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/l8 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.)

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ABSTRACT

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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 inflorescense 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 northwest 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 northwest 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 northwest 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. tricoccum, 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 Omar^3.

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. Accented by environmentally sensitive alleles conditioning resistance in the differential varieties, data comparisons under differing environments 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.

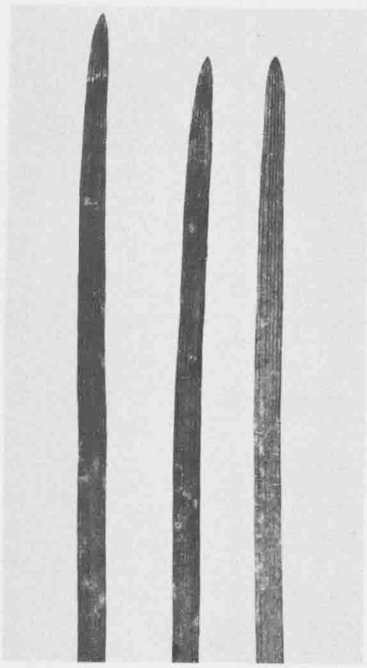
MATERIALS AND METHODS

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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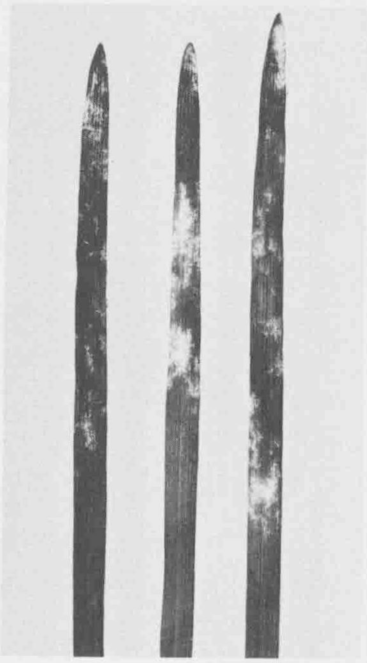

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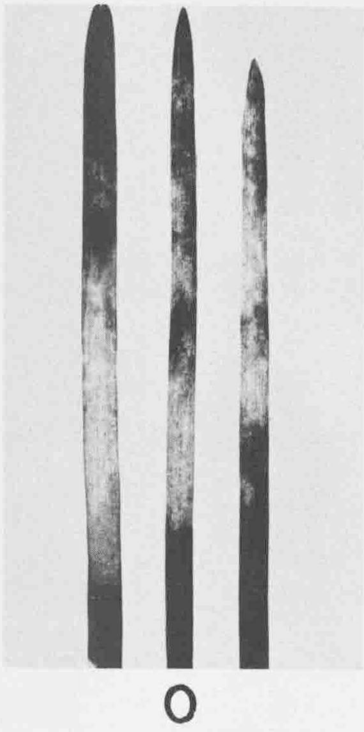

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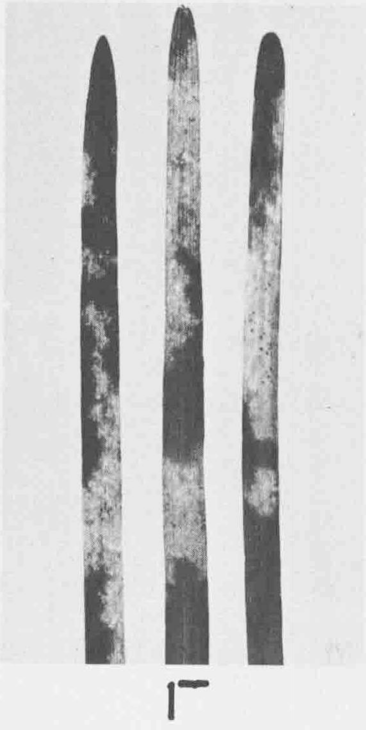

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Figure 1. (cont'd) Stripe rust infection types used in physiological race identification.