Physiologic Specialization and Pathogen Aggressiveness in Stripe Rust

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ABSTRACT

Urediospores of stripe rust, *Puccinia striiformis* were collected from graminaceous hosts throughout the northwestern region of the USA. These were used to inoculate wheat and barley cultivars selected for their potential ability to differentiate genes for virulence among rust isolates. Many cultivars were unable to discriminate among any of the isolates under a controlled environment. By using wheat cultivars 'Chinese 166', 'Druchamp', 'Leeds', 'Moro', 'Medeah', 'President Riverain', 'Marfed', and 'Red River 68', 11 *P. striiformis* races were distinguished. The aggressiveness of 13 stripe rust isolates was measured by the ability of urediospores to germinate at 5, 10, 15 and 20 C. Two isolates, both virulent on Chinese 166, germinated at all temperatures.

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Additional key words: physiological race, genes for virulence.

Stripe rust of wheat (*Triticum aestivum* L. em Thell.), caused by *Puccinia striiformis* West. contributes to losses in cereal crops throughout the world. The damp, cool climates of coastal or intermountain areas favor the organism. Severe losses were recorded in the Pacific Northwest area of the USA during the early 1960's when yields were reduced up to 60% (9).

Pathogenic specialization in stripe rust has been studied extensively in Europe (2, 14), Great Britain (7), Canada (8) and in the western USA (1, 10). Race determination has been based largely upon the differential hosts established in Europe by Gassner & Straib (3). As new genes for virulence were detected in the pathogen population, continued differentiation was accomplished by adding supplemental cultivars.

Preliminary studies indicated that the European differential set was inadequate to differentiate stripe rust races prevalent in the northwestern USA. Cultures were found which had not been reported in Europe (10). Recently a system was proposed for differentiating races of stripe rust in the USA using seven wheat cultivars (6). The list of differential hosts was intended to be flexible allowing additions or deletions as more information on pathogenicity of stripe rust was obtained.

The ability of stripe rust urediospores to germinate at certain cardinal temperatures has been considered to be one measure of aggressiveness (7). The purpose of this investigation was to study aggressiveness as an aid in describing races of stripe rust and to provide new information about genes for virulence in stripe rust in the northwestern USA.

MATERIALS AND METHODS.–Spring and winter wheat cultivars of international origin are planted annually in nurseries throughout the world and evaluated for response to the stripe rust pathogen. Locations in northwestern USA include: Bozeman, Montana; Bonners Ferry, Idaho; Moscow, Idaho; Pullman, Washington; and Corvallis, Oregon. Visits were made to these nurseries in 1967, 1968 and 1969, at which time the cultivars were rated for resistance by two types of stripe rust readings: severity, the average percentage of leaf area infected; and infection type, an expression of host-pathogen interaction. Descriptions and designations of the different infection types were given earlier (5). Stripe rust isolates were obtained from the above locations and also from wheat cultivars at Logan, Utah.

In comparing disease readings from the various nurseries, certain cultivars exhibited infection type differences among locations. Twenty-one rust collections were obtained from these cultivars and 16 were increased upon leaves of susceptible wheat seedlings as monospore isolates. The procedure of obtaining single-spore isolates was aided using a glass capillary tube melted and drawn to a needle point at one end. Individual urediospores (viewed at X 30) adhering to the glass point were transferred to leaf surfaces. Methods of incubation and other methods of inoculation have been described (11). Lantern chimneys covered with cloth at one end, were placed over the sporulating plants. After collection, the urediospores were stored at 5 C and 50% relative humidity until they could be lyophilized (12).

Cultivars which exhibited differences in infection type at field nursery locations were harvested, grown under isolation covers, and inoculated at the seedling stage with urediospores representing the regional collections. In addition, 17 barley (*Hordeum vulg* L.) cultivars, and 18 tetraploid *Triticum* sele from the Montana State University Experiment Station were tested as differential candidates.

All tests were conducted in controlled environment chambers to minimize any differences in infection type due to the local environment. The temperature profiles, incubation periods, and rust ratings used were described earlier (4, 5). Host-pathogen interaction was observed 14 and 21

days after inoculations on the plants grown under two temperature profiles, 15/24 C and 2/18 C (night/day), respectively.

To study aggressiveness of the isolates, some of which differed in genes for virulence, urediospores were dusted on microscope slides coated with 0.25% Ion Agar No. 2 (Code No. L-12; Colab, Inc.; Chicago, Illinois). They were placed at 100% relative humidity and incubated at 5, 10, 15 and 20 C in darkness for 24 hr. Two slides of each isolate were prepared and 400 spores on each slide were assayed. A germination percentage was calculated daily for each isolate over a period of 6 days.

RESULTS.—*Selection of differentials and designation of races.*—It is not certain that all the genes for virulence present in the northwestern USA were represented in the 16 stripe rust isolates. The objectives were to obtain a representative and workable sample of isolates from the rust population and to select host differentials best able to distinguish isolates of that sample.

Many of the nearly 200 hosts tested did not discriminate among rust isolates when tested at either temperature profile. Slight differences observed among some cultivars when inoculated with different isolates were not considered to be sufficiently distinct to be reliable. The 15/24 C temperature profile conditioned the highest level of host resistance in

TABLE 1. Infection type on differential cultivars of wheat used to describe races of *Puccinia striiformis* collected in the northwestern USA[a]

	Differential and infection type[b]						
	1	2	3	4	5	6	7
State of origin and race designation	Lemhi	Chinese 166	Heines VII	Moro	Suwon 92/Omar	Druchamp	Riebesel
Group I							
Montana 2,4,7,5,6,3 / 1[c]	3[d]	00	1	00	0	1⁻	00
Montana 2,4,7,5,6,3 / 1[e]	3	00	1	00	0	0	00
Group II							
Montana 2,4,7,5,6 / 3, 1	3	00	3⁻	00	0⁻	1	00
Montana 4,7,2,5,6 / 3,1	3	0⁻	3⁻	00	0⁻	0⁻	00
Idaho 2,4,5,7,6 / 3,1	3	00	3	00	00	1⁻	00
Utah 2,4,5,7,6 / 3,1	3	00	3⁻	00	00	1	00
Group III							
Idaho 2,7,5,6 / 3,4,1	4	00	3	3	0⁻	1	00
Group IV							
Montana 4,7,2,5 / 3,6,1	3	0⁻	3⁻	00	0	3⁻	00
Montana 2,4,5,7 / 6,3,1	4	00	3	00	00	3⁻	00
Montana 2,4,7,5 / 6,3,1	3	00	3	00	0⁻	3⁻	00
Idaho 2,4,7,5 / 3,6,1	3	00	3⁻	00	0⁻	3	00
Washington 2,4,5,7 / 6,3,1	3	00	3	00	00	3⁻	00
Oregon 2,4,7,5 / 6,3,1	3	00	3	00	0⁻	3⁻	00
Group V							
Montana 4,7,5 / 3,6,2,1	3	3	2	00	0⁻	2	00
Oregon 4,7,5 / 3,6,2,1	4	3⁻	2	00	0⁻	2	00
Washington 4,7,5 / 3,6,2,1	3	3⁻	2	00	0⁻	2	00

[a] Determinations were conducted at 2/18 C, night/day.

[b] CI or PI numbers include: Lemhi (11415), Chinese 166 (11765), Heines VII (201195), Moro (13740), Suwon 92/Omar (13749), Druchamp (13723), Riebesel (295999).

[c] Races avirulent on differential cultivars are represented by numbers to the left of the slash (/); races virulent are listed on the right. Each designation lists cultivars with lowest infection type to the left, the highest to the right.

[d] Infection types used were: 00 = small necrotic symmetric flecks; 0⁻ = larger necrotic flecks, usually nonsymmetrical; 0 = necrosis involving larger areas of the leaf without pustulation; 1⁻ = similar to the 0 type, but eventually forming very small pustules around necrotic lesions; 1 = necrosis with small pustules; 2 = necrosis and chlorosis with larger pustules; 3⁻ = necrosis and chlorosis with normal pustules; 3 = no necrosis but chlorosis with normal pustules; 4 = no necrosis or chlorosis with normal pustules. Each infection type represents an average taken from primary leaves of seedling cultivars.

[e] An albino isolate which produces white instead of orange urediospores.

most cases. Using the seven differentials selected by Line et al. (6), five race groups may be distinguished from the 16 isolates (Table 1).

Examination of other differential candidates throughout this investigation revealed the usefulness of additional cultivars and correspondingly raised questions about the continued use of some of those previously used. Using a revised list of eight differentials, 11 stripe rust races may be distinguished (Table 2). Each candidate was tested over a period of 3 years as many as nine times with a given isolate at both temperature profiles. Those cultivars found suitable for use as differentials responded with clear, consistent, and reproducible infection readings.

TABLE 2. Tentative physiological races of *Puccinia striiformis* present in the northwestern USA[a]

	Differential and infection type[b]							
	1	2	3	4	5	6	7	8
Race designation	Chinese 166	Druchamp	Leeds	Moro	Medeah	President Riverain	Marfed	Red River 68
MAES 1[c]								
1,4,3,8,5,2,6 / 7[d]	00[e]	1	0	00	1⁻	1	3	0
MAES 2								
1,4,8,3,5,2 / 6,7	00	1⁻	0⁻	00	0⁻	3	3	00
4,5,1,2,3,8 / 6,7	0⁻	0⁻	0	00	00	3	3	0
MAES 3								
1,4,2,6,8 / 3,5,7	00	1⁻	3	00	3	1⁻	3	1⁻
1,4,2,6,8 / 3,5,7[f]	00	0	3	00	3	0	3	1⁻
MAES 4								
1,3,8,5,2 / 6,4,7	00	1	0⁻	3	0	3⁻	3	0⁻
MAES 5								
1,4,3,2,5 / 6,8,7	00	1	1⁻	00	1	2	3	3⁻
MAES 6								
4,8,3,7,5 / 2,6,1	3	2	0	00	1	2	1⁻	0⁻
MAES 7								
1,4,3,5 / 2,6,7,8	00	3⁻	1⁻	00	1	3⁻	3	4
1,4,3,5 / 6,7,2,8	00	3	1⁻	00	1⁻	3⁻	3⁻	3
MAES 8								
4,8,5,7 / 2,3,6,1	3	2	2	00	1⁻	2	1⁻	0
MAES 9								
1,4,8,3 / 5,2,6,7	00	3⁻	1⁻	00	2	3⁻	3	0
MAES 10								
4,1,8 / 6,2,3,7,5	0⁻	3⁻	3⁻	00	3	2	3⁻	0⁻
1,4,8 / 3,5,6,2,7	00	3	2	00	2	2	3	0
1,4,8 / 2,3,5,6,7	00	3⁻	3⁻	00	3⁻	3⁻	3	1⁻
MAES 11								
4,3,5 / 2,6,8,1,7	3	2	0	00	1⁻	2	3	3⁻

[a]Determinations were conducted at 2/18 C, night/day.

[b]CI or PI numbers include: Chinese 166 (11765), Druchamp (13723), Leeds (13768), Moro (13740), Medeah (5140), President Riverain (174687), Marfed (11919), Red River 68 (14193).

[c]MAES = Montana Agricultural Experiment Station.

[d]Races avirulent on differential cultivars are represented by numbers to the left of the slash (/); races virulent are listed on the right. Each designation lists cultivars with lowest infection type to the left, the highest to the right.

[e]Infection types used were: 00 = small necrotic symmetric flecks; 0⁻ = larger necrotic flecks, usually nonsymmetrical; 0 = necrosis involving larger areas of the leaf without pustulation; 1⁻ = similar to the 0 type, but eventually forming very small pustules around necrotic lesions; 1 = necrosis with small pustules; 2 = necrosis and chlorosis with larger pustules; 3⁻ = no necrosis but chlorosis with normal pustules; 4 = no necrosis or chlorosis with normal pustules. Each infection type represents an average taken from primary leaves of seedling cultivars.

[f]An albino isolate which produces white instead of orange urediospores.

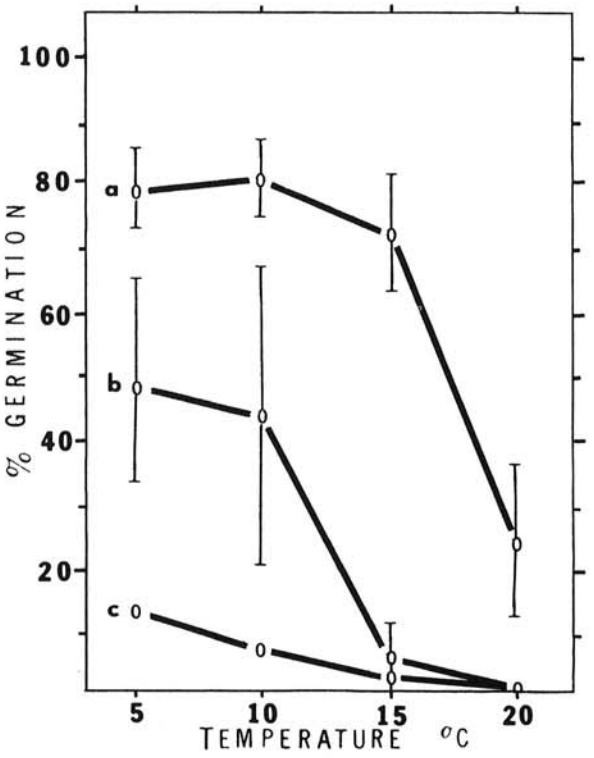

Fig. 1. Ability of urediospores representing different stripe rust races to germinate at four temperatures: **(a)** races MAES (Montana Agricultural Experiment Station) 6 and 8; **(b)** races MAES 1, 2, 3, 4, 5, 9, 10, 11;and **(c)** albino isolate of race MAES 3. Vertical lines indicate the range of mean germination values between designated races at a given temperature.

During the preliminary testing of a field rust collection and prior to monospore culturing, it was noticed that two different infection types, "3" and "00", appeared upon a single leaf of the cultivar Medeah. Monospore isolates were obtained which gave two races, MAES 2 and MAES 10. These presently have the isolate designation 4,5,1,2,3,8 / 6,7 and 4,1,8 / 6,2,3,7,5 and are different in respect not only to the differential Medeah but also to Druchamp and Leeds.

In the past it has been suggested that only one race existed in the Pacific Northwest which was virulent on Chinese 166. Using the differentials in Table 2 it was possible to determine the presence of two different race groups both virulent on Chinese 166. Race MAES 8 contains genes for virulence which are different than those in race MAES 11, yet both were isolated from Chinese 166 in Oregon and Washington, respectively. Not until the revised differential set (Table 2) was employed could these two races be distinguished.

After repeated tests at both temperature regimes, the barley and tetraploid wheat candidates were found to be unreliable as differential hosts.

Aggressiveness.—The rust isolates evaluated for aggressiveness include all those listed in Table 2 with exception of all isolates comprising race MAES 7 and isolate 4,1,8 / 6,2,3,7,5 of race MAES 10. The daily germination averages were grouped in Fig. 1 since germinability for some races was similar at corresponding temperatures. Urediospores of races MAES 6 and 8 which germinated the best over the whole temperature range, and especially at 20 C, were both virulent on Chinese 166.

DISCUSSION.—The fact that the majority of host candidates selected on the basis of their reaction at different field locations were not suitable as differential hosts was not surprising. Evaluation of these hosts at controlled environments indicated that most of the observed differences in the field were due to varying temperature regimes. The influence of the environment, especially temperature, on development of stripe rust is widely known and has led to unreliable and variable results even where the tests were conducted in the greenhouse (7, 8). Ideally, differential host candidates should be either highly resistant, or highly susceptible, to specific rust isolates. Hosts giving an intermediate reaction often indicate the presence of temperature sensitive, additive genes and precise environmental control is required for reproducible results (13). Furthermore, there is inherent difficulty with different researchers evaluating intermediate infection types. It was not possible to totally eliminate all differentials giving intermediate reactions in this study, but those few remaining proved to be most reliable in repeated tests. Since most of the cultivars containing temperature-sensitive additive genes condition the greatest resistance at a relatively high temperature regime, a low regime of 2/18 C was used in this work. This tended to place more emphasis on the "major effect" genes for resistance. In addition to repeatedly evaluating plant response under controlled environments this study was strengthened by the fact that field locations were sampled over three growing seasons for the presence of different races and approximately 200 host cultivars were tested at both temperature regimes as candidate differentials. Since a diversity of host germ plasm was present in the international wheat nurseries, the opportunity to find different genes for virulence was maximized. Single spore isolates were used throughout this study.

It was interesting that two of the races determined in this study were notably aggressive in that they germinated better at all temperatures and particularly at 20 C. These characteristics were associated with virulence to Chinese 166 and may contribute to increased survival in a population of different races.

It is recognized that the proposed set of differentials will probably not be long-lasting. The interaction of host and pathogen is a dynamic relationship. However, the determination of the gene pool for virulence in an area is a necessary first step and a prerequisite for a breeding program for disease resistance. The races determined in this study have been preserved and are currently being used to screen potential parental materials and candidate wheat cultivars for resistance to stripe rust.

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