher	8.9 y	8.5 y	9.0 y	9.5 y
Line 244-6	7.7 z	6.0 z	6.8 z	7.4 z

[a] Glasshouse tests at 20 C. Inoculations at the flowering stage. Values calculated from two tests with 4 or 5 replicates per test. Tissue segments were of equal length. Values for each plant part followed by the same letter do not differ significantly ($P = 0.05$). Prelude and line 243-8 differ significantly from line 244-6 ($P = 0.01$). Prelude differs from Thatcher ($P = 0.05$). Line 243-8 differs from Thatcher ($P = 0.01$). Values followed by different lowercase letters differ significantly ($P = 0.05$).

DISCUSSION

Because the selection of adult plants for slow rusting is complicated and costly, attempts have been made to use seedlings. Musick (8) reported that slow rusting of wheat seedlings with leaf rust was correlated with lengthening of the incubation period (time from inoculation to first spore production). Heagle and Moore (3) demonstrated that, in oats that rusted slowly with crown rust, the development of disease in seedlings was characterized by fewer infections, restricted hyphal growth, delayed onset of sporulation, and smaller uredia. Clifford (2) showed that *Puccinia hordei* on slow-rusting barley produced smaller colonies, fewer uredia that opened readily, and fewer spores. Differences in the receptivity of wheat seedlings to infection by stem rust were observed by Mont (7). Mycelial colonies produced by the stem rust pathogen in seedlings of slow stem rusting accessions of *Avena sterilis* were smaller than those in fast-rusting plants (15). Parlevliet (9–11) proved that the development of *P. hordei* on partially resistant barley was associated with a longer latent period and lower infectibility.

In our tests with seedlings, the latent period was longer and areas of mycelium were smaller in wheats that rust slowly in the field than in wheats that rust rapidly. The slow rusters could be distinguished from the fast rusters by the number of infections produced by inoculation of adult plants, but differences in numbers of infections on seedlings were inconsistent. Thus we tentatively concluded that, in tests with seedlings, either the latent period or the size of uredia would be a promising characteristic for selecting slow-rusting wheats. Because our tests involved only a few host lines inoculated with a single race of rust, additional work should be done before seedlings are used exclusively for selecting slow-rusting lines of wheat.

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