



Physiological race determination and environmental factors affecting the development of infection type in stripe rust (*Puccinia striiformis* West.)
by Raymond Bradford Volin

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Plant Pathology
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Abstract:

Isolates of stripe rust, *Puccinia striiformis*, were collected from numerous graminaceous hosts throughout the northwestern region of the United States. Host candidates were chosen for their potential ability to differentiate genes for virulence in the rust cultures. The hosts were inoculated as seedlings using monospore isolates of stripe rust and tested under controlled environments at two temperature profiles, 2/18 and 15/24 C (night/day). Many varieties were unable to discriminate any of the isolates under a controlled environment.

Wheat cultivars Chinese 166, Druchamp, Leeds, Moro, Medeah, President Riverain, Marfed and Red River 68 were selected as differentials. They were capable of distinguishing eleven races from the cultures.

Different races were tested on plant lines which possessed minor genes for resistance to stripe rust. Plant selections with one, two and three minor genes sustained moderate to high levels of incompatibility to all available races suggesting nonspecific or horizontal resistance.

In order to determine the possible origin of physiologic races, isolates differing in genes for virulence and color were increased together on a single host to encourage anastomosis, nuclear reassortment and possible parasexualism. A heterokaryon was produced which expressed genes for virulence which were different than in other races determined in the study.

As an aid in following possible nuclear reassortment, a technique was developed to differentially stain nuclei within urediospore germ tubes. Nuclear number was determined to be dependent on germination time.

The aggressiveness of 13 stripe rust isolates was measured by the ability and extent of urediospore germination at 5, 10, 15 and 20° C. Races 6 and 8, both virulent on Chinese 166, were most aggressive at all temperatures.

PHYSIOLOGICAL RACE DETERMINATION AND ENVIRONMENTAL FACTORS AFFECTING
THE DEVELOPMENT OF INFECTION TYPE IN STRIPE RUST
(PUCCINIA STRIIFORMIS WEST.)

by

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ABSTRACT

Isolates of stripe rust, Puccinia striiformis, were collected from numerous graminaceous hosts throughout the northwestern region of the United States. Host candidates were chosen for their potential ability to differentiate genes for virulence in the rust cultures. The hosts were inoculated as seedlings using monospore isolates of stripe rust and tested under controlled environments at two temperature profiles, 2/18 and 15/24 C (night/day). Many varieties were unable to discriminate any of the isolates under a controlled environment.

Wheat cultivars Chinese 166, Druchamp, Leeds, Moro, Medeah, President Riverain, Marfed and Red River 68 were selected as differentials. They were capable of distinguishing eleven races from the cultures.

Different races were tested on plant lines which possessed minor genes for resistance to stripe rust. Plant selections with one, two and three minor genes sustained moderate to high levels of incompatibility to all available races suggesting nonspecific or horizontal resistance.

In order to determine the possible origin of physiologic races, isolates differing in genes for virulence and color were increased together on a single host to encourage anastomosis, nuclear reassortment and possible parasexualism. A heterokaryon was produced which expressed genes for virulence which were different than in other races determined in the study.

As an aid in following possible nuclear reassortment, a technique was developed to differentially stain nuclei within urediospore germ tubes. Nuclear number was determined to be dependent on germination time.

The aggressiveness of 13 stripe rust isolates was measured by the ability and extent of urediospore germination at 5, 10, 15 and 20 C. Races 6 and 8, both virulent on Chinese 166, were most aggressive at all temperatures.

INTRODUCTION

Stripe rust of wheat (Puccinia striiformis West., Puccinia glumarum (Schm.) Erikiss, and Henn.) has long been recognized as one of the main fungus diseases of cereal crops. In comparison to other rusts which attack cereals, stripe rust is adapted to lower temperatures. It is able to parasitize susceptible hosts during the relatively cool environment characteristic of coastal and intermountain regions. Infection may occur early in the growing season during initial stages of crop development with the intensity of infection diminishing gradually with rising temperatures. Damage to the crop may be severe especially when the inflorescence of the plant is infected.

The occurrence of stripe rust is world-wide and has caused considerable losses in Europe, the Near East, Eastern Asia, India, North Africa and in intermountain areas of the northwestern United States. It has been reported to be distributed in the highlands of South America and Mexico.

Many cereal varieties have been produced with resistance to stripe rust. However, only a few seasons after distribution of resistant varieties, these also were attacked by stripe rust. As host varieties were developed with resistance the rust also adapted by natural processes of evolution, favoring its survival. Formerly resistant varieties succumbed to the advance of new physiological races specifically adjusted to their new host.

Virulence patterns have noticeably shifted in the pacific north-west region of the U. S. During the period 1958-1961, over 50% of all wheat (Triticum aestivum L.) produced in northwestern counties was from the variety Omar . Large acreages of monovarietal culture allowed large increases in virulent rust genotypes. By 1962 Omar was responsible for only 34% of the total wheat production and by 1968 Omar contributed less than 3%. In 1968, production from a new variety, Moro , which was resistant to stripe rust, had begun to increase. During that year, however, it was observed that a virulent race had overcome Moro's resistance and was able to form enough inoculum for wide dispersal. The Moro resistance was sustained only two seasons. Since 1962, the variety Gaines has been widely grown in the north-west region. It is moderately susceptible to stripe rust but its high yield potential contributes to its continued importance to wheat production in the area.

During the same period of Omar production in the pacific north-west regions, western Montana wheat production was not without its problems. The hard red winter varieties, Westmont and Itana , were grown in the western area of the state. In 1962, Westmont accounted for 56% of the total wheat acreage in northwestern Montana and Itana 42% of the total in southwestern Montana. Large acreages of few varieties again favored the pathogen. A favorable environment, susceptible hosts and sufficient inoculum caused the severe stripe rust

epiphytotic of the 1960's.

Crop losses due to stripe rust have periodically been quite large through the northwestern United States. During the late 1950's and early 1960's the intermountain and coastal regions sustained considerable crop damage from stripe rust attack. In 1962, losses in western Montana wheat crops were estimated at \$1,827,805 with \$1,018,080 of this occurring in Gallatin County alone. The year following, losses were over \$2,000,000 in infected areas of Montana. Since this severe epiphytotic, losses have been considerably smaller, under \$500,000 per year in western Montana. The sowing of wheat varieties having measurable resistance to stripe rust has been a major factor contributing to the decrease in crop loss both in Montana and the northwest region. In some areas, however, such varieties are undesirable from other disease and agronomic standpoints.

The mechanisms by which changes of virulence may occur in P. striiformis are restricted because of the absence of pycnial and aecial stages. It has been shown by growing rust cultures in isolation for long periods that mutations for pathogenicity do not occur frequently. Under conditions of mass increase of spores on susceptible field plants, mutant factors may be expressed through nuclear exchange between physiological races. Heterokaryosis alone may account for new races. If the parasexual cycle is completed in mitotic recombination, many new races could arise--races possessing new genes

for virulence with altered aggressive characteristics favoring survival.

The present study was prompted by the need for identification and characterization of the existing stripe rust virulence pool in the northwestern area of the United States. The objectives were two-fold: (1) to select a group of host differentials for use in the identification of specific genes for virulence that are present within the stripe rust population in the northwestern United States and (2) to study the influence of some environmental factors affecting host-parasite interaction.

Stripe rust race identification is quite new in the northwestern United States. As isolates differing in genes for virulence are identified, the breeding and selection of resistant plant lines is able to take a new direction. Resistance becomes more specifically defined with designated genes allowing plant selection for identifiable sources of resistance. Through race classification, conclusions may be drawn regarding prevalence, occurrence and geographic distribution of races in an area.

The influence of the environment upon the compatibility of host and parasite is of prime importance, and all information on the subject is valuable. The intent of this study was to establish some environmental parameters for the study of stripe rust which could be controlled, and that would produce the most consistent and reliable

experimental results. As communication is continued among stripe rust researchers, it is intended that these parameters, though perhaps subject to revision, may point toward more uniform results.

REVIEW OF LITERATURE

I. The occurrence of stripe rust.

Stripe rust is an important rust disease of wheat and grasses in regions of moderate temperature and rainfall. Lupton and Macer (1962) reported its occurrence in northwestern Europe including Great Britain where it has been found in most years upon susceptible wheat varieties. The disease has also been reported in India and China (Manners 1950) (Fang 1944), Japan (Kajiwara, Ueda, Iwata 1964), Europe (Allison and Isenbeck 1930) (Gassner and Straib 1930) and in North and South America (Newton, Johnson and Brown 1933) (Johnson and Newton 1946).

The discovery of stripe rust on wheat near Sacaton, Arizona by Dr. F. Kølpin Ravn of Denmark indicated the disease could cause crop losses in intermountain regions of the United States (Carleton 1915). At about the same time the rust was found in southern California and later in Washington, Oregon, Idaho, Utah and Montana (Humphrey 1924) (Hungerford 1923) and (Bever 1934).

II. The pathogen.

Puccinia striiformis West. is a fungus of the class Basidiomycetae and is parasitic upon graminaceous hosts. The name Puccinia glumarum (Schm.) Erikiss. and Henn. was often used in early literature and is still frequently used in Europe. Common names include stripe, yellow or glume rust.

The requirement for cool temperatures, especially during the early phases of infection restricts this disease to coastal or mountainous

regions. The pathogen may overwinter both as urediospores or as mycelium (Hungerford 1923) (Sharp and Hehn 1963). Infection is established much the same as in stem rust, Puccinia graminis (Pers.) except with lower temperature optimums. The mycelium of stripe rust may proliferate throughout the host tissue from the successful establishment at one infection center. The uredia coalesce to form a linear infection pattern between leaf veins, hence the name stripe rust. Plant parts attacked include leaves, leaf sheaths, awns and glumes. Dickson (1956) may be consulted for a more complete description of the disease. Arthur (1934), Johnson and Newton (1946), Britton and Cummins (1956) and Dickson (1956) listed a number of hosts upon which stripe rust has been noted.

Severe losses, resulting from reduced crop yield and test weight may occur when early season conditions are optimal for disease development. Losses exceeding 50% were reported by Domashova (1959) in Russia and by Pope, Sharp and Fenwick (1963) in the pacific northwest area of the United States. Batts and Elliot (1952) estimated losses of 20% in England. The potential threat of this disease was reflected when Purdy and Allen (1963a) stated, "Stripe rust is presently the most important disease threatening wheat production in the Pacific Northwest."

Some of the earliest work comparing the morphological development of infection structures of a large number of rusts, including

stripe rust, was done by Eriksson (1905) and Pole Evans (1907). The anatomical distinctness of certain infection structures are often used as a means of species differentiation.

III. Physiological specialization in stripe rust.

The occurrence of form species of Puccinia glumarum (Schm.) was recorded by Eriksson (1894). Five forma speciales were distinguished: f. sp. tritici, hordei, secale, elymi, and agropyri. He was unable to secure infection of barley and rye with the f. sp. tritici or of wheat with f. sp. hordei. The successful infection of wheat by f. sp. secale indicated, however, that some f. sp. were more strictly specialized than others. The discovery by Newton and Johnson (1936) that certain varieties of Hordeum vulgare and certain species of Agropyron and Elymus were susceptible to the tritici form of P. glumarum led them to question Eriksson's earlier division of the rust. They believed that stripe rust should be considered as a species consisting of a series of closely related specialized forms not adhering to a form species concept. In addition, it was shown by Gassner and Straib (1931) and many others since that P. striiformis is specialized into physiological forms which have been identified by the reactions of differential host varieties.

Not long after the discovery of stripe rust in the United States, Hungerford and Owens (1923) made field collections of P. glumarum in the western United States from wheat, barley, rye, spelt, emmer and

33 wild grasses. It was shown by artificial inoculation that the rust would also infect 26 additional grass hosts. The f. sp. tritici was pathogenic on rye, barley and 47 wild grasses.

Rudorf (1929) tested a number of wheat varieties to P. glumarum types found in Germany and found some varieties which were susceptible to the rust types of Germany which Hungerford (1923) had classified resistant. He used rust types found in the U. S. The conclusion was drawn that United States and German collections of stripe rust differed physiologically.

Allison and Isenbeck (1930) established the existence of races in P. glumarum tritici. Four races were isolated from wheat in Europe using ten differential hosts. Wilhelm (1931) isolated five races from various parts of Europe with ten differential hosts all different from those used by Allison and Isenbeck (1930).

Specialization in P. glumarum was studied extensively in Europe by the German researchers Gassner and Straib (1931, 1932, 1934). A series of 11 wheat varieties were selected by Gassner and Straib (1932) as differentials. They included: Michigan Amber, Ble'rouge d'Ecosse, Strubes Dickkopf, Webster, Holzapfels Früh, Vilmorin 23, Heines Kolben Carstens V, Spalding's prolific, Chinese 166 and Rouge prolifique barbu. Triticum dicoccum var. triccoccum, Fong Tien barley and Heils Franken barley were added later along with Estanquela 75 barley and Petkuser rye. Using reaction types specified by Gassner

and Straib (1932), these researchers had identified 47 physiologic races from Europe, Asia and North and South America by 1939.

Bever (1934) used the European differential set in addition to a few supplemental hosts and established the presence of two physiological races of stripe rust in the United States, one of which had not been found in Europe earlier.

Physiological forms of the rust were isolated for the first time in Canada by Newton, Johnson, and Brown (1933). Newton and Johnson (1936) reported that one of the two physiological forms was common in Europe. The other form had not previously been reported in Europe. In England, Manners (1950) used 16 differentials employed by Straib (1939) to isolate 13 races from 254 collections made from a wide host range.

The original varieties used to differentiate the physiological races of stripe rust were selected for use in Germany nearly forty years ago. Since that time, it has been found necessary to add differentials or in some parts of the world to select an entirely new set to differentiate between the present races. Fuchs (1966) examined some 400 wheat varieties for suitability as additional rust differentials. She was able to identify approximately 60 races through her studies in Brunswick, Germany.

Purdy and Allen (1963b) were able to show that three stripe rust field collections from California, Montana and Washington represented

three distinct pathogenic types of the fungus. Wheat varieties were used which were different from the European differential series. Three years later Purdy and Allen (1966) demonstrated the presence of two distinct races based on the reaction of the wheat selection Suwon 92 x Omar³.

Sharp (1962) (1965) reported a physiologic race in Montana undescribed in North America or Europe while Tollenaar and Houston (1967) indicated that only one race was present in California. A new race pathogenic on the wheat variety Moro (C.I. 13740) was reported from a collection made in Idaho by Beaver and Powelson (1969).

IV. The influence of environment

The recognition that P. striiformis is more sensitive than other cereal rusts to environment has caused much concern among researchers. Straib (1940) reported that temperature during sporulation influenced germination potential of urediospores. That optimum and maximum temperatures for germination varied with physiological races was shown by Newton and Johnson (1936) and Manners (1950).

Zadoks (1961) and Manners (1950) indicated the importance of temperature, relative humidity, irradiation, and inoculum quality upon compatibility. Difficulties arose in comparing Braunschweig, Cambridge, and Wageningen rust data collected in greenhouses. Accentuated by environmentally sensitive alleles conditioning resistance in the differential varieties, data comparisons under differing envi-

ronments have greatly confused efforts in race identification (Macer 1963).

Newton and Johnson (1936) and Manners (1950) indicated that reactions to stripe rust on a number of hosts, including some selected by Gassner and Straib (1932) as differentials, were strongly influenced by environmental conditions. Relatively high temperatures increased the resistance of some hosts to some races but decreased that of others. Low temperatures increased the susceptibility of some differential hosts to certain races.

The influence of environment, particularly temperature and light, during the preinoculation phase was studied by Sharp (1962) (1965). The differential effects of diurnal temperatures on compatibility of infection in many pure-line wheat varieties indicated the importance of a controlled environment.

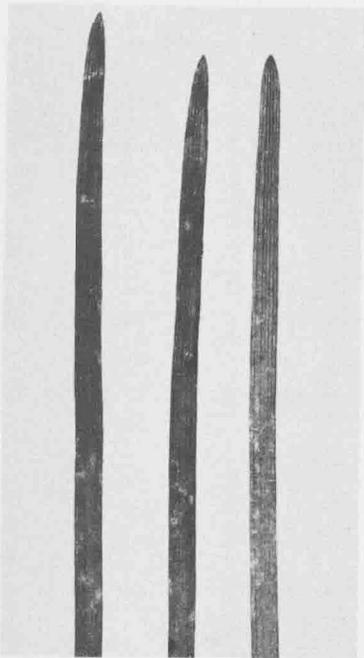
MATERIALS AND METHODS

I. Evaluation of international stripe rust nurseries.

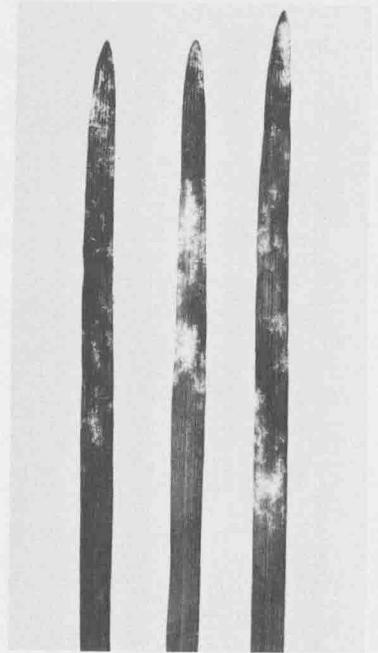
Spring and winter wheat nurseries are planted annually at various international locations. Entries, submitted by world-wide contributors, are evaluated for resistance to stripe rust and other diseases. Nurseries were sown in 1967, 1968 and 1969 at Corvallis, Oregon; Pullman, Washington; Moscow, Idaho; Bonners Ferry, Idaho and Bozeman, Montana. Each nursery for the year 1967, 1968 and 1969 was evaluated by two types of stripe rust readings: severity - average percentage of leaf area infected and infection type - expression of host parasite interaction.

A modified scheme of Gassner and Straib (1932) was used to score the degree of host-parasite compatibility. Resistant: 00 = no uredia, small chlorotic flecks only; 0⁻ = no uredia, chlorosis not spanning the width of the leaf; 0 = no uredia, medium to large areas of chlorosis, necrosis; 1⁻ = uredia few, isolated and minute, chlorosis, necrosis. Intermediate: 1 = uredia few and minute, chlorosis, necrosis; 2 = uredia few and scattered, chlorosis, necrosis; 3⁻ = uredia moderate, chlorosis, isolated necrosis. Susceptible: 3 = uredia abundant, chlorosis; 4 = uredia very abundant, no chlorosis. The designations, with exception of 3⁻ are displayed in Figure 1. Because of host effects caused directly by physiological or environmental factors infection types 00, 0⁻, 1⁻ or 3⁻ were not used in field evaluations.

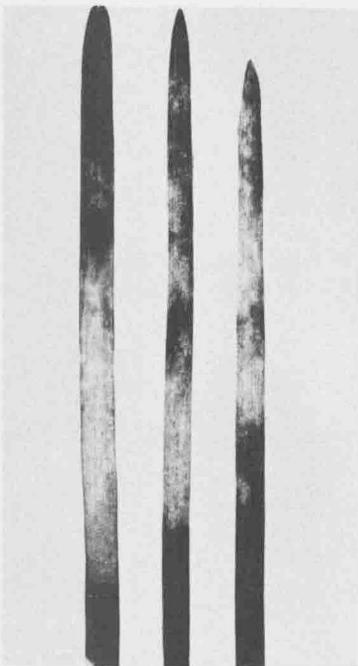
Figure 1. Stripe rust infection types used in physiological race identification.



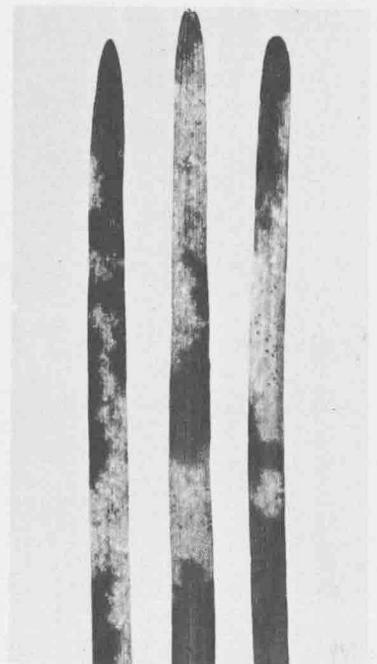
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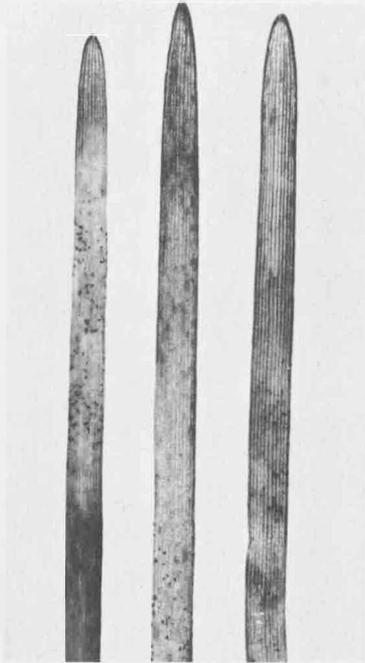


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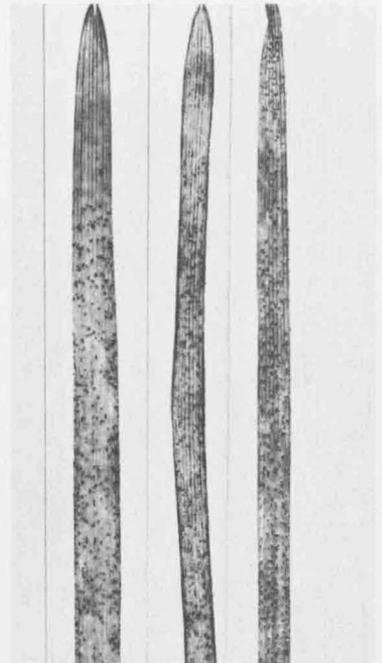


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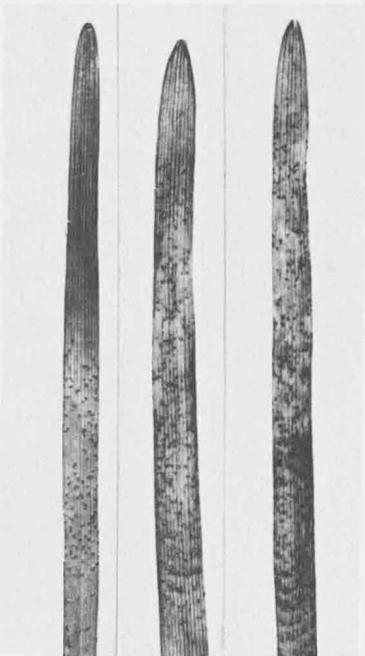
Figure 1. (cont'd) Stripe rust infection types used
in physiological race identification.



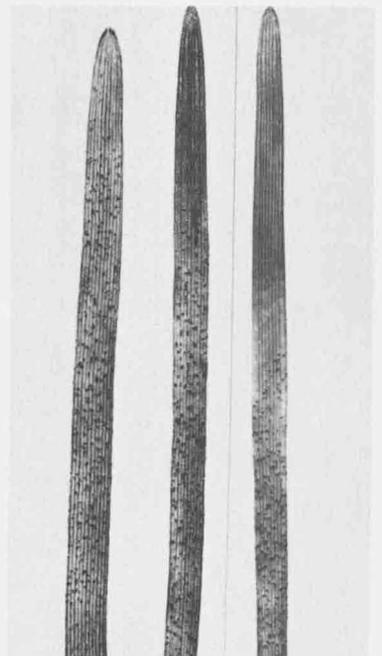
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II. Collection and increase of isolates.

In comparing disease readings from the various nurseries, certain varieties exhibited infection type differences between locations. Stripe rust collections were made from some of these wheat varieties as well as from other hosts in the area. Collections were conducted in such a way as to widely sample the array of susceptible hosts. The purpose was to increase the likelihood of obtaining as many different physiological races as possible. Collections were also made from locations in the northwest region apart from the International Stripe Rust Nurseries (I.S.R.N.).

A number of the rust collections were increased in the laboratory for use in additional tests. Table I gives the designation, location and host source of isolates obtained. Due to lack of space and time, only 16 isolates were studied extensively.

Figure 2 geographically illustrates the location at which the described isolates were collected.

The collections were increased as single spore isolates as soon as possible after collection. Triticum aestivum L. var. Lemhi, a stripe rust-susceptible spring wheat, was used as the rust increase host. However, where possible, the isolate was increased upon the variety from which it was collected. The host plants were grown in 10 cm clay pots and placed in one of two

Table I. Designation and sources of isolates of Puccinia striiformis (West.) used in physiological race identification studies.

No.	Isolate designation	Location of collection	Host from which collected	Monospore isolate
1	B-1	Bozeman, Montana	<u>Triticum aestivum</u> L. var. Itana	No
2	B-W(a)	Bozeman, Montana	<u>T. aestivum</u> var. Witchita	Yes
3	BF-H	Bonnars Ferry, Idaho	<u>T. spp.</u> var. Heines VII	Yes
4	BF-Mo	Bonnars Ferry, Idaho	<u>T. aestivum</u> var. Moro	Yes
5	BF-Om	Bonnars Ferry, Idaho	<u>T. aestivum</u> var. Omar	No
6	C-We	Creston, Montana	<u>T. aestivum</u> var. Westmont	No
7 ^a	C-We(00) C166	Creston, Montana	<u>T. aestivum</u> var. Lemhi	Yes
8 ^a	C-We(3) C166	Creston, Montana	<u>T. aestivum</u> var. Chinese 166	Yes
9	CC-I	Coffee Creek, Montana	<u>T. aestivum</u> var. Itana	No
10 ^b	CC-I(00) Me	Coffee Creek, Montana	<u>T. aestivum</u> var. Lemhi	Yes
11 ^b	CC-I(3) Me	Coffee Creek, Montana	<u>T. spp.</u> var. Medeah	Yes

^a C-We(00)C166 and C-We(3)C166 are subisolates of C-We which produce an infection type of "00" and "3", respectively, on Chinese 166.

^b CC-I(00)Me and CC-I(3)Me are subisolates of CC-I which produce an infection type of "00" and "3", respectively, on Medeah.

Table I. (cont'd.)

No.	Isolate designation	Location of collection	Host from which collected	Monospore isolate
12	Cvl-Cl66	Corvallis, Oregon	<u>T. aestivum</u> var. Chinese 166	Yes
13	KH-Ar	Kings Hill, Montana	<u>Agropyron repens</u> (L.) Beauv.	Yes
14	Ln-72640	Logan, Utah	<u>T. aestivum</u> Breeding line selection of vars. Gaines X NB-5-3-3	Yes
15	M-Ad	Moccasin, Montana	<u>Agropyron</u> <u>dasystachyrum</u> (Hook.) Scribn.	No
16	Mo-B	Moscow, Idaho	<u>T. spp.</u> var. Bezostozia	No
17	Msl-We	Missoula, Montana	<u>T. aestivum</u> var. Westmont	Yes
18	P-Bu	Prosser, Washington	<u>T. aestivum</u> var. Burt	No
19	Pn-B	Pullman, Washington	<u>Hordeum vulgare</u> var. Cambrinus	No
20	Pn-Br	Pullman, Washington	<u>T. aestivum</u> var. brevor	No
21	Pn-Cl66	Pullman, Washington	<u>T. aestivum</u> var. Chinese 166	No
22	SB-40-1	Burns, Oregon	<u>Bromus marginatus</u> Nees.	Yes

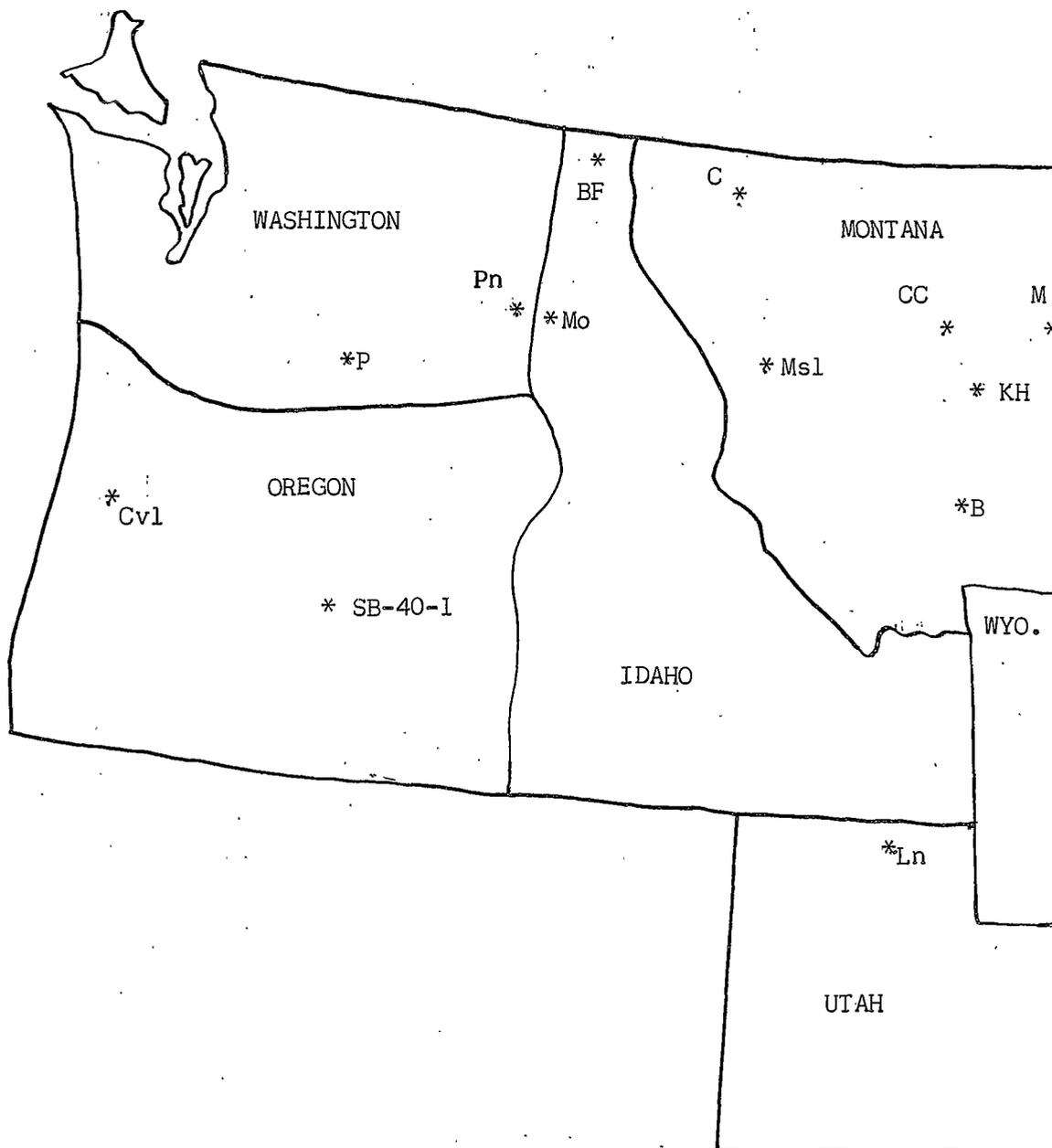


Figure 2. Location at which isolates of stripe rust were collected.

environmental chambers rigidly controlled and programmed for temperature, relative humidity and light. Environmental specifications were described by Lewellen and Sharp (1968).

Urediospores were dusted on the primary leaves of seedlings. The plants were then placed in the dark in dew chambers at 5 C for a 24 hour period. Upon removal they were returned to the environment chambers. Lantern chimneys, covered with cloth at one end, were placed over the sporulating plants and after collection the spores were stored at 5 C and at 50% RH until they could be lyophilized (Sharp and Smith 1952).

III. Evaluation of candidate differential varieties.

Varieties which exhibited differences in infection type in the 1967, 1968, and 1969 I.S.R.N. field trials were harvested and grown under isolation covers. Seedling plants were inoculated (as described earlier) with urediospores representing the regional collections. Such tests were conducted in controlled environment chambers to minimize any differences in infection type which heretofore could have been due to local environment. Temperature profiles and incubation periods were the same as described by Lewellen and Sharp (1968). The infection type designations were described earlier. Host candidates were evaluated at two temperature profiles using isolates listed in Table I. No less than two tests were conducted with any one variety at a given temper-

ature regime. Hosts tested are listed in Table II. In addition to rust readings, observations were taken as to host hypersensitivity, incubation periods and other features associated with host-parasite interaction.

IV. Physiological race determination.

Under a controlled environment it was soon apparent that many varieties could not meet the requirements necessary for race differentiation. Hosts not usable at either temperature profile were discarded in favor of those which showed promise as differentials. These were tested most rigorously and certain of them were selected on the basis of their discriminative ability. Physiological races, differing in factors conditioning virulence could then be differentiated.

V. Evaluation of plant lines selected from P.I. 178383 X Itana.

By inoculation, selection, continuous selfing and diallel cross-analysis at various temperature regimes Lewellen, Sharp and Hehn (1967) selected lines of P.I. 178383 X Itana possessing one, two and three minor genes. These minor genes were shown to be additive in their effect and conditioned a higher level of resistance at 15/24 than at 2/18 C. Wheat lines containing one, two, and three minor genes conditioned mean infection types 2, 0 and 0⁻ at 15/24 (night/day) and 3, 2 and 0 at 2/18 (night/day), respectively, when inoculated with rust culture B-I (ATCC PR No. 35) of P.

Table II. Host varieties tested to isolates stripe rust isolates.

Acker Manns V. 3/15 (P.I. 192257)
Alba (P.I. 191303) (C.I. 13256)
Allies (P.I. 174591)
Ankar (P.I. 192416)
Argentina Selection 7688 (P.I. 282911)
Austral 14, T334 (Switzerland)

Barleta Benvenuto (C.I. 14196)
Bersee (P.I. 168661)
Bigo
Birgitta 0865 (P.I. 192413)
Ble rouge d'Ecosse
Bon Fermier
Bretagne Desprez (P.I. 174609)

Cambrinus
Cappelle (P.I. 229563)
Cappelle Desprez (P.I. 260897)
Carstens V
Chartres Desprez (P.I. 174614)
Chile Selection 3814, 908 X Frontana (A8) 3 X Frontana X Yaqui
2 X Kentana 48 X Africe #43, Ch-7300-lp-lp-40-2p-lp
Chinese 166 (P.I. or C.I. 11765)
Chinese Spring Addition
C.I. 1237
C.I. 13645
Cleo
Compair
Crest
Criewener 104 (P.I. 192473)

Diosecka 777 (P.I. 182850)
Dippes Triumph
Druchamp (C.I. 13723)

Ecuador Selection 150
Emir
Epidor (P.I. 174629)
Equator (C.I. 6833)

F25, (P.I. 83249) (C.I. 10758)
Falco
Felix

Table II. (cont'd.)

Fiume (P.I. 278361)
Flamingo
Flevina

G-78303 (Greece)
Gem (P.I. 299422)
Gentil Roos (P.I. 174639)
Golden (C.I. 11063)

H15, P.I. 83346 (C.I. 10855)
Hansa (P.I. 264271)
Harvest Queen
Harvest Queen X Kawvale (C.I. 12284)
Hatif Inversable (P.I. 125087)
Hatvani 5613 (P.I. 192239)
Hector (Oregon)
Heines VII (P.I. or C.I. 201195)
Heines VII X 6 Redmond, Corvallis 61-1228
Heines Koga (P.I. 180618)
Heines Kolben
Hohenheimer (C.I. 11458)
Hohenheimer (Washington 562005)
Hope X Timstein
Hybride 40 (P.I. 174654)
Hybrid 46
Hybride 46 (P.I. 174655)

ID 0001 (Idaho)
Idaho 53-57 (Idaho)
Inia 66
Isobamba 4777, II-4777-2b-1b-10t-1t (Columbia)

Juanita X ND 333 (North Dakota)

K17 P.I. 83512 (C.I. 11021)
Kirik 228-1 (Turkey)

Lakota
Langdon/60-243 (North Dakota)
Leda
Lee
Leeds (C.I. 13768)
Lemhi (P.I. or C.I. 11415)

Table II. (cont'd.)

Little Club
Little Joss (C.I. 6730)
Lucas

Manella
Marfed
Marival (P.I. 174668)
Medeah (C.I. 5140)
Mendel (P.I. 191312)
Meteor (P.I. 167418)
Mexico Selection-Pitic 62 X Yaqui 54 2X Tezanos Pintos
Precoz X Nainari 60E, II-18734-8M-1T-3Y-2C.
Mexico Selection-Sonora 64A 2X Tezanos Pintos Precoz
X Nainari 60, 18889-4M-4Y-2M-1Y.
Michigan Amber
Mildress
Moro (C.I. 13740)
Moskowskaia (P.I. 191275)

Navarro 291 (P.I. 191170)
Nord Desprez (P.I. 167419)
Noresie 66
Nudif TP3
Nudif TP12
Nudif TP241
Nudif TP250
Nudif TP257
Nugaines

Omar 2X C.I. 12804 X Renacimiento 3X Omar, 66-11809
(Washington)

Opal
Orca

Peko
Persian
P.I. 178383
P.I. 273992 (Ethiopia)
Point Cailloux (P.I. 174684)
Polonium (P.I. 174683)
Poncheau, 262228 (P.I. 297325)
Portugal 90, C-7921 (P.I. 116231)
President Riverain (P.I. 174687)

Table II. (cont'd.)

Probstdorfer Kolbenweizen (P.I. 184859)
Professeur Journee D.I.V. 2430 (P.I. 282905)
Purple Stem (P.I. 53590)

RIC P.I. 83388 (P.I. or C.I. 10897)
Red River 68
Redman
Riebesel 47-3
Riebesel 47-51 (Netherlands) (P.I. 295999)
Rimpaus Bastard II (P.I. 180588)
Roma (P.I. 132862)
Rouge Prolific Barbu (C.I. 11774)
Rubis

Scandia (P.I. 184362)
Selkirk
Sillon d'Or Div. 4138 (P.I. 174692)
Sind 174 (P.I. 57987) (C.I. 7300)
Sonora 37
Strubes Fruh (P.I. 191306)
Suwon 92 (Washington)
Suwon 92 X 4 Omar (656, Wa 4962) (Washington)
Suwon 92 X Omar (C.I. 13749)
Suwon X² Omar (Oregon)

Tadorna
Tehuacan-Pitic X Barrigon Yaqui 2 X² Tehuacan,
D-14571-3R-12T-2R-1R

Topper
Triticum spelta album
Triticum spelta' a saharensense
Triticum timopheevi var. nigrum Div. 7747 (P.I. 282933)
Turkey Selection (C.I. 10100)

Union

Vilmorin 23 (P.I. 125092), Div 2424, (C.I. 11773)
Viloria (P.I. 191229)
Virman (Italy)

Table II. (cont'd).

Wegiarska 2/11 P.I. 129531

Includes varieties of the European Yellow Rust Trial, excludes wheat tetraploids (Table XIII) and Montana barley varieties (Table XIV).

striiformis. In determining the specificity or nonspecificity of the minor genes, the host lines were inoculated with five monospore rust isolates (No. 3, 4, 12, 13, 14) and six nonmonospore isolates (No. 1, 9, 16, 19, 20, 21 Table I). These were known to possess different genes for virulence. Plants were tested as seedlings under earlier described procedures and environmental conditions. Disease reaction at both temperature profiles was observed.

VI. Heterokaryosis and parasexual recombination.

Due to the absence of the pycnial and aecial stages in P. striiformis, somatic recombination and/or mutation must account for the production of new races of stripe rust presently known. To study these mechanisms, urediospores representing three monospore isolates which differed in genes for virulence were separately placed on Lemhi seedlings and each was increased in association with an isolate containing a mutant marker gene for color. The B-W (a) mutant isolate (Table I) though devoid of yellow pigments, was pathogenic and tests indicated it contained genes for virulence that differed from the three yellow isolates BF-Mo, Cvl-C166 and KH-Ar (Table I). By allowing association and increase of the paired isolates on a susceptible host, attempts were made to induce nuclear reassortment (heterokaryosis) through anastomosis of germ tubes or by hyphal fusion. Verifi-

cation of this occurrence was tested by evaluating the isolates along with the two parent races, under controlled environment at two temperature profiles, on selected differential hosts.

To establish that anastomosis could occur, urediospores of different races were dusted on 0.25% Ion Agar No. 2 (Code No. L 12 Colab, Inc., Chicago, Ill.). After 8 hours at 10 C under 100% R H the media surfaces were examined for the occurrence of germ tube fusions.

If heterokaryosis is to account for the production of new races of stripe rust, some cells would likely contain more than two nuclei of different genetic constitution. Also the presence of one large, diploid nucleus could indicate the parasexual cycle is involved. Numerous methods were used unsuccessfully for cytological study of urediospores and the determination of nuclear number. Success was finally achieved through modifying the technique prescribed by Little and Manners (1969). Since the urediospores would not germinate on Difco water agar, ion agar was used. The following schedule was found to be the most successful:

- (1) Allow the spores to germinate for 5-6 hr. on 0.25% ion agar on a microscope slide at 10 C.
- (2) Fix in half-strength Schaudin's fluid (100 ml sat. aq. solution mercuric chloride, 50 ml absolute alcohol,

150 ml distilled water). Add 2% glacial acetic acid (total volume basis).

- (3) Rinse carefully by several changes in distilled water and allow the slides to dry for 6-24 hours.
- (4) Rinse again in distilled water and mordant in freshly prepared 4% ferric ammonium sulfate (iron alum) for at least 4 hours.
- (5) Rinse in distilled water and stain in freshly prepared, half-oxidized, Heidenhain's haematoxylin (Baker and Jordan 1953).

Haematoxylin - 5 gm (Matheson, Coleman and Bell)

Sodium iodate - 0.5 gm

Distilled water - 950 cc

Alcohol (96%) - 50 cc

Staining is accomplished in 6-12 hours.

- (6) Rinse in distilled water.
- (7) Differentiate in 4% iron alum by inspection.

The length of time needed for differentiation is very critical - usually 3-4 minutes. Too long a period will cause the nuclei to destain and too short a period will cause insufficient differentiation.

- (8) Rinse in distilled water and dry. After clearing with Permout (Fisher) a cover slip may be applied.

Germ tube nuclei of several isolates were examined using this method. The effect of incubation time upon nuclear number was also studied.

VII. Aggressiveness of rust isolates.

The germination ability of stripe rust urediospores at certain cardinal temperatures becomes a measure of the aggressiveness of the isolate or race (Manners, 1950). Several isolates, some of which differed in genes for virulence, were allowed to germinate at 5, 10, 15 and 20 C. Spores were dusted on surfaces of 0.25% Ion Agar and placed at 100% RH. Duplicates of each isolate were incubated in the dark for 24 hours at each of the four temperatures. A total of 400 spores per slide were counted from which a germination percentage was calculated. Each isolate was evaluated daily over a period of six days.

RESULTS

I. Influence of temperature and inoculation date.

Environmental components affect host-pathogen relationships, and temperature is among the most important. Infection type readings were made 14 and 21 days after inoculations on the plants grown at 15/24 and 2/18 C, respectively. When seedlings grown at both temperature profiles were inoculated with a given isolate, infection types on identical varieties were often different. Results are shown in Tables III - VIII for a number of candidate differentials grown at two temperature profiles and tested with isolates B-I, BF-Mo, CC-I(00)Me, Cvl-C166, KH-Ar and SB-40-1. Other isolates were tested in like-manner, however, the results are not presented because those shown are representative. Varieties were tested at least twice under each temperature profile and to each isolate. When possible, tests comparing host reaction at the two different temperature profiles were conducted simultaneously. Throughout this study, care was exercised to keep all isolates representative and free of mixtures. Fresh spores were used wherever possible; but, in some cases, it was necessary to use lyophilized spores which had been collected on different dates.

All spores were increased under controlled environmental conditions. The infection type designated represents the average reaction shown on 10-15 primary leaves.

Table III. Host reaction at 2/18 C (L) and 15/24 C (H) to stripe rust isolate B-I.

Host.	L	H
Gentil Rooso	3-	2
H15 P.I. 83346	1	0
Vilmorin 23	3	3-
Chartres Desprez	3	1-
Harvest Queen X Kawvale	3	3-
Little Joss	3	1
Cappelle	1-	2
Hybride 40	3	3-
Suwon 92	0	1
Sonora 37	1-	2
G-78303	1	3
Equator	1	1-
Strubes Fruh	1	2
Nord Desprez	2	3
Acker Manns	3	2
Birgitta	3	1
Criewener 104	0	2
Moskowskaja	3	3-
Mendel	0	2

Table IV. Host reaction at 2/18 C (L) and 15/24 C (H) to stripe rust isolate BF-Mo.

Host	L	H
Crest	3	2
Nugaines	3	3-
Langdon	4	3-
<u>Triticum pyramidale</u> No. 113964	0	2
Heines VII	3	2
Alba	1-	3
Viloria	3-	2
Hybride 46	3	1

Table V. Host reaction at 2/18 C (L) and 15/24 C (H) to stripe rust isolate CC-I(00)Me.

Host	L	H
Viloria	1	2
Professeur Journee	1	3
Bersee	0	3-
H15 P.I. 83346	2	0
R.I.C. P.I. 83388	3	3-
K17 P.I. 83512	3-	1
Vilmorin 23	0	3-
Harvest Queen X Kawvale	3	2
Heines VII X ⁶ Redmond	3	2
Cappelle	0-	2
Druchamp	0-	2
Hybride 46	2	1
Heines VII	3-	2

Table VI. Host reaction at 2/18 C (L) and 15/24 C (H) to stripe rust isolate Cvl-C166.

Host	L	H
Diosecka 777	3	1
Hatvani 5613	3	1
Strubes Fruh	3-	1
Acker Manns	3-	2
Ankar	3-	1
Birgitta	3	1
Mendel	3	2
Heines VII	2	1-
Druchamp	2	0
F25	2	1

Table VII. Host reaction at 2/18 C (L) and 15/24 C (H) to stripe rust isolate KH-Ar.

Host	L	H
Gentil Rooso	3	2
Cappelle	0	1
Chartres Desprez	3	1-
G-78303	1	3
Hansa	3	3-
H15 P.I. 83346	0	2
Hector	1	3
Heines VII X ⁶ Redmond	3	2
Little Joss	4	3-
Navarro	4	3-
Professeur Journee	2	3
R.I.C. P.I. 83388	3	2
Poncheau	2	0
Epidor	0	2
Alba	3-	1

Table VIII. Host reaction at 2/18 C (L) and 15/24 C (H) to stripe rust isolate SB-40-1.

Host	L	H
Lakota	1	3-
Viloria	2	1-
Virman	1	3
President Riverain	2	0
Roma	2	1-
Bersee	1	0
Hybride 46	1	0
Sonora 37	2	1-
<u>T. pyramidale</u> No. 113964	2	1-
Leeds	1	0
Druchamp	2	0
Alba	1-	0-
Lee	1	0
Heines VII	3	1
Scandia	3-	1-

The plants grown at 15/24 generally were more resistant than those grown at 2/18. This was noted especially with tests using isolate Cv1-C166. Varieties listed in Table VI all showed higher levels of resistance at 15/24 than at 2/18. In some tests, hosts were more susceptible at 15/24 such as Crie Werner 104 and Mendel in Table III, Alba in Table IV and Bersee, Vilmorin 23, Cappelle and Druchamp in Table V. Such results indicate that host-pathogen responses are influenced by temperature and emphasize that comparative studies in stripe rust must all be conducted under identical temperature profiles.

The date of inoculation was shown to be associated with varietal response to rust infection. Wide ranges of reaction within varieties gave evidence of the unstable nature of many of the hosts. Tables IX, X, XI indicate reaction changes at different dates of inoculation, in studies conducted at the 2/18 temperature. Data from tests of only three isolates B-I, Cv1-C166 and KH-Ar are shown and was representative of other isolates evaluated in a similar manner. Varieties exhibiting instability with one isolate often were unstable when tested to other isolates. The range of variability was noticeably demonstrated by Navarro, Virman, Roma and Vilmorin 23 in Table IX, by Vilmorin 23, Allies and Scandia in Table X and by Bersee, Vilorina and Bretagne Desprez in Table XI and precluded these varieties as important differentials. Host re-

Table IX. Variability of host reaction associated with inoculation date.^{a/}

Host	Mo.	9	10	11	7	9	10	11	1	4	3
	Day	5	12	17	19	3	30	21	15	8	4
	Year	67	67	67	68	68	68	68	69	69	70
Hector		2	2			1-					
Hohenheimer		2		3							
Navarro		2	0		3	1					
Professeur Journee		1-		3-							
Virman		1-	0-		0	0		2	0-	1-	2
Allies			3						1	0	
Point Cailloux				3			1-				
Roma				0-	2	0	0	1-	2	0	1-
Vilmorin 23			3		3	3	3-	1-	1	0-	3-

^{a/} Testing was conducted at 2/18 C with isolate B-I.

Table X. Variability of host reaction associated with inoculation date.^{b/}

Host	Mo.	11	1	4	12	1	4	5	6
	Day	25	15	3	2	28	8	18	10
	Year	68	69	69	69	70	70	70	70
Allies		3	1	3-					
Roma		1-	1-	3-		3-			
Sonora 37		1	1	0					
Vilmorin 23		00	0-	0-		1-		2	3-
Viloria		3-	2	1				2	3-
Virman		2	1	1-		3	3		3-
Scandia					00	2			

^{b/} Testing was conducted at 2/18 C with isolate Cv1-C166.

Table XI. Variability of host reaction associated with inoculation date.^{a/}

Host	Mo.	5	7	9	11	1	4	3
	Day	3	22	3	25	15	4	6
	Year	68	68	68	68	69	69	70
Allies		3			3-	2	1-	
Bersee		2		1-			0-	
Bretagne Desprez		1	1	3		1	1	
G-78303		0	1	1				
Hector		1-	2	1				
President Riverain		3		3	2	2	1	3-
Professeur Journee		3-	1					
Roma		3		3-	1-	1	0	3-
Viloria		3		3	1	1	0	
Virman		3-		1-	2	2	1-	3

^{a/} Testing was conducted at 2/18 C with isolate KH-Ar.

Table XII. Variability of host reaction associated with inoculation date.^{b/}

Host	Mo.	8	9	10	6
	Day	31	25	23	12
	Year	67	67	67	68
Hector		0		3	
Hohenheimer		1			3
Navarro		2			3
Professeur Journee		1		3-	
Viloria		1		3	1
Virman		1		3	
Allies			1-	3-	3-
Heines VII X ⁶ Redmond			1-		3
Bretagne Desprez			0	2	3
Point Cailloux			0-	1	3
Roma				2	0-

^{b/} Testing was conducted at 15/24 C with isolate B-I.

action also varied according to inoculation date in tests conducted at 15/24. Representative results are presented in Table XII for varieties tested with B-I, isolate. The inability of many varieties to produce consistent reactions to stripe rust when inoculated with a homogeneous spore source at different dates makes them undesirable as differentials.

Also undesirable as differentials were wheat cultivars which were resistant (infection types 00, 0⁻ or 0) or completely susceptible (infection types 3 or 4) to all rust isolates which were available (Tables XIII and XIV). Testing was conducted at both 2/18 and 15/24 C.

II. Tetraploid wheat and barley selections.

The observation of Singh and Swaminathan (1959) that genes for resistance to the rust diseases occur predominantly in the A and B genomes prompted the testing of tetraploid ($2n=28$) wheats. Eighteen tetraploid and two hexaploid selections from the Montana State University Experiment Station were inoculated with rust isolates B-I, BF-H and Cv1-C166 (Table XV). The tests were conducted simultaneously on three sets of seedlings at 2/18. On the basis of this test, a few of the selections gave evidence of being promising differentials. All were intermediate to susceptible to B-I isolate while a few were resistant to isolates BF-H and Cv1-C166. Repeated testing at different dates and temperatures to

Table XIII. Hosts with major factors conditioning resistance to stripe rust isolates tested from 1967 through 1970.^{a/}

Argentina selection 7688	Suwon X ² Omar
Riebesel	Suwon 92 X ⁴ Omar
Rouge Prolific Barbu	Marival
Juanita	Mexico selection - Pitic 62
Isobamba	Chinese Spring Addition
Heines Koga	Hatif Inversable
Kirik 228-1	Purple Stem
Polonium	Heines Kolben
Epidor	

a/ Varieties were tested at 2/18 C and 15/24 C and exhibited infection type ≤ 0 to all isolates.

Table XIV. Hosts without detectable factors conditioning resistance to stripe rust isolates tested from 1967 through 1970.^{b/}

Austral	Lemhi
Birgitta	P.I. 273992
C.I. 13645	Omar X Rensimieto X Omar
Hybride 40	Portugal 90
I.D. 0001	Tehuacan
Idaho 53-57	Wegierska

b/ Varieties were tested at 2/18 and 15/24 C and exhibited infection type ≥ 3 to all isolates.

Table XV. Tetraploid wheat selections tested with isolates of stripe rust at 15/24 C.

Selection	P.I. or C.I. No.	Isolate		
		B-I	BF-H	Cvl-C166
<u>Triticum polonicum</u>	185309	3	3-	3-
	225334	2	0:1	00
	125356	3	3	3
<u>Triticum turanicum</u>	-----	3	3-	3
	254196	3	3-	3
	254212	3	3-	4
	68287	2	3-	3-
<u>Triticum pyramidale</u>	113951	3	00	00
	113964	3-	0:1	00:2
	117423	3	0:2	0
	115812	3	3-	3
<u>Triticum turgidum</u>	-----	3	3-	4
<u>Triticum persicum</u>	94757	3	3-	3
	94753	1	0	3-
	78813	3	3-	2
<u>Triticum sphaerococcum</u> ^{a/}	40941	0:2	2	2
Purple Ethiopian	7867	3-	0-	1
	7847	3-	1	3
	7849	3	3	3-
Selection 5815 ^{a/}	5815	3	1	1-

^{a/} Hexaploid selections.

these and other isolates indicated that the selections were highly influenced by extrinsic factors and hence were unreliable as differentials.

Barley cultivars have been used successfully in Europe to differentially determine stripe rust races. Presently, six varieties of barley, Bigo, Topper, Cambrinus, Emir, Union and C.I. 1237 are used in the European Yellow Rust Trial. Barley is an important cereal in Montana and the northwest region and its resistance or immunity to stripe rust under field conditions has been encouraging. A collection of 17 barley varieties were tested to different isolates of stripe rust under controlled environments. Cultivars were tested at 2/18 to three stripe rust isolates, BF-Mo, Cvl-C166, and B-I, and at 15/24 to isolate B-I. Results indicated only two selections, Hypana and Traill, possessed discriminative abilities (Table XVI). Additional tests using the two varieties proved that these were unreliable as differentials. All the barley varieties were very hypersensitive at both temperatures and were consequently disregarded as differentials.

Table XVI. Reaction of barley varieties to three isolates of stripe rust at 2/18 C.

Host Variety	Isolates and mean infection types			
	BF-Mo	Cv1-C166	B-I	B-I ^a
Betzes	0-	0	0-	00
Compana	0	0	0	1
Conquest	0	0	1-	0
Decap	0	0	0	0-
Firlbecks III	0	0	0	00
Freja	0	0	1-	1-
Horsford	-	0	1-	0
Hypana	0	1-	1-	2
Ingrid	0-	0	0	0-
Manchuria	1:2	1	3	3
Piroline	0-	0	-	0-
Prior	0:1-	1-	1	1
Proctor	0	0	00	00
Titan	0	0	1	0-
Traill	1-	0	2	1-
Unitan	0	-	0	0
Vantage	0	0	0	-

^a/ Evaluated at 15/24 C.

III. Results of the European Yellow Rust Trial.

In cooperation with European stripe rust researchers, varieties of the European Yellow Rust Trial were planted at Bozeman, Montana in 1969 and 1970. The field plot was inoculated in the spring with the stripe rust isolate endemic to the Bozeman valley. Readings were taken during the time the grain was heading, or stage 10.5 according to the Feekes growth scale for estimating the stages of development of wheat (Large, 1954). Infection types and severity are listed in Table XVII for winter and spring trial varieties. The low severities may have been due to the extremely low spring temperatures and the hot summers characteristic of 1969 and 1970. Infection type differences between years for certain varieties were noted. This was probably due to environmental influences being different from year to year. It is unlikely that seed lots were altered. Since the trial was inoculated with rust spores collected from the field, it is possible that different races could account for the differences exhibited.

To broaden the search for useable stripe rust differentials, varieties of the 1969 European Yellow Rust Trial were tested at 2/18 to isolates B-I, BF-Mo, CC-I(00)Me, Cvl-C166, KH-Ar, Mo-B, and Msl-We. The entries tested are listed in Table XVIII. Most varieties were unable to discriminate between the isolates,

Table XVII. Field reaction of varieties of the European Yellow Rust Trial to stripe rust at Bozeman, Montana 1969 and 1970.

Wheat Variety	1969		1970	
	Infection Type	Severity ^{a/}	Infection Type	Severity ^{a/}
<u>Winter habit</u>				
Felix	0	0	0	0
Cappelle Desprez	3	10	2	5
Bon Fermier	0:1	tr	2	10
P.I. 178383	0:1	tr	0	0
Cleo	0	0		
Falco	0:1	tr		
Chinese 166	0	0	0	0
Michigan Amber	4	80	3	80
Tadorna	0	0	0	0
Mildress	0	0	0:1	tr
Heines VII	0:1	tr	2	10
Rubis	3	80	3	70
Dippes Triumph	0	0	2	5
Manella	0:1	tr	3	5
Leda	0	0		
Harvest Queen	3:4	50	3	80
Alba	0	0		
Flamingo	0	0	0	tr
Flevina	0	0	0	tr
Persian	3:4	50	3	50
Carstens V	0	tr	2	5
Lucas	4	40	3	80
Nudif TP3			0	0
<u>Triticum spelta album</u>			0	0
Ble rouge d' Ecosse			2	20
Riebesel 47-3			0	0
Hybride 46			0	0
Strubes Dickkopf			-	-
Nudif TP12			0	0

^{a/} Severity was recorded as per cent of infection according to the modified Cobb scale. Below 5% severity, trace (tr) was used.

Table XVII. (cont'd)

Wheat Variety	1969		1970	
	Infection Type	Severity ^{a/}	Infection Type	Severity ^{a/}
<u>Spring habit</u>				
Heines Kolben	0:1	1	0	tr
Opal	0	0	0	tr
Little Club	4	60	3	30
Peko	0	tr	0	tr
Hope X Timstein	0	tr	1	1
Selkirk	0	0	0	tr
Redman	2	20	1	tr
Orca	0	0	0	0
Bigo	0	0	0	0
Union	0	0	0	0
Emir	0	0	0	0
Cambrinus	0	0	0	0
Topper	2	tr	0	0
C.I. 1237	0	0	3	20
Compair			0	tr
Nudif TP241			0	tr
Nudif TP250			0	0
Inia 66			2	10
<u>Triticum spelta</u>				
<u>a' saharensis</u>			3	70
Noresie 66			1	tr
Nudif TP257			1	tr

^{a/} Severity was recorded as per cent of infection according to the modified Cobb Scale. Below 5% severity, trace (tr) was used.

Table XVIII. Reaction of some 1969 entries of the European Yellow Rust Trial to isolates of stripe rust at 2/18 C. ^{a/}

Wheat Variety	Isolate						
	B-I	BF- Mo	CC-I (00)Me	Cvl- Cl66	KH- Ar	Mo- B	Msl- We
Felix	2	0	2	1-	00	i	2
Cleo	0	0	1	0-	0-	3-	2
Falco	1-	0	i	0	0-	i	2
Tadorna	0-	0-	0	1-	00	0-	1
Mildress	00	00	i	00	00	-	0
Dippes Triumph	3-	3	3	3-	1	3	3
Manella	1	0	0	0-	1-	2	1-
Flamingo	1	00	0-	1	1-	0-	1-
Flevina	1-	0	0-	0	0	1-	2
Carstens V	2	3-	3-	3	3-	3	3
Peko	1	0-	0-	0-	0	0	1-
Hope X Timstein	1	1-	0	1-	0	1	2
Selkirk	3-	2	1	3-	3-	2	2
Orca	00	00	00	0-	0	00	0-
Opal	1	1	2	1	1	2	3-
Cambrinus	00	00	i	1-	00	00	i
Topper	3	3-	3-	1	1-	3-	3-
Emir	i	00	00	00	00	00	00
Union	00	00	00	00	0-	00	00
C.I. 1237	i	i	i	0-	0-	00	i

^{a/} i=immune; no visible reaction.

however, attention was directed to six selections which appeared promising. Certain isolates were differentiated by Felix, Cleo, Falco, Manella, Flevina, and Hope X Timstein. Repeated testing at 2/18 indicated that infection types often varied quite notably with the date of inoculation. Such varieties were less desirable as differentials for that reason.

IV. Physiological race determination.

In 1969, an agreement was tentatively established which defined certain criteria which could be used by northwest stripe rust researchers in the study and reporting of stripe rust races. Cooperators selected seven wheat varieties comprising a numbered list of First Category differentials and eight wheat cultivars comprising an unnumbered list of Second Category differentials.^{a/} Hosts were selected which had exhibited satisfactory race discriminative abilities when used by the contributor in tests conducted prior to 1970.

First Category differentials:

1. Lemhi (P.I. or C.I. 11415)
2. Chinese 166 (P.I. or C.I. 11765)
3. Heines VII (P.I. or C.I. 201195)
4. More (P.I. or C.I. 13740)
5. Suwon 92/Omar (P.I. or C.I. 13749)
6. Druchamp (P.I. or C.I. 13723)
7. Riebesel 47-51 (P.I. or C.I. 295999)

^{a/} Cooperators were E.L. Sharp, Montana State University; R.F. Line, Washington State University; R.L. Powelson, Oregon State University.

Second Category differentials:

Golden (C.I. 11063)
President Riverain (P.I. 174687)
Heines Kolben
Medeah (C.I. 5140)
Alba (C.I. 13256)
Vilmorin 23 (P.I. 125092)
Lee
Turkey selection (P.I. 178383)

The main conditions discussed and reported by Line et al. (1970) were: (1) in reports and publications all stripe rust isolates will be tested on the First Category differentials; (2) the isolate description is based on the reaction of these varieties listing the number of those most resistant on the far left and most susceptible on the far right. A slash (/) is used to separate numbers that represent cultivars on which the race is avirulent from numbers that represent cultivars on which the race is virulent. Avirulence is established and represented by hosts having infection types equal to or less than 1. If there is more than a trace of sporulation, with or without chlorosis or necrosis, the pathogen is considered virulent; (3) infection types will be determined on the first, or on the second, or on both first and second leaves of seedlings; (4) all reported testing will be at a temperature profile of 2/18 C with a 12 hour photoperiod at not less than 1,000 ft-c and (5) Second Category differentials will be tested but not necessarily reported or be

a part of the isolate designation. The First and Second Category differentials were tested with 16 rust isolates (Tables XIX and XX). Due to a limited seed supply, some of the Second Category differentials were not tested to all isolates.

In the First Category differentials Lemhi and Riebesel were chosen representing universal susceptible and resistant hosts, respectively. No isolate studied thus far has been virulent on Riebesel. Chinese 166 was an important differential as its resistance was overcome only by isolates C-We(3)Cl66, Cv1-Cl66 and Pn-Cl66. Culture BF-Mo was the only isolate virulent on Moro. Heines VII was of only marginal use because its performance strongly suggested it had no specific gene or genes capable of differentiating the regional isolates. Tests with Druchamp indicated it probably has specific genes with modifying factors conditioning intermediate responses. Suwon 92/Omar was not important in this study because its resistance was not overcome by any of the isolates.

The isolate description was most definitive when the differentials making up the description were specifically arranged in the designation according to the degree of susceptibility (or resistance). Often, however, the order is based only on small differences between host reactions and is best used only for comparative purposes among cooperators. To separate races using

Table XIX. Reaction of tentatively selected northwest regional differential hosts to isolates of stripe rust at 2/18 C.

First Category Differentials

Isolate and Description	Chinese	Heines	Moro	Suwon	Dru-	Ri-	
	Lemhi	166		VII	92/Omar	champ	ebesel
	1	2	3	4	5	6	7
B-1	3	00	1	00	0	1-	00
2,4,7,5,6,3/1							
B-W(a)	3	00	1	00	0-	0	00
2,4,7,5,6,3/1							
BF-H	3	00	3	00	00	1-	00
2,4,5,7,6/3,1							
BF-Mo	4	00	3	3	0-	1	00
2,7,5,6/3,4,1							
C-We(00)C166	3	00	3-	00	0-	1	00
2,4,7,5,6/3,1							
C-We(3)C166	3	3	2	00	0-	2	00
4,7,5/3,6,2,1							
CC-I(00)Me	3	0-	3-	00	0-	0-	00
4,7,2,5,6/3,1							
CC-I(3)Me	3	0-	3-	00	0	3-	00
4,7,2,5/3,6,1							
Cv1-C166	4	3-	2	00	0-	2	00
4,7,5/3,6,2,1							
KH-Ar	4	00	3	00	00	3-	00
2,4,5,7/6,3,1							
Ln-72640	3	00	3-	00	00	1	00
2,4,5,7,6/3,1							
Mo-B	3	00	3-	00	0-	3	00
2,4,7,5/3,6,1							
Msl-We	3	00	3	00	0-	3-	00
2,4,7,5/6,3,1							
Pn-B	3	00	3	00	00	3-	00
2,4,5,7/6,3,1							
Pn-C166	3	3-	2	00	0-	2	00
4,7,5/3,6,2,1							
SB-40-1	3	00	3	00	0-	3-	00
2,4,7,5/6,3,1							

Table XX. Reaction of tentatively selected northwest regional differential hosts to isolates of stripe rust at 2/18 C.

Second Category Differentials

Isolate	Gölden	President Riverain	Heines Kolben	Me-deah	Alba	Vilmorin 23	Lee	P.I. 178383
B-I	3	1-	0	3	1-	3	2	00
B-W(a)	3	0	0	3	1-	2	2	00
BF-H		3	0	0-	0	0:3	1-	00
BF-Mo	4	3-	0-	0	1-	1-	1-	1
C-We(00)C166		2		1		1		00
C-We(3)C166		2		1		0:1		00
CC-I(00)Me		3	0	00	1-	0		00
CC-I(3)Me		2	0	3	1-	3		00
Cv1-C166		2	0-	1-	0	0:2	1-	00
KH-Ar		3-	0	1	3-	0:2	0	00
Ln-72640		1		1-		1:3-		00
Mo-B		2		2		0:2		00
Msl-We		3-	0-	3-	3-	3-	1	00
Pn-B		3-		2		1-:2		00
Pn-C166		2		1-		0-		00
SB-40-1		3-	0	1-	1-	0:3-	1	00

the regional First Category differentials, the number arrangement within avirulent/virulent categories was disregarded thereby distinguishing five races: (race 1) B-I and B-W(a); (race 2) BF-H, C-We(00)C166, CC-I(00)Me and Ln-72640; (race 3) BF-Mo; (race 4) C-We(3)C166, Cv1-C166 and Pn-C166; and (race 5) CC-I(3)Me, KH-Ar, Mo-B, Msl-We, Pn-B and SB-40-1.

Tests involving the Second Category differentials (Table XX) indicated President Riverain and Medeah had discriminatory specific genes. Only isolates B-I, B-W(a) and Ln-72640 were avirulent on President Riverain. Isolates B-I, B-W(a), CC-I(3)Me, Mo-B, Msl-We and Pn-B were virulent on Medeah. The major resistance gene of P.I. 178383 was overcome by the BF-Mo isolate, however, measurable resistance still remained. Limited testing at 2/18 with Golden, Heines Kolben, Alba and Lee precluded any judgments about their usefulness. In all tests with Lee, notable physiological hypersensitivity developed at both temperature profiles. At 15/24, Golden was susceptible or intermediate and Heines Kolben was resistant to all isolates. Vilmorin 23 contained modifying factors conditioning intermediate responses to nearly all isolates. Variability within the seed lot suggested a mixture of genotypes.

Since the selection of the seven First Category differentials in 1969 by regional stripe rust workers, the current

study revealed the usefulness of certain additional cultivars as differentials. On the basis of extensive evaluation, the following were used to selectively differentiate races of stripe rust throughout the northwest: Chinese 166, Druchamp, Leeds, Moro, Medeah, President Riverain, Marfed and Red River 68. With exception of Leeds, Marfed and Red River 68, the hosts represented the First or Second Category differentials of the selected regional group. The reaction of these eight hosts to 16 rust isolates are given in Table XXI. Average infection types are shown for tests at both 2/18 and 15/24. Varieties were tested to an isolate at 2/18 as many as nine different times during this study to verify its usefulness. Some varieties were not tested at 15/24. A number of the hosts had temperature sensitive factors conditioning infection type, especially Druchamp and President Riverain. Plants grown at the higher profile showed more physiological hypersensitivity which often was confused with the true rust infection type. To best utilize the discriminatory ability of the hosts the 2/18 temperature profile was best.

Chinese 166 and Moro are meritorious differentials for reasons earlier described. Using the avirulent/virulent criteria regionally established, at 2/18 Druchamp was resistant to seven isolates, Leeds to ten isolates, Medeah to ten isolates and President Riverain to only three. Only cultures Cv1-C166 and

Table XXI. Reaction of differential hosts to isolates of stripe rust at 2/18 and 15/24 C.

Isolate and Description ^{a/}	Chinese 166 1	Druchamp 2	Leeds 3	Moro 4	Medeah 5	President Riverain 6	Marfed 7	Red River 68 8
B-I 1,4,2,6,8/3,5,7	00/00	1-/1-	3/2	00/00	3/2	1-/0	3/	1-/
B-W(a) 1,4,2,6,8/3,5,7	00/00	0/0-	3/3-	00/00	3/2	0/0-	3/	1-/
BF-H 1,4,8,3,5,2/6,7	00/00	1-/0-	0-/0-	00/00	0-/0-	3/3-	3/	00/
BF-Mo 1,3,8,5,2/6,4,7	00/00	1/1-	0-/0	3/3-	0/1	3-/2	3/	0-/
C-We(00)C166 1,4,3,2,5/6,8,7	00/00	1/1-	1-/1	00/00	1/1	2/2	3/	3-/
C-We(3)C166 4,8,3,7,5/2,6,1	3/3-	2/1	0/0-	00/00	1/1	2/	1-/	0-/
CC-I(00)Me 4,5,1,2,3,8/6,7	0-/00	0-/0-	0/0-	00/00	00/1-	3/3-	3/	0/
CC-I(3)Me 4,1,8/6,2,3,7,5	0-/00	3-/	3-/	00/00	3/	2/	3-/	0-/
Cv1-C166 4,8,5,7/2,3,6,1	3/3-	2/	2/2	00/00	1-/1-	2/1-	1-/	0/
KH-Ar 1,4,3,5/2,6,7,8	00/00	3-/	1-/	00/00	1/	3-/2	3/	4/
Ln-72640 1,4,3,8,5,2,6/7	00/00	1/0	0/0	00/00	1-/1	1/0	3/	0/
Mo-B 1,4,8,/3,5,6,2,7	00/00	3/1	2/1	00/00	2/1	2/1	3/	0/
Msl-We 1,4,8/2,3,5,6,7	00/00	3-/	3-/	00/00	3-/	3-/	3/	1-/
Pn-B 1,4,8,3/5,2,6,7	00/00	3-/	1-/	00/00	2/	3-/	3/	0/
Pn-C166 4,3,5/2,6,8,1,7	3/3-	2/	0/0	00/00	1-/	2/	3/	3-/
SB-40-1 1,4,3,5/6,7,2,8	00/00	3/0	1-/0	00/00	1-/	3-/0	3-/	3/

^{a/} Isolate description based on infection types at 2/18 C.

C-We(3) Cl66 were avirulent on Marfed. Isolates C-We(00)Cl66, KH-Ar, Pn-Cl66 and SB-40-1 were able to overcome the resistance of Red River 68.

In Table XXII, isolates were compared only on the basis of resistance or susceptibility of the host. The isolate description allowing numbers to be specifically arranged within avirulent/virulent categories was not considered. Using this set of differentials, 11 different races were identified. Isolates B-I and B-W(a) possessed common virulence factors. Other isolates which had common virulence factors were: (1) BF-H and CC-I(00)Me, (2) CC-I(3)Me, Mo-B, Msl-We, (3) KH-Ar and Sb-40-1. All other isolates were different and made up the seven remaining races. Tentative race numbers were assigned according to the number of virulence genes expressed.

V. Itana X P.I. 178383 minor gene plant lines.

Earlier work with the minor gene lines indicated that the lines containing one, two and three minor genes conditioned low, moderate and high resistance, respectively, when inoculated with rust culture B-I (race 3). As isolates having different genes for virulence were discovered it was important to determine the reaction of these minor-gene lines to the diverse rust genotypes. It was found earlier in this study that the major genes conditioning resistance in P.I. 178383, Chinese 166, Moro and other

Table XXII. Reaction of differential hosts which separate isolates at 2/18 C.^{a/}

Isolate	Chinese 166	Druchamp	Leeds	Moro	Medeah	President Riverain	Marfed	Red River 68	Tentative Race Number
	1	2	3	4	5	6	7	8	
Ln-72640							S		1
BF-H						S	S		2
CC-I(00)Me						S	S		2
B-I			S		S		S		3
B-W(a)			S		S		S		3A
BF-Mo				S		S	S		4
C-We(00)C166						S	S	S	5
C-We(3)C166	S	S				S			6
KH-Ar		S				S	S	S	7
SB-40-1		S				S	S	S	7
Cv1-C166	S	S	S			S			8
Pn-B		S			S	S	S		9
CC-I(3)Me		S	S		S	S	S		10
Mo-B		S	S		S	S	S		10
Msl-We		S	S		S	S	S		10
Pn-C166	S	S				S	S	S	11

^{a/} "S" = susceptibility at infection type ≥ 2 .

No symbol = resistance at infection type ≤ 1 .

wheats were attacked by some specific isolates of the northwest region.

In determining the specificity or nonspecificity of the minor genes, the host lines were inoculated with 11 rust cultures known to possess different genes for virulence. The plant lines were described by letters and numbers assigned by the Montana Agricultural Experiment Station (Table XXIII). The letters identify Montana as the State of selection, the first three digits refer to the field number, the fourth digit indicates the number of major genes conditioning host-parasite compatibility and the fifth indicates the number of minor genes conditioning resistance. Seedlings of a given plant line, tested at 2/18 and 15/24 C were inoculated at the same date with all isolates.

All 11 isolates produced infection types on the minor gene lines similar to those for isolate B-I (Table XXIII). Culture BF-Mo was capable of overcoming the major gene from P.I. 178383, however, the three remaining minor genes sustained high resistance at 15/24 and moderate resistance at 2/18.

The minor gene lines have been evaluated in the field under local epidemic conditions for two years, and have shown similar infection types to those obtained on seedlings at 15/24. The line containing only one detectable minor gene may eventually develop infection type 3 in the field, but severity of infection

Table XXIII. Reaction of Itana X P.I. 178383 minor gene lines to stripe rust at 2/18 (L) and 15/24 (H).

Isolate	Minor gene line									
	P.T. 178383		MT. 00703		MT. 00902		MT. 01301		Itana	
	L	H	L	H	L	H	L	H	L	H
B-1	0-	00	0	0	1-	1-	1	1	4	3
BF-H	0-	00	0	0	0	0	1-	1-	4	3
BF-Mo	1	1-	1-	0	1-	1-	3-	2	4	3
CC-I	00	00	0	0	1-	1-	0	1-	4	3
Cv1-Cl66	0-	00	0	0-	0	0	1-	1	4	3
KH-Ar	0-	00	0-	1-	0-	0	1-	1-	4	3
Ln-72640	00	00	0-	1-	1-	0	0	1-	4	3
Mo-B	0-	00	0	00	0	0	0	1	4	3
Pn-B	0-	00	1-	0	0	0	1-	1-	4	3
Pn-Br	0-	00	1-	0	1	1	2	1-	4	3
Pn-Cl66	0-	00	0	1-	0	0	1-	1	4	3

increases at a much slower rate than for completely susceptible checks. Preliminary trials also indicate that the grain yield of this line is not noticeably reduced by stripe rust, while susceptible checks, selected from the same cross, were reduced more than 50 per cent in yield.

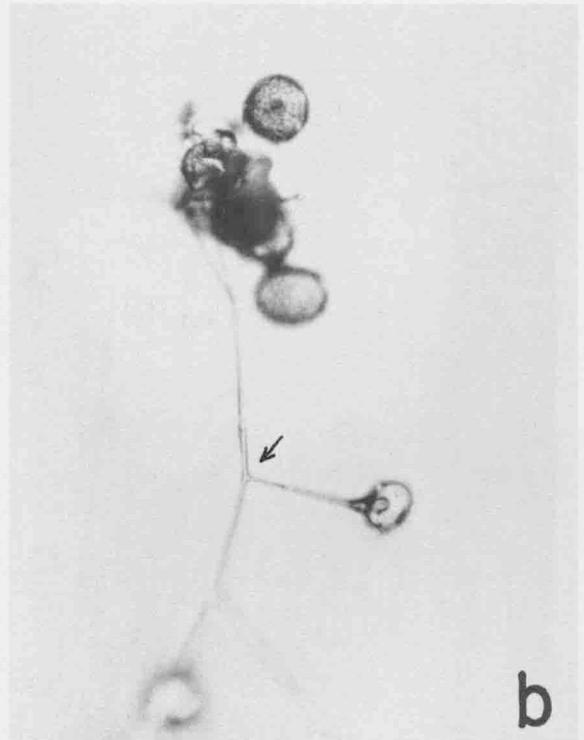
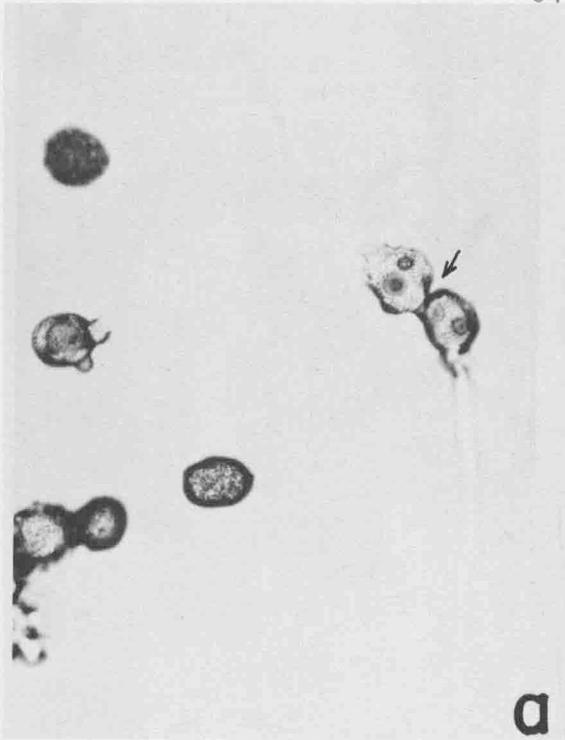
VI. Anastomosis, heterokaryosis and nuclear studies.

The fusion, or anastomosis, of genetically different hyphae or urediospore germ tubes allows nuclear reassortment in higher fungi. This is perhaps the most important asexual mechanism producing virulence changes in rusts. To induce anastomosis on artificial media spores of races 4 and 3A (albino) were mixed and dusted on 0.25% Ion Agar. After incubation the agar surface was examined for germ tube fusions. Cultures were distinguished visibly by the spore color.

Many tube pairs from separate spores were seen in contact; however, they were held together only by what appeared to be surface tension or cohesion. They were easily drawn apart at the juncture by slight evaporative forces applied over the media.

Infrequently, germ tubes were seen firmly in contact. Figure 3.(b and c) illustrates two events where germ tubes, originating from two different urediospores were fused. It could not be determined if the spores were from the different isolates because both spores and one germ tube were void of cell contents.

Figure 3. Anastomosis in races 3A and 4 between (a) nodular projections on spores. X200, (b) two germ tubes. X200 and (c) two germ tubes. X400. (d) Germ tube of race 8 showing one nucleus (stained with Heidenhain's haematoxylin). X200.



Other infrequent occurrences were observed where the cytoplasm of genetically different spores of races 3A and 4 was visibly transferred from one tube to another. Attempts to separate the joined tubes in Figure 3.c resulted in fracture some distance from the point of anastomosis.

Urediospores which produced germ tubes also frequently were seen with raised bubble-like projections from many of the germ pores around the spore. Periodically spores were seen tightly joined by these nodular points (Figure 3.a). It could not be verified if actual cytoplasmic and/or nuclear exchange occurred through these bridges. However, that the event could occur seems highly probable.

Since anastomosis was verified between germ tubes of two isolates on agar media, experiments were arranged to induce similar events using a host plant. Races 3A and 8 were mixed and increased on approximately 200 plants of the universal susceptible wheat variety Lemhi. Spores were collected and inoculated on differential varieties. The absence of changes in pathogenicity indicated the lack of an effective heterokaryon. The above races were mixed in two attempts to induce a detectable heterokaryon. Likewise, races 4 and 7 were each mixed with 3A and twice increased on Lemhi. Pathogenicity tests in each instance indicated the absence of a culture with altered virulence.

A combination of isolates BF-Mo (race 4) and B-W(a) (race 3A) in a third attempt produced a pathogenicity change on differential host Red River 68. Yellow pustules of type 3 formed on six leaves in combination with 00 and 0- infection types. On the basis of previous tests with the parental isolates only types 00 and 0- were expected on the differential. Inoculum from Red River 68 was applied again on eight differentials and type 3 to 4 yellow pustules developed on Druchamp, Moro, President Riverain, Marfed and Red River 68. Varieties Chinese 166, Leeds and Medeah exhibited types 00, 0, and 1, respectively. The ability of the assumed heterokaryon to overcome genes for resistance in the five mentioned differentials substantiates the occurrence of nuclear reassortment between isolates. The isolate designation, according to criteria established regionally, would become 1, 3, 5/2, 4, 6, 7, 8 - distinctly different than any listed in Table XXI. The derived heterokaryon maintained virulence on Marfed, a characteristic common to both parental cultures, (races 3A and 4), however, the acquired virulence on Druchamp, Moro and President Riverain was not a property common to both parents. Most interesting is the fact that Red River 68, previously resistant to both isolates used in the combination, became susceptible to the derived type.

The prospect that another isolate entered the mixture causing contamination was a factor which cannot be discounted. The entry of race 7 could cause pathogenicity changes identical to those noted. Extreme care was exercised to prevent unwanted mixtures, therefore, the probability of contamination, though not impossible, was regarded as unlikely. Time did not allow mono-spore isolation of the new race, but this method, associated with an assay of pathogenicity could be used to verify the presence of a heterokaryon.

The determination of nuclear number in stripe rust urediospores is useful as a supplement to other means of verifying the presence of a heterokaryon. After several unsuccessful attempts to differentially stain nuclei of ungerminated spores it was discovered that after germination, nuclei within the germ tube could be readily stained. Tests were designed to ascertain the optimal germination time giving accurate nuclear differentiation. Spores of race 8 were allowed to germinate at 10 Cx on agar for time intervals from 5 to 18 hr. The distribution of nuclear number as a function of germination time is shown in Table XXIV. Data for times less than 5 hr are not shown. At these short time intervals very few nuclei were visible.

Nuclei at the 6-hr interval were most clearly seen and varied in number from one to five per cell as evidenced by

Table XXIV. The number of nuclei within urediospore germ tubes after 5 to 18 hours incubation at 10 C.^{a/}

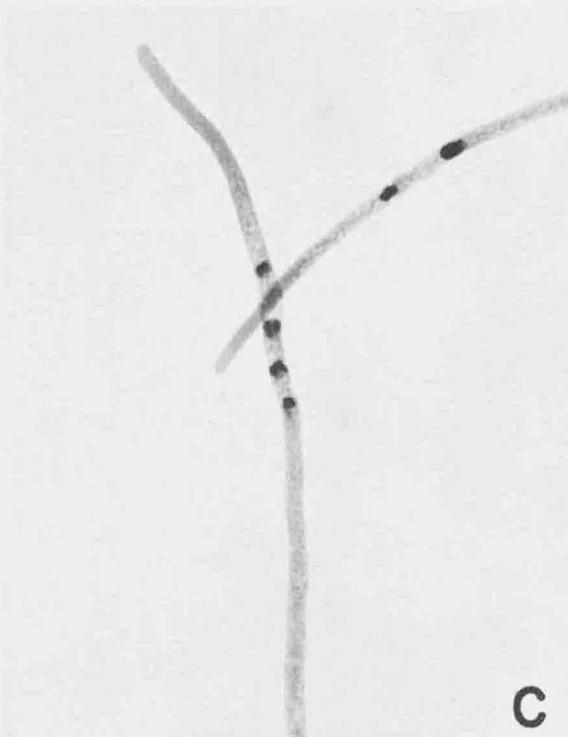
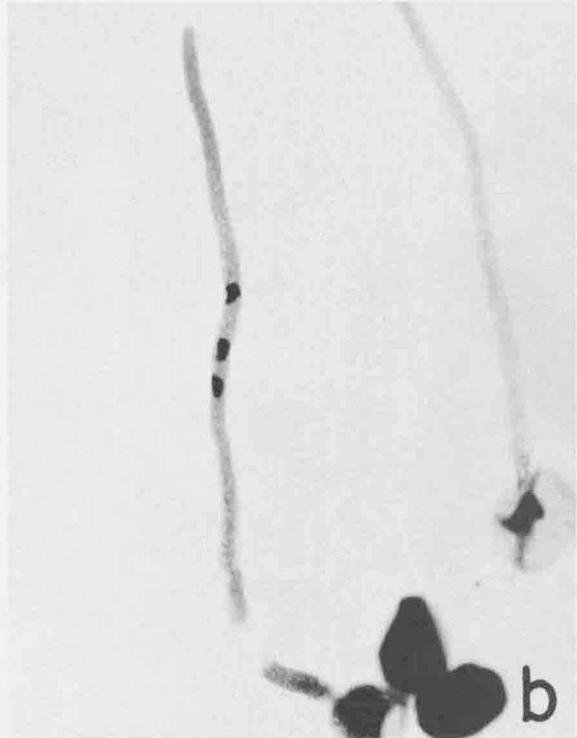
Incubation Time (Hr.)	No. of Germ Tubes Exhibiting Nuclei					Total
	1	2	3	4	5	
5	98	1018	7	0	0	1123
6	39	738	22	2	1	802
7	62	439	0	0	0	501
9	91	84	0	0	0	175
11	23	391	0	0	0	414
14	29	275	9	1	0	314
16	45	265	2	0	0	312
18	<u>5</u>	<u>88</u>	<u>4</u>	<u>0</u>	<u>0</u>	<u>97</u>
TOTAL	392	3298	44	3	1	3738

^{a/} Urediospores were germinated on ion agar. Nuclei were stained using Heidenhain's haematoxylin.

Figures 3.d and 4.a-d. The single nucleus in Figure 3.d was slightly longer than other paired nuclei and appeared to be bound by only one membrane. A number of these were seen at all time intervals. A majority of tubes studied contained two nuclei (Figure 4.a). Only a few had more than two (Figure 4.a-d and Table XXIV). At time intervals of 5 to 7 hr nuclei were associated and clearly visible. They were separated from the spore by a distance of approximately two-thirds the length of the tube and remained apart from the densely stained cell matrix in the tip (Figure 4.d). At incubation times exceeding 7 hr nuclear resolution became increasingly difficult. Frequently the nuclei were within the densely stained cytoplasm in the tube tip and could not be differentiated. Also the stain combined very poorly with nuclei at intervals over 7 hr.

A contingency chi-square analysis was used with actual score data (Table XXIV) to test the hypothesis that nuclear number was independent of germination time. A chi-square value of 439.58, based on 28 degrees of freedom indicates the probability that the observed deviations are due to chance is 1 per cent or less. The number of nuclei within a germ tube is dependent on germination time. Since the 9-hr and 6-hr times contributed excessively to significance, the hypothesis was tested again, omitting the data from these intervals. A chi-square value of 83.72

Figure 4. Germ tubes of race 8 showing (a) two nuclei, (b) three nuclei, (c) four nuclei and (d) five nuclei. Stained with Heidenhain's haematoxylin, X200.



indicated significant departures from expectation at the 1% level for 20 degrees of freedom. When only the 9-hr data were omitted, the chi-square value was 101.57 and was significant at the 1% level for 24 degrees of freedom. Using this statistical test it was determined that the number of nuclei within urediospore germ tubes was very highly influenced by incubation or germination time.

Differences in aggressiveness between isolates of the pacific northwest region was shown by testing the germination abilities of 13 isolates at four temperatures. Germination was in total darkness, under 100% R H and temperatures were controlled within 3 C. Races investigated included:

1. (Ln-72640)
2. (BF-H)
2. (CC-I(00)Me)
3. (B-I)
- 3A. (B-W(a))
4. (BF-Mo)
5. (C-We(00)C166)
6. (C-We(3)C166)
8. (Cv1-C166)
9. (Pn-B)
10. (Mo-B)
10. (Msl-We)
11. (Pn-C166)

Daily germination tests were conducted at each temperature for each isolate over a six-day period and averaged in Figure 5. The highest and lowest percentage contributing to each temperature average is delimited by the vertical lines. There were

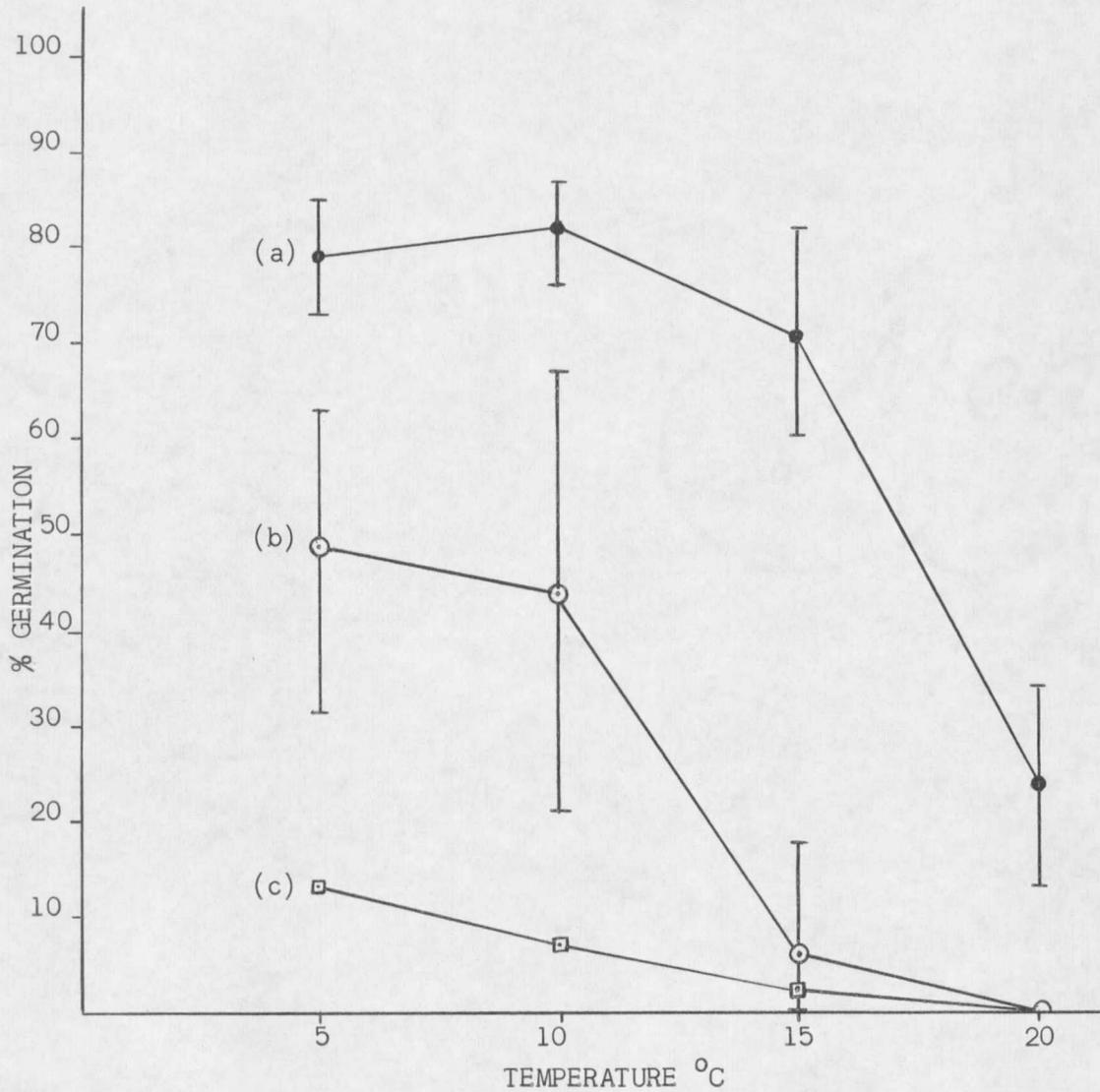


Figure 5. Germination ability of urediospores representing different stripe rust isolates at four temperatures: (a) races 6 and 8, (b) races 1,2(BF-H), 2(CC-I(00)Me), 3,4,5,9,10(Mo-B),10 (Msl-We),11 and (c) 3A. Vertical lines indicate the range of germination percentage associated with each mean.

two races, 6 and 8, which demonstrated the ability to germinate at 13% and 34%, respectively, at 20 C. They also germinated significantly higher than the others at 15, 10 and 5 C (Figure 5-a). Their virulence on Chinese 166 was a characteristic common to both. All other isolates germinated at less than 0.8% at 20 C.

The germination percentages for ten isolates were averaged giving the intermediate line-b on Figure 5.

The albino isolate (race 3A) was least aggressive over the whole temperature spectrum (Figure 5-c).

DISCUSSION

Since the discovery by Flor (1956) of complementary genetic systems in the flax-Melampsora lini interaction, the mode of action of other host-pathogen complexes is often speculated to follow a gene-for-gene relationship. The infection types observed in the present study were undoubtedly due to interactions between host and pathogen, but since the fungus cannot be crossed it was not possible to establish a corresponding gene-for-gene system between them.

Lewellen, Sharp and Hehn (1967) found two types of resistance factors which conditioned disease reaction by studying selection lines of winter wheat varieties Itana X P.I. 178383. A single, major gene and a number of minor, additive genes were determined. In the current study, the high resistance in other varieties with a major gene was not successfully overcome until it was challenged by rust collections of the northwest region. It was found that the single, major gene of P.I. 178383, also present in Moro, was attacked by an isolate endemic to northern Idaho. Similarly, a major gene in Chinese 166 was overcome by cultures obtained outside the Bozeman area. That these two host genes were different was verified by Lewellen, Sharp and Hehn (1967). Rust races were specific in that the one compatible with Moro was incompatible with Chinese 166. The inverse relationship was also true. Therefore, we are able to propose with a fair measure of assurance that stripe rust does contain genes for virulence (or avirulence) which complement inactive (or active) genes in the host.

The interaction of such genes results in an infection type that we may observe.

In contrast, the minor host genes studied by Lewellen, et al. (1967) appeared to be nonspecific in their mode of action toward different pathogen genotypes. They were capable of conditioning useable levels of resistance in additive combinations and were quantitative in their behavior. A high level of resistance was obtained in plant lines with three minor genes; intermediate infection types resulted when only one detectable minor gene was present. Such reactions were greatly influenced by environment, especially temperature.

In this study the sustained resistance of minor gene plant selection lines of P.I. 178383 X Itana indicated that minor genes, not necessarily restricted to P.I. 178383 or Itana, may be used to a great advantage in a plant breeding program to produce selections with resistance to stripe rust. Such additive factors are best incorporated without the accompanying presence of major, specific-resistance genes that are dominant or epistatic. Resistance may be followed quantitatively. Ultimately, the varieties produced should have long-lasting resistance irregardless of whether they are non-specific or not. If some minor genes are subsequently found to be specific, varietal resistance would erode much less rapidly because of the many complementary genes in the parasite which would need to mutate to virulence. Any alteration in pathogenicity would be difficult to

detect because of these small, step-wise changes.

The performance of a majority of cultivars tested throughout this study indicated many of them possessed only minor genes. Compatibility between host and parasite was measured by infection types of the intermediate category and was extremely subject to environmental influences. In many cases, specific major genes capable of discriminating rust isolates appeared to be absent.

In addition, a number of hosts appeared to possess major genes for race specific determinations, but also had minor genes conditioning resistance. The result was a moderately resistant to moderately susceptible reaction depending on the number of genes involved. The rust pathogen, capable of overcoming the major gene or genes, was unable to overcome the resistance conditioned by the minor gene. The reaction was also variable depending upon environment.

For clear, discrete physiological race determination a differential should be free of minor genes. The development of plant lines with race specific major genes in a common genetic background would provide best results for race determination and would make it possible to follow virulence changes in the rust population. Plant breeders could also use combinations of the major genes which would confer multigenic resistance to agronomically acceptable varieties.

Nearly 200 varieties were tested in the present study and eight were selected for use as differentials. Even some of the eight

indicated the presence of minor genes, but these genes were relatively ineffective at the 2/18 C profile, therefore, the specificity of the major gene could be determined and used in race identification.

The importance of using a common temperature profile for the determination of stripe rust races cannot be over-emphasized. The reduced effect, in most cases, of the minor genes at 2/18 C makes it the most desirable profile. Furthermore, other environmental measurements, such as light intensity and light periodicity as well as relative humidity, should be closely duplicated between locations of study.

It is difficult to assess the precise effect of extrinsic atmospheric factors upon complex host-pathogen interactions. In Germany, Schröder (1964) found that the germination potential of fresh urediospores of several stripe rust races was associated with atmospheric pressure. Sharp (1967) indicated that high concentrations of large air ions, were related to air pollution, and resulted in reduced spore germination. Experiments were not specifically designed in this study to measure such effects; however, germination counts were observed to be extremely low on certain dates. Periods of poorest germination and prolonged incubation times were observed in this study during winter months and especially under conditions of temperature inversions. Limitations arise in the control of such ubiquitous entities; yet it is possible that they influence host-

parasite relations (Sharp, 1967).

The ability of Puccinia striiformis to change to new physiologic forms is well known. The observation by Little and Manners (1969) of fusion between spore germ tubes of different races indicated that heterokaryosis is a factor in causing virulence changes in stripe rust. Without the aid of sexual processes genetic alterations may be brought about in this manner. Experiments were designed in this study to induce heterokaryosis using isolates which differed in genes for color and pathogenicity. Assuming a gene-for-gene relationship between host and pathogen, it was possible to select isolates in which nuclear reassortment would most likely be detected. Isolate pairs which attacked the least number of common differentials were chosen because they should give the highest frequency of detectable heterokaryons. The albino race 3A was used as a color marker in these studies. Since other cereal rusts investigated are heterothallic, the hypothesis that P. striiformis is heterothallic was adopted. A viable race must then have a "+" and a "-" nucleus to be viable (Figure 6).

A genetic model may be set up for three independently segregating virulence factors and a factor for spore color. In Table XXV, avirulence and yellow spore color is dominant. Capital and lower case letters C, M and R are pathogen genes conditioning infection on Chinese 166, Moro and Red River 68. The yellow color allele is designated by the letter Y, and the white (recessive) characteristic is

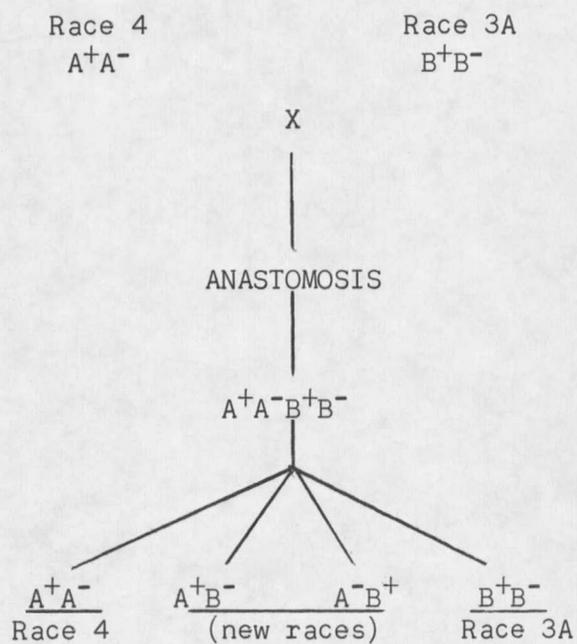


Figure 6. The occurrence of new biotypes from races 4 (BF-Mo) and 3A (B-W(a)) of stripe rust by nuclear re-assortment.

shown by y.

Table XXV. Distribution of factors for virulence in the nuclei of races 3A (B-W(a)) and 4 (BF-Mo) assuming virulence and yellow spore color to be dominant.

Race	Nucleus			
	A ⁺	A ⁻	B ⁺	B ⁻
4(BF-Mo)	cmRy	CmRY	--	--
Possible heterokaryotic dikaryons:				
(aa)	cmRy	--	--	cMRy
(bb)	--	CmRY	Cmry	--
3A(B-W(a))	--	--	Cmry	cMRy

The model may be explained as follows: Changes in virulence or color would not be observed if either parent culture were homozygous dominant at complementary loci. Heterokaryon (aa) in Table XXV was not observed but would have been virulent on Chinese 166 and would have produced white uredia offering definite proof of nuclear exchange. This would also have indicated that the allele for yellow color in race 4 was heterozygous. The observed heterokaryon (bb) could be verified by its pathogenicity change in association with Red River 68. The R allele was heterozygous in both parent cultures. The absence of white uredia on Red River 68 does not indicate that the allele for color in race 4 was heterozygous as shown. Virulence on Moro was common to both heterokaryon (bb) and parental race 4 (BF-Mo).

Only three virulence factors were chosen for illustration. Factors following similar patterns could be used for the other five differentials. It is also appropriate to consider that all heterokaryons are not necessarily dikaryotic. A spore may have more than two nuclei and function quite satisfactorily. During several uredial generations virulence factors dissociate as the pathogen stabilizes toward the dikaryophase (Nelson, et al. 1955).

To substantiate the existence of a heterokaryon, it would be necessary to produce single spore isolations from Red River 68 to be tested on other varieties, including the differentials. Host reactions using the monospore culture could be compared to those of the original parent cultures. Time did not allow this procedure in the present study and research is being continued in this area.

A study of the nuclear condition of urediospores was conducted as a technique to supplement the determination of heterokaryons by pathogenicity. The occurrence of three or more nuclei at significantly higher frequencies than in parent cultures could verify nuclear reassortment. Important for consideration is that nuclear number stabilizes showing a more frequent dikaryotic ($n+n$) condition with each succeeding generation. Also the frequency of nuclear number may very likely be associated with normal mitotic events within a growing germ tube. The importance of assaying nuclear number early in the germination phase was shown. At later times mitotic events are

probably more frequent. Also as germination time increased, the nuclei became increasingly difficult to differentiate.

More heterokaryons were not detected possibly because of the small number of hosts used for the assay of pathogenicity. Completion of the parasexual cycle in mitotic recombination would allow many new strains to be produced (Tinline and MacNeill, 1969). Certainly the inability to detect heterokaryotic dissociates or parasexual recombinants does not disprove their presence or importance in nature.

The possible involvement of incompatibility factors has not yet been mentioned; however, it certainly may be a factor causing certain heterokaryons to be inviable or even prevent anastomosis between certain isolates (Nelson, et al. 1955).

SUMMARY

1. Collections of stripe rust Puccinia striiformis were made throughout the northwest region of the United States from a variety of hosts.
2. Candidate host differentials were inoculated with monospore rust cultures and observed under controlled environments.
3. The host candidates were assayed, using two temperature profiles 2/18 and 15/24 C (night/day), for their ability to discriminate physiological races. Most varieties possessed minor genes which conditioned a higher level of resistance at 15/24 than at 2/18.
4. Eight differential varieties of wheat were selected and at 2/18 C. were able to differentiate the collected isolates into 11 races.
5. Plant selection lines with minor genes conditioning resistance to stripe rust were tested using the 11 races. Sustained resistance to all the races indicated that minor genes may be nonspecific and could be a favorable source of long-lasting field resistance.
6. Races differing in genes for virulence were mixed to encourage nuclear reassortment and a heterokaryon with changed pathogenicity.
7. Staining of nuclei within germ tubes revealed that the number of nuclei varied significantly with germination times of 5 to 18 hours.
8. The aggressive ability of rust races was measured by allowing spore germination at temperatures of 5, 10, 15 and 20 C. Races 6 and 8 germinated better at all temperatures. They were superior

to all races in that they were able to produce germ tubes at 20 C;
whereas, the other races were inhibited at that temperature.

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