The Relationship of the *Sr6* Gene to Slow Rusting in Wheat

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Scientific Journal Series Paper 10,565, Minnesota Agricultural Experiment Station.
Accepted for publication 3 May 1981.

ABSTRACT

Cox, D. J., and Wilcoxson, R. D. 1982. The relationship of the *Sr6* gene to slow rusting in wheat. Phytopathology 72: 178-181.

In field studies, wheat lines that possessed the *Sr6* gene generally developed stem rust more slowly than lines that did not. The *Sr6* gene conditions slow rusting in conjunction with other minor genes as the temperature fluctuates about 24 C or when genes for virulence are present in the stem rust population. Heritability estimates indicated that the slow-rusting trait can be incorporated into a breeding population. Histological observations of the development of the stem rust pathogen in flag leaves of plants indicated that the greatest effect of the *Sr6* gene was in its ability to suppress growth of the pathogen after penetration had been achieved.

Disease development has been measured by determining the area under the disease progress curve. Wilcoxson et al (13) found that areas under the disease progress curve were a reliable means of data summation for stem rust studies. They further indicated that this method could be used as a means of identifying cultivars with a slow-rusting type of resistance when the cultivars do not differ in major genes that would make them incompatible to a large portion of the rust races present.

The *Sr6* gene may influence the slow development of wheat stem rust as suggested by Skovmand et al (9,10), who found that many lines that possessed this gene rusted slowly. It was most effective against stem rust when associated with other genes that conditioned slow rusting. Our study provides additional information on the relationship between *Sr6* and slow rusting and on the behavior of the stem rust pathogen on slow and rapid rusting lines of wheat that either did or did not possess *Sr6*.

MATERIALS AND METHODS

Materials. Four lines of wheat (*Triticum aestivum* L.), developed by Skovmand et al (9,10), were selected because they possessed genes that conditioned slow rusting, and the gene *Sr6*, in various combinations. Line 347-7 (S+), derived from Kenya 58 × Marquis, rusted slowly and carried *Sr6*. Lines 23-9 (S−) and 24-2 (F−), derived from Kenya 58 × Idaed 59, rusted slowly and rapidly, respectively, and neither possessed *Sr6*. Line 205-2 (F+), derived from Kenya 58 × Prelude, rusted rapidly and possessed *Sr6*. The lines were tested to detect which of the *Sr* genes present in the parents were carried by the lines. *Sr6* was the only *Sr* gene identified. The presence of three of the *Sr* genes possessed by Marquis, *Sr18*, *Sr19*, and *Sr20*, was not tested because avirulence does not occur in the natural stem rust population of North America. The lines were in the F_6 when selected. Lines 23-9, 24-2, and 205-2 were mated with 347-7, and progenies of the three matings were advanced to the F_4 by single seed descent.

Field studies of stem rust development. From each mating, 96 F_4 lines and the parents were planted in hills in the field in a randomized block design with three replicates at St. Paul and Rosemount, MN, on 25 April 1977. The hills were spaced 30 cm apart with 10 seeds per hill. Border hills of Era, a stem rust resistant spring wheat, were sown around the entire experiment.

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0031-949X/82/02017804/$03.00/0

When in the boot stage, the wheat plants were inoculated with uredospores of race 15-TLM of *Puccinia graminis* Pers. f. sp. *tritici;* ie, 500 mg of uredospores per liter of Soltrol® 170 were sprayed on each plot with an ultralow-volume sprayer. At temperatures greater than 24 C, race 15-TLM is virulent on wheat with the gene *Sr6* due to this gene's temperature sensitivity. The race 15 complex was the most abundant race of stem rust in the north central region during the mid-1970s (8). When plants at St. Paul were inoculated, some had a trace of stem rust from a nursery in a nearby field. The plots at both locations were irrigated on 4 June 1977.

Stem rust severity was estimated on each hill 2 wk after inoculation at both locations, and at weekly intervals thereafter for 4 wk by using a modified Cobb's scale (6). The area under the disease progress curve (AUDPC) was calculated from the weekly rust severity ratings via the Fortran IV subroutine AREA (10). The AUDPC was used to distinguish wheat lines that differed in ability to retard stem rust development.

Analyses of variance were used to detect the significance of variation in the AUDPC. The combined location analysis of variance for lines within each cross was used to estimate the genetic variance (11) from which heritability of the rusting trait was estimated (4).

Seedlings of parental lines and their F_5 progenies were evaluated for presence of *Sr6*. One-week-old seedlings were inoculated with uredospores of race 15-TLM, kept in a dew chamber overnight at 21 C, and then maintained in a greenhouse at 20–23 C. Fourteen days after inoculation, infection types were noted and the presence or absence of *Sr6* was recorded (7,12).

Histology of *P. graminis tritici* in greenhouse-grown plants. The germination of uredospores, formation of appressoria, and the production of uredia were studied in plants of the parental lines 347-7 (S+), 205-2 (F+), 24-2 (F−), 23-9 (S−) infected with *P. graminis tritici* race 15-TLM. Test plants were grown in a greenhouse at about 18 C in natural light supplemented with fluorescent light. When in the flag leaf to early heading stages of growth, the plants were inoculated with fresh uredospores in a settling tower where the plants were rotated for 5 min in a cloud of uredospores. The inoculated plants were kept in a dark moist chamber for 8 hr at 21 C, after which the leaf surfaces were dried slowly for approximately 8 hr. During the last 2 hr that the plants were in the moist chamber, they were exposed to about 8,070 lux (750 ft-c) of light from fluorescent lamps. After removal from the moist chamber, the plants were placed in greenhouses at 18 or 27 C.

The percentage of germinated uredospores and the percentage of germ tubes that formed appressoria were determined on flag leaves inoculated in a cloud containing 30 mg of uredospores. This was done with two sets of plants. From the first set, five leaves per line were collected 5 hr after inoculation. From the second set, three

leaves per line were collected 24 hr after inoculation from the plants were grown at 18 or 27 C. Leaf pieces 7.5 cm^2 (total area) from the central portion of each leaf, were taped onto glass slides, placed in moist petri plates for about 12 hr, stained with cotton blue and acid fuchsin (1), and examined with a microscope at ×200. Thus, the two sets of leaves were examined about 17 and 40 hr after inoculation. One hundred spores were examined on each leaf.

In tests where the uredia on flag leaves were counted, the plants were inoculated in clouds of uredospores created from 10 or 20 mg of spores. Flag leaf segments 20 cm^2 (total area) from plants grown at 18 and 27 C were examined on alternate days during the 9-day period, 6–14 days after inoculation. A total of 24 plants from each line were examined: three plants per line at two temperatures in four trials.

RESULTS

Field studies of stem rust development. Stem rust severity was less than 1% at St. Paul on 7 June 1977, 4 days after inoculation; the rust apparently had developed from natural inoculum, but stem rust was not present at Rosemount until 17 June. On 17 June, 14 days after inoculation, when plants were almost 8 wk old, stem rust severity was 1–10% at St. Paul and 0–5% at Rosemount. On 30 June, stem rust severity was 20–70% at St. Paul and 1–30% at Rosemount. Rust severities were also noted on 7 July, but by that date, plants were either dead from stem rust or mature. Only infection types that indicated susceptibility to *P. graminis* f. sp. *tritici* were observed on the lines.

The AUDPC varied with lines in each mating and location. Mean squares for among lines within each mating and for the interaction of locations with lines within each mating were significant. Variation due to locations, to matings, and to the interaction of locations × matings was significant also.

The mean AUDPC varied with the parental lines (Table 1). Line 347-7 (S+) and line 23-9 (S−) rusted more slowly at both Rosemount and St. Paul than did the other two. All lines rusted more rapidly at St. Paul than at Rosemount.

The AUDPC also varied for each mating at both locations (Fig. 1 and Table 2). Frequency distributions for AUDPC and transgressive segregation for progenies are shown in Fig. 1. In each cross, *Sr6* segregated (the presence of this gene is indicated in the histograms by cross-hatching). Progeny distributions were skewed.

TABLE 1. Mean area under the stem rust progress curve of the parental lines of wheat infected with *Puccinia graminis* f. sp. *tritici* at Rosemount and St. Paul, MN

	Mean area ± SE[z]	
Lines	Rosemount	St. Paul
347-7 (S+)	84 ± 28 a	609 ± 40 b
205-2 (F+)	304 ± 26 b	841 ± 19 c
23-9 (S−)	123 ± 72 a	503 ± 27 a
24-2 (F−)	629 ± 31 c	948 ± 62 d

[z]Values based on nine hills (three replicates per cross, three crosses per location). At each location, means followed by a different letter are different, according to Duncan's multiple range test ($P = 0.05$).

TABLE 2. Mean and range for area under the stem rust progress curve for F_4 progeny of three wheat crosses infected with *Puccinia graminis* f. sp. *tritici* at two locations

	Rosemount		St. Paul	
Mating	Mean ± SE[y]	Range	Mean ± SE[y]	Range
S+ × F+	215 ± 13 b	4–752†[z]	734 ± 10 b	476–1,097
S+ × S−	94 ± 11 a	0–623†	386 ± 32 a	4–1,014†
S+ × F−	224 ± 17 b	4–1,032†	796 ± 11 c	406–1,161†

[y]Values based on 96 lines per cross, average of three replicates per location. Means followed by a different letter are significantly different according to Duncan's multiple range test, $P = 0.05$.
[z]Dagger (†) indicates the distribution varied from a normal distribution.

Lines that indicated slow rust development predominated, that is, lines with AUDPC at the low end of the distribution. The only exception was for the mating of S+ × S− at St. Paul where the distribution of progenies did not vary significantly from a normal distribution. Since 87% of the slow rusting lines contained *Sr6*, the slow rusting trait of these lines may be due to an interaction of minor genes and a major gene.

The relationship between the *Sr6* gene and slow development of stem rust was investigated by grouping the lines from each mating according to the allelic state at the *Sr6* locus. The mean AUDPC for the group of lines with *Sr6* (homozygous and heterozygous state) was significantly lower than that for the group with *sr6* in each mating (Table 3). As when all lines were considered, the stem rust

TABLE 3. Mean and range of area under the stem rust progress curve for groups of F_4 wheat lines of three crosses that possessed the dominant or the recessive allele of *Sr6* locus

		Presence of a dominant allele		Homozygous recessive		
Mating	Location	Mean area ± SE	Number of F_4 lines	Mean area ± SE	Number of F_4 lines	LSD ($P = 0.05$)
S+ × F+	Rosemount	194 ± 15	75	283 ± 16	21	43
	St. Paul	718 ± 11	75	789 ± 18	21	42
S+ × S−	Rosemount	63 ± 10	66	161 ± 26	30	55
	St. Paul	305 ± 35	66	565 ± 55	30	128
S+ × F−	Rosemount	128 ± 16	56	359 ± 22	40	53
	St. Paul	754 ± 10	56	836 ± 28	40	58

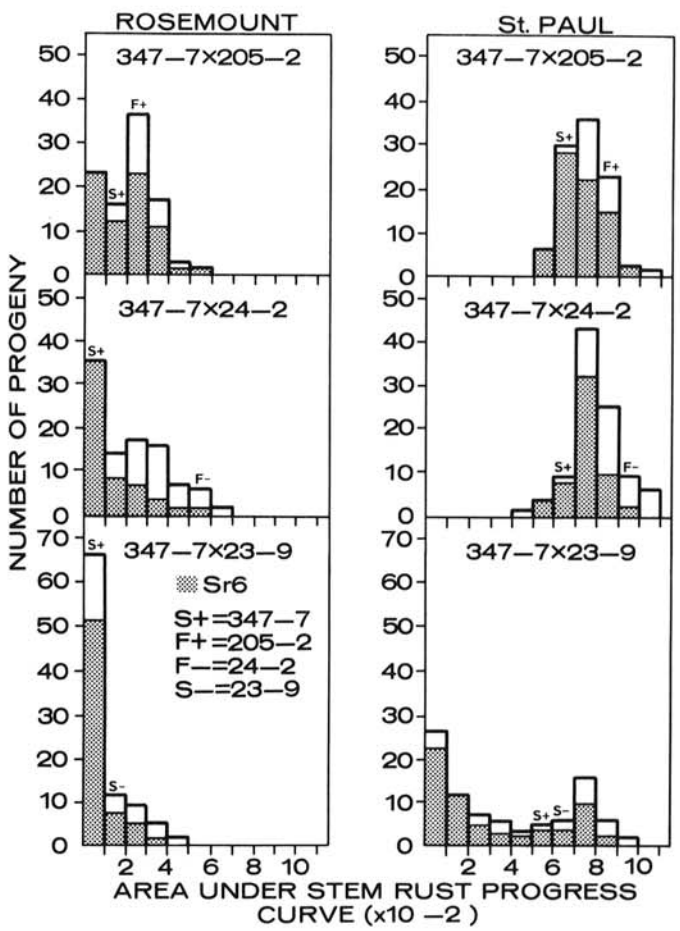

Fig. 1. Frequency distributions for area under the stem rust progress curve of wheat progenies derived from three matings at Rosemount and St. Paul. The area under the curve for the parents is indicated by a letter above certain bars. Cross-hatching indicates the presence of the *Sr6* gene for specific resistance.

progress curves for lines containing *Sr6* also were skewed, with the greatest portion of lines being at the lower end of the distribution (Fig. 1).

The number of lines observed in each allelic group is listed in Table 3. The expected phenotypic ratio of F_4 lines containing the dominant allele to F_4 lines homozygous recessive for *Sr6* for the matings of S+ × S− and S+ × F− should have been 9:7. The lines from S+ × S− fit a 3:1 ratio, but those from S+ × F− fit the expected 9:7 ratio. The F_4 lines from the mating of S+ with F+ fit a 3:1 ratio, which indicated that some of the F+ plants used as parents were homozygous for *sr6*.

Heritability of the rusting character on a per plot basis was 42% for the mating S+ × F+ and 44% for the matings of S+ with F− and S−. This statistic is a measure of the ability to incorporate the trait (in this case, the minor genes conditioning a slow rusting response) into a breeding population. The relative amount of rusting for a line at the two locations was shown by a correlation of 0.65 for readings at the two testing sites when computed across the three matings.

The races of *P. graminis tritici* present in the plots at St. Paul and Rosemount were different (Table 4). Of 41 isolates of *P. graminis tritici* collected at Rosemount, 17% were virulent on lines containing *Sr6*, 77% of 44 isolates from St. Paul were virulent on lines with *Sr6*. Thirty of the isolates from Rosemount and two from St. Paul were of race 15-TLM, the race used to inoculate the field plots.

Histology of *P. graminis tritici* in greenhouse-grown plants. About 70% of the uredospores germinated on the leaves regardless of the allele at the *Sr6* locus. On all wheat lines, about 30% of the germ tubes formed appressoria on plants harvested 24 hr after inoculation, whereas 15% formed appressoria on plants harvested 5 hr after inoculation.

No uredia were observed at 18 C 6 days after inoculation (Fig. 2), whereas they were visible on plants at 27 C. Lines without *Sr6* (lines 23-9 and 24-2) usually had a larger number of uredia per flag leaf than those that had *Sr6* (lines 347-7 and 205-2). At 18 C, line 24-2, a line that rusted rapidly, had a greater number of uredia per flag leaf than 23-9 and 347-7, lines that rusted slowly, irrespective of whether the plants were inoculated with 10 or 20 mg of uredospores.

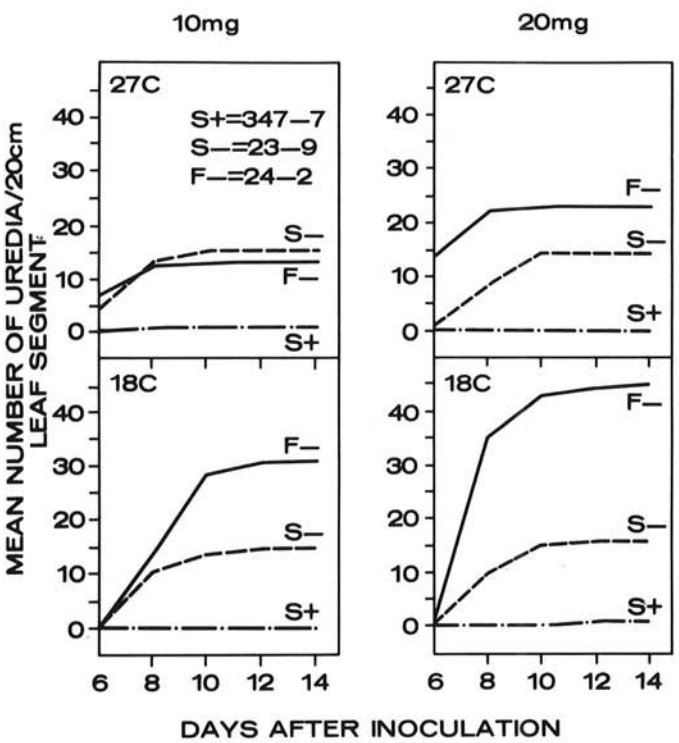

Fig. 2. Mean number of uredia per 20 cm^2 leaf segment of flag leaves of slow- and fast-rusting lines with and without the *Sr6* gene, 6, 8, 10, 12, and 14 days after inoculation.

DISCUSSION

The two groups of lines from each mating, differentiated according to the allelic state of *Sr6*, can be considered isogenic populations as they have equivalent genetic backgrounds and differ at the *Sr6* locus. By comparing isogenic populations, it was possible to establish whether the gene for specific resistance was actively reinforcing the slow rusting trait (2). The *Sr6* gene seemed to enhance the slow rusting trait of wheat lines in each mating we studied. *Sr6* did not guarantee slow rusting, however. Several lines rusted rapidly even though they possessed *Sr6*, which suggests the gene acts in concert with other unidentified genes. These observations on the relationship of *Sr6* to slow rusting support the conclusions of Skovmand et al (10).

There may have been two reasons for the stem rust epidemic being more severe at St. Paul than at Rosemount. First, rust infection was initiated a week earlier at St. Paul than at Rosemount due to natural inoculum, and second, the races at St. Paul were virulent on *Sr6*, whereas at Rosemount they were avirulent on *Sr6* at temperatures below 24 C (5). The greater proportion of races avirulent on *Sr6* at Rosemount could account for the greater effect of *Sr6* in reducing AUDPC among F_4 lines from the three matings at Rosemount than at St. Paul.

Differences in AUDPC were due to the differential effect of races on the total amount of rust. The number of slow rusting lines was calculated as the number of lines from each mating that rusted more slowly than the slow rusting parent of that mating. Of the 288 lines tested, 35% rusted slowly at Rosemount, whereas at St. Paul, 27% rusted slowly. Yet, at a given location, the allelic groups of *Sr6* still differed in AUDPC for each mating. Therefore, *Sr6* was effective in conditioning some degree of resistance regardless of the virulence of the rust population. The hypersensitive reaction or fleck characteristic of specific resistance was not indicated, since only susceptible reactions were observed. There were only 4 days during the 5-wk period when disease severity notes were being taken at Rosemount that the maximum temperature was below 24 C. Forsyth (3) stated that a susceptible reaction was obtained when light warm periods (16 hr, 24 C) were alternated with shorter dark cold periods (8 hr, 16 C). Thus, a resistant reaction was not produced by the host-pathogen complex thereby allowing the pathogen to grow when temperatures in the field exceeded 24 C. The temperature sensitive gene, *Sr6*, was not completely ineffective above temperatures at which avirulent races are able to produce susceptible type pustules. The phenomena observed could be one of fewer or smaller pustules and may or may not be specific to avirulent races.

The observations on the development of stem rust on flag leaves in the greenhouse indicated that the pathogen germinated about equally well on lines that rusted slowly or rapidly. The greatest effect of the host on the pathogen was in suppressing the growth of the pathogen after penetration had been achieved. This was apparent in the data on the numbers of uredia that formed during the 2-wk period after penetration. This type of resistance, which has

TABLE 4. Identified races of *Puccinia graminis* f. sp. *tritici*, their virulence or avirulence on wheat lines containing the gene *Sr6*, and the number of identifications in collections made within F_4 lines from Rosemount and St. Paul, MN

Race[1]	Virulence upon lines containing *Sr6*	Rosemount		St. Paul	
		Number identified	%	Number identified	%
11-32-113-RKQ	+	0	0	11	25
11-32-113 RTQ	+	0	0	16	37
15-TLM	−	30	73	2	4.5
15 TNM	−	0	0	7	16
151 QSH	+	7	17	7	16
151 QCB	−	3	7.5	1	1.5
56 MBC	−	1	2.5	0	0

[1]The number refers to the race as identified on the standard differential cultivars (12). The letters refer to the race as identified on single-gene differential lines (7).

been called general resistance, is characterized by a longer latent period, fewer infections, and less sporulation.

The effectiveness of the resistance conditioned by the *Sr*6 locus was apparently enhanced by genes conditioning slow stem rust development. These genes conditioning the slow stem rust reaction may be minor genes; thus, quantitative inheritance is indicated. Another alternative is that *Sr* genes that are ineffective in conditioning a resistant reaction may be effective in providing what would be looked upon as a form of general resistance.

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