The Relative Survival Ability of Pathogenic Types of Puccinia striiformis in Mixtures

J. F. Brown and E. L. Sharp

Postdoctoral Research Associate and Professor, respectively, Department of Botany and Microbiology, Montana State University, Bozeman 59715.

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ABSTRACT

The relationship between the relative survival ability and the number of genes for virulence in two pathogenic types of *Puccinia striiformis* was studied on susceptible wheat varieties, using an albino mutant as a marker. The relative survival ability and the host range of the albino culture was identical with that of the wild type from which it was derived. When urediospore mixtures of a rust isolate from Bonner's Ferry, Idaho, and an albino isolate derived from a collection made at Bozeman, Montana, were used to inoculate the susceptible varieties Lemhi and Itana for successive uredial generations, the pathogenic type with the widest host range (Bonner's Ferry) predominated at 2 C/18 C (night/day) and 15 C/24 C (night/day) temperature regimes, regardless of the original proportions of urediospores of each pathogenic type in the mixtures. However, despite the competitive advantage shown by the Bonner's Ferry culture, the albino culture persisted in low frequencies (about 1%) after seven successive uredial generations. When the wheat variety President Riverain was inoculated with a mixture of the two pathogenic types, a marked increase in susceptibility to the albino isolate was observed. This increase in susceptibility was apparently not related to exchange of nuclei between the two pathogenic types. Phytopathology 60:529-533.

Changes in the incidence of physiologic races of cereal rusts in the field are well known, and under laboratory conditions several workers have observed that some races tend to predominate in mixtures, even on varieties equally susceptible to all races in the mixtures. Watson (20) studied races 17, 19, 34, 56, and 147 of *Puccinia graminis tritici,* singly and in association, and found that race 34 always maintained itself or increased in frequency, whereas race 147 was always eliminated from the mixture after several uredial generations on a susceptible host. Essentially similar findings have been reported by others working with *P. graminis tritici* (3, 9, 10, 11, 13, 23, 24), *Puccinia recondita* (8), *Phytophthora infestans* (17, 18), *Tilletia caries* and *Tilletia foetida* (14), and *Diplodia zeae* and *Diplodia macrospora* (7).

Watson (21) concluded that races of *P. graminis tritici* with a wide host range, and therefore having the most factors for virulence, were unable to maintain themselves in a population when grown on susceptible varieties in association with races having a narrower host range. This contention is supported further by the results of Black (2) for *P. infestans* and by Flor (5, 6) for *Melampsora lini.* Thus, several workers believe that races with the most genes for virulence are not always the fittest to survive, and that, when grown on susceptible cultivars, there is a negative correlation between survival ability and the number of specialized genes for virulence in the race. Indeed, van der Plank (19) considered that unnecessary virulence reduced fitness to survive, and may prevent highly virulent races from becoming abundant in the field.

Nevertheless, some evidence, particularly that obtained in the laboratory, indicates that survival ability is unrelated to the presence of genes for virulence. Loegering (13) presented evidence indicating that the ability of races to multiply in mixtures is not due to observable differences in virulence alone. He suggested that relatively minor ecological factors may combine to affect the differential development and survival of races in mixtures that occur commonly under natural conditions. The results of Katsuya & Green (11) showed that the relative survival ability of races 56 and 15B of *P. graminis tritici* was dependent on factors such as temperature regime and inoculum density. Recently, it was shown (23) that under greenhouse conditions, regardless of the initial proportions in mixtures of races 21 Anz 7 and 21 Anz 1, 2, 3, 7 (nomenclature used by Watson & Luig (22) to designate races found in the Australian and New Zealand geographic area) of *P. graminis tritici,* the race with the greatest number of genes for virulence predominated after four uredial generations.

The conflicting nature of the data available, together with the lack of published information concerning the relationships between survival ability and virulence of the stripe rust fungus (*Puccinia striiformis* West.), prompted this investigation which describes some of the factors which influence survival of races of *P. striiformis* in mixtures.

Materials and Methods.—Seedlings of the wheat varieties Lemhi and Itana were grown in 4-inch clay pots within walk-in environment chambers rigidly controlled and programmed for temperature, light, and relative humidity. Diurnal temperature profiles of 2 C/18 C (night/day) and 15 C/24 C (night/day) were programmed to simulate natural diurnal fluctuations during spring-autumn and summer seasons, respectively. In both environment chambers, light intensities were increased step-wise from 300 to 1,800 ft-c to a maximum of 3,500 ft-c during the middle of the photoperiod, and decreased through a similar range. Photoperiods were 12 hr, and relative humidity was about 95% during the dark period and 65% during the light.

The wheat varieties Lemhi and Itana were chosen because Lemhi is believed to possess neither major nor minor genes for resistance (12), whereas Itana, although susceptible at the temperatures used in these experiments, shows some resistance at higher temperatures.

The original cultures of rust inoculum used in this study were collected in fields near Bozeman, Montana, (B1) and Bonner's Ferry, Idaho (BF1). Assays on selected host varieties indicated a single biotype within each collection. After increase on a susceptible variety, cultures of each collection were lyophilized by the method of Sharp & Smith (16). The BF1 collection, in terms of several host varieties, appeared to have more genes for virulence than the B1 collection (Table 1).

During laboratory experiments an albino culture (B1a) arose, apparently by mutation from B1. On the basis of infection types on more than 50 different wheat varieties, including those listed in Table 1, the albino mutant had the same virulence as B1.

The following mixtures of urediospores of known proportions (by wt) were prepared and inoculated onto wheat seedlings at the rate of about 70 urediospores/cm^2 of leaf surface: (i) 53:47 mixture B1:B1a; (ii) 91:9 mixture B1:B1a; (iii) 99:1 mixture B1:B1a; (iv) 23:77 mixture BF1:B1a; (v) 73:27 mixture BF1:B1a; (vi) 96:4 mixture BF1:B1a.

The albino isolate was used because the number of pustules produced by each culture could be counted directly without having to inoculate differential hosts to distinguish between the two pathogenic types. However, for the albino to be useful, it was necessary to show that the survival ability of the albino type was identical with that of the wild type from which it was derived. Therefore, experiments were made to compare the relative survival abilities of the B1a and B1 collection both in the laboratory and in the field.

Primary leaves were inoculated, using a settling tower and dew chamber incubation described elsewhere (15). After inoculation, plants were placed in a dark dew chamber kept at 7 C for 24 hr before being returned to their respective environments. After initial sporulation at the two temperature regimes, a further generation was inoculated by brushing urediospores from the diseased seedlings onto healthy plants. Immediately after inoculating a new set of plants, the number of white and yellow pustules on the primary leaves of the spore donor plants was determined by counting, with the aid of a stereomicroscope, the number of pustules on a 3-cm section of each leaf, commencing 1 cm below the leaf tip. In each urediospore generation, three pots each containing 10 plants were inoculated for each treatment. The data were subjected to an analysis of variance, and the means were compared with the LSD method as recommended by Balaam (1).

TABLE 1. Reactions on wheat varieties which differentiate isolates of *Puccinia striiformis* made at Bozeman, Montana (B1), and at Bonner's Ferry, Idaho (BF1)[a]

	Mean infection type[b]	
Wheat var.	B1	BF1
Viloria	0	3
Virman	0	3
President Riverain	0	3
Roma	0	3
Tehuacan	1	3
Hector	1	3
Navarro	2	4
Marival	0	2

[a] Plants were grown at 2 C/18 C (night/day) in both the preinoculation and postinoculation phases.

[b] 0 = Necrosis involving the entire width of the leaves, no pustules. 1 = Necrosis and chlorosis with a few small pustules. 2 = Necrosis and chlorosis with moderate development of pustules. 3 = Chlorosis only, abundant pustules. 4 = No necrosis or chlorosis, abundant pustules.

RESULTS.—*Relative survival ability of wild and albino isolates of the Bozeman collection.*—Urediospore mixtures of known proportions were inoculated onto leaves of the wheat varieties Lemhi and Itana, which were kept at either the 2 C/18 C or the 15 C/24 C temperature regime. The number of pustules of the albino and yellow wild collection were counted at each of several successive uredial generations.

With the exception of Lemhi kept at 15 C/24 C, there were no significant changes in the proportion of pustules of each culture in different generations (Table 2). On Lemhi grown at 15 C/24 C, the proportions of pustules of each culture in the third and seventh generation for the 53% original mixture were significantly different to the other generations. The reason for these differences is uncertain. However, despite the differences, the results suggest that the albino type was able to compete favorably with the wild type from which it was derived. The only other factor which influenced the proportion of pustules of each type was the proportion of each type in the original urediospore mixture, an expected result.

To investigate the relative survival ability of the two isolates under field conditions, a urediospore mixture of both was inoculated onto Lemhi wheat in a field plot at the Central Montana Research Station at Moccasin during 1968. This locality was selected because stripe rust epidemics occurred infrequently and data obtained might less likely be confounded by the presence of naturally occurring inoculum.

Two rows of Lemhi wheat (3 ft long) within the center of a field plot 36 × 40 ft were inoculated with a mixture of stripe rust isolates B1 and B1a. The higher germination potential of the B1 isolate resulted in more initial infections of the wild type, but the albino isolate was able to compete well with the wild type in succeeding generations. In the initial infection area, all leaves were examined and it was found that 61% of pustules were of the wild type. At subsequent periods of examination, 10 tillers were examined at each of eight compass points. Counts taken at various time periods after inoculation, over a 4-week period, showed that the percentage of wild type pustules were 50%, 48%, 48%, and 63% at 3 ft, 4 ft, 10 ft, and 16 ft from

TABLE 2. Comparison of the relative survival ability of the wild type isolate (B1) of *Puccinia striiformis* in mixtures with its albino mutant (B1a)[a]

Wheat var. and temp. regime	Original urediospore mixture	% Wild type pustules						
		Generation no.						
		1	2	3	4	5	6	7
Lemhi 15 C/24 C	53	73	80	68	83	86	83	92
	91	99	97	92	97	98	99	98
	99	99	100	99	100	99	100	100
Lemhi 2 C/18 C	53	75	67	62	77	76		
	91	95	94	97	99	95		
	99	99	99	99	99	99		
Itana 15 C/24 C	53	77	81	76	86	85	86	94
	91	99	98	96	98	97	99	99
	99	98	97	98	99	100	100	99
Itana 2 C/18 C	53	62	61	66	83	74		
	91	94	93	94	98	95		
	99	98	100	100	100	100		

[a] Avg for three replicates, each consisting of 10 plants.

the inoculation center, respectively. Many individual leaves contained infections with both the yellow B1 isolate and the albino mutant. Environmental conditions for rust development were somewhat marginal, prevalence never reached 100% for any of the examination periods, and a definite downwind spread pattern was apparent. Observations of adjoining susceptible wheat plots indicated a complete absence of natural stripe rust infection.

Relative survival of two pathogenic types of Puccinia striiformis as affected by wheat variety.—An experiment was made to determine whether or not a wider range of virulence was related to relative survival ability, and to determine if the results might vary with host variety. For this purpose, urediospore mixtures of known proportions of the BF1 and B1a isolate were inoculated onto leaves of Lemhi and Itana wheats. The number of pustules of each isolate was recorded in each of seven successive uredial generations. The plants were kept at 15 C/24 C both before and after inoculation.

The proportion of BF1 pustules increased with number of generations (Table 3). It was apparent that, regardless of the original proportions in the mixture, the BF1 pathogenic type had a much greater survival ability than the B1a type, which almost disappeared after seven uredial generations.

The susceptible variety of wheat on which the rust was grown had a significant effect ($P < .01$) on the survival of races of *P. striiformis* in mixtures. When seedlings were initially inoculated with a 23% mixture of BF1, the proportion of BF1 pustules was greater on Itana during the early generations than on Lemhi (Table 3). However, when plants were initially inoculated with either a 73 or 96% mixture of BF1, the variety on which the rust was grown did not influence the survival ability of the pathogenic types in the mixtures. Thus, there was an interaction ($P < .05$) between wheat variety and the proportions of the two types in the initial mixture that influenced survival ability of *P. striiformis*.

As one might expect, the initial proportion of the isolate in mixtures had a marked effect on the proportion of each isolate at successive generations. The percentage of BF1 pustules in the 23% initial mixture was significantly different ($P < .05$) from the other mixtures until the seventh generation. However, although the 96% initial mixture gave 97% and 94% BF1 pustules in the first generation on Lemhi and Itana, respectively, the B1a isolate persisted in low proportions for at least seven generations.

Effect of temperature regime on relative survival ability.—Seedlings of the wheat variety Lemhi were inoculated with urediospore mixtures of known proportions of BF1 and B1a, and kept at either the 2 C/18 C or the 15 C/24 C temperature regime. The number of pustules of each pathogenic type was recorded in each of five generations.

At both temperature regimes, the proportion of BF1 pustules increased with generation number (Table 4). It is apparent that, regardless of the original propor-

TABLE 3. Relative survival ability of the Bonner's Ferry (BF1) and the albino Bozeman isolate (B1a) of *Puccinia striiformis* as affected by host variety[a]

Wheat var.[b]	Generation	% Pustules of BF1[c]		
		% In original mixture		
		23	73	96
Lemhi	1	43a	81a	97NSD
	2	46a	73a	94
	3	66b	91b	99
	4	78c	93bc	99
	5	92d	98d	100
	6	86cd	95bcd	96
	7	97e	97cd	100
Itana	1		82a	94NSD
	2	76ab	79a	97
	3	59a	92b	99
	4	83bc	96bc	99
	5	93d	99cd	100
	6	94cd	98cd	99
	7	93cd	99d	98

[a] Mean of 3 replicates each consisting of 10 plants.
[b] Plants at 15 C/24 C both prior to and following inoculation.
[c] Letters indicate significant differences at the 5% probability level for each treatment.

TABLE 4. Effect of temperature on the relative survival ability of the Bonner's Ferry isolate (BF1) in mixtures of known proportions with the albino Bozeman isolate (B1a) of *Puccinia striiformis* on Lemhi[a]

Temp. regime	Generation	% Bonner's Ferry pustules[b] % In original mixture 23	73	96
15 C/24 C	1	43a	81a	97NSD
	2	46a	73a	94
	3	66b	91b	99
	4	78c	93b	99
	5	92d	98c	100
2 C/18 C	1	34a	95NSD	100NSD
	2	59a	97	98
	3	70ab	97	98
	4	80b	98	98
	5	83b	97	99

[a] Mean of 3 replicates each consisting of 10 plants.
[b] Letters indicate significant differences at the 5% probability level for each treatment.

tions of urediospores in the mixture, the proportion of pustules of BF1 either increased or remained constant. In no case did the proportion of pustules of BF1 decrease.

The temperature regime in which plants were kept had a significant effect ($P < .01$) on the proportion of pustules of each culture at different generation times. When seedlings were initially inoculated with a 73% mixture of BF1, the proportion of BF1 pustules did not change with successive uredial generations when plants were kept at the low temperature regime. In contrast, on plants kept at the high temperature regime, the proportion of BF1 showed an increase with generation number. Thus, there was a significant interaction ($P < .05$) between temperature regime and the proportions of each pathogenic type in the initial mixture which influence the survival ability of *P. striiformis*.

During this study an unexpected observation was noted. When collections BF1 and B1a were inoculated onto the wheat variety President Riverain alone, BF1 produced infection type 3, whereas B1a produced infection type 0 (Table 1). However, when seedlings were inoculated with a mixture of the two pathogenic types, both produced type 3 infection types. When a single pustule of the albino type was isolated from the mixture and reinoculated alone onto President Riverain, the resulting infection type was 0.

DISCUSSION.—Obviously, the ability of a given physiologic race of a wheat rust to survive in the field is influenced by the varieties of wheat grown. Varieties possessing major genes for resistance will have a selective effect against those races of the fungus which do not contain genes for virulence capable of overcoming the genes for resistance in the host. Thus, a close correlation exists between the varieties of wheat grown and the prevalence of rust races capable of infecting the predominant varieties (21). However, when the selective effects of major resistance genes in the host are removed, such as exists when varieties that are uniformly susceptible to all races are grown, certain races of rust still tend to predominate. Presumably, some races possess characters that give them a competitive advantage over associated races that are eventually overrun in the population. Katsuya & Green (11) point out that the Canadian races 15B-3 and 15B-5 of *P. graminis tritici* occurred in trace amounts for several years, but despite their virulence on the predominant variety Selkirk, apparently disappeared in the field. The reasons for such differences in relative survival ability among races of rust is uncertain. It seems likely that characteristics such as the number of successful infections produced per unit of inoculum, the number of viable spores produced per infection, the duration of sporulation, the generation time, longevity of spores, and the response of the particular race to environment would contribute toward the relative survival ability of rust races on a susceptible host.

Several authors have suggested that an inverse correlation exists between relative survival of rust races and the number of genes for virulence present (3, 5, 6, 19, 21). However, the evidence presented in this paper does not support this hypothesis. The stripe rust culture showing the widest host range (BF1), and, therefore, presumably possessing the greater number of genes for virulence, had a greater relative survival ability on the susceptible varieties Lemhi and Itana than the pathogenic type showing a narrower host range (B1a). The findings of Katsuya & Green (11), who showed that temperature is an important factor regulating the competitive abilities of races 15B-1 (Can.) and 56, indicates further that a correlation does not necessarily exist between virulence and survival ability. Furthermore, they found that race 56 predominated when plants were lightly infected and race 15-1 predominated in heavy infections. These results, together with the report (11) of the displacement of race 56 in recent years by strains of race 15B with added virulence on certain resistant varieties of durum wheat, questions the proposal that there is always a negative correlation between survival ability and the number of genes for virulence present.

Despite the competitive advantage of collection BF1 of *P. striiformis*, the albino culture (B1a) persisted in low frequencies, and even after seven uredial generations it had not been completely overrun. Whether a stable equilibrium between the two pathogenic types was reached when the albino culture persisted with about 1% frequency, or whether the albino culture would have eventually been eliminated from the mixture, is uncertain.

Apparently the albino culture of *P. striiformis* had lost none of its competitive ability when compared with the wild collection from which it was derived. Furthermore, the ability of the albino mutant to survive for several generations in the field and to compete successfully with the wild collection is somewhat surprising in view of the contention that the pigment in the walls of urediospores protects the protoplasm from the injurious action of ultraviolet radiation (4).

The change in susceptibility of the wheat variety President Riverain to collection B1a when a mixture of B1a and BF1 was inoculated onto seedlings is an interesting observation. It appears that the simultaneous infection by the two pathogenic types of the fungus, and not the exchange of nuclei, rendered the host susceptible to a biotype to which it was normally resistant. It is uncertain whether an interaction between rust races similar to that which was observed in this study is a common phenomenon. If so, it may explain why some races of rust continue to persist for long periods in the absence of susceptible cultivars (11, 22, 23).

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