FURTHER STUDIES ON COMPETITION AMONG PHYSIOLOGIC RACES OF THE LEAF RUST OF WHEAT

by

CLARENCE IMEL

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INTRODUCTION

Competition is a phenomenon that has often been observed among the various fungi. Many workers have observed it in their studies of the facultative parasites. On the other hand the understanding of competition among the obligate parasites is limited, this limitation being due, in a large part, to the specific host-parasite relationship between the two plants.

A series of studies conducted at the Kansas Agricultural Experiment Station, Manhattan, Kansas, by Irish (20) indicated that competition existed among certain races of leaf rust. The studies reported herein are an extension of those investigations.

The present investigation deals with one segment of the many phases of study that have been conducted with the leaf rust of wheat in the United States. Many of the species of parasitic fungi have been shown to be composed of physiologic races, which account for differences in susceptibility of a single variety to different cultures of the same organism. During the past 30 years much research has been conducted in that field in an effort to gain a better understanding of the phenomenon involved. Wheat leaf rust is a good example of this specialization, with 129 different races noted to date.

These races often are more or less limited in distribution, that is, few are of world wide occurrence, and different races are found in abundance in different parts of the world. In Kansas a very common physiological race of the fungus *Puccinia rubigo-vera tritici* is race 9, while in Mexico the race which is most abundant is race 11. Race 11 is seldom found in the fields of Kansas, and never is a dominant race in the principal wheat growing areas of the United States.

The origin of new physiologic races in the United States is not known. The leaf rust of wheat is a heteroecious parasitic fungus, and only the asexual stages occur on the wheat plant. New races arise through matings on the sexual plant host, but the susceptible alternate hosts Thalictrum flavum and Thalictrum delavayi of Europe, Asia Minor and Western Asia, and the eastern Asiatic Isopyrum fumarioides, are not found in the United States. Here the fungus is carried on from one generation to the next by the asexual urediospores, in the presence of a favorable environment. American workers have come to the conclusion that new races arise in this country by means other than sexual. Johnston (22) reported an aberrant form of Puccinia triticina Erikss. that probably arose through mutation. This race is different from other races, and is continuous for type.

REVIEW OF LITERATURE

Leaf rust of wheat, Puccinia rubigo-vera tritici Erikss, has long been associated with that plant. Some 35 centuries ago, Moses spoke of rust and smut, and of the harm that they did to the food crops of his people. Throughout the Bible there is mention of the harm that rust did to the growing crops, and of the famine and pestilence that often resulted from this disease; II Paralipomenon 6:28 (Chronicles), and Amos 4:9. Nonchristian peoples set up gods of rust, one of these being known as Robigus.

Aristotle and Theophrastus described this disease about 350 B. C.

During the last part of the 19th century the various rust species became generally recognized as distinct. But it is only during the last 20 years that there has been a marked and rising appreciation of the economic importance of the leaf rust of wheat. In 1718 wheat leaf rust was recognized as a botanical curiosity in England. Today, with the full realization of the economic importance of this disease, there is an ever increasing amount of work being performed in the field of leaf rust studies.

Classification of the rust fungi was not seriously attempted until near the end of the 18th century. Persoon (38) recognized the rusts as a distinct group of fungi, and divided those known to him into several genera. Hooks, according to Johnson and Newton (21), prior to the time of Persoon, had published pictures of the teliospores of a rust in his "Micrographia." This was published in 1665. In 1729, Micheli (Johnson and Newton, 21) made a beginning at rust nomenclature when, in his "Nova Plantarum Genera," he gave the name Puccinia to a rust that was later transferred to the genus Gymnosporangium.

By 1853 and 1854 the Tulasne brothers, according to Johnson and Newton (21), had shown that some rusts were polymorphic, and by 1865 Anton de Bary, as reviewed by Johnson and Newton (21), proved that some species of rust spend part of their life cycle on one host species, and part of it on another. This condition he called "heteroecism." It was only after this work by the Tulasnes and de Bary that workers realized that a single rust might possess more than one spore form, and more than one host.

This work showed that two previously accepted species of rust might merely represent different phases of the life cycle of a single rust.

Eriksson and Henning (16) and Eriksson (15) demonstrated that there existed within some morphological species well defined strains that could be differentiated from one another by physiological criteria. Working with rust in Sweden they showed that there were several of these specialized strains, each limited to one or a few crop species, and they designated them as formae speciales. In the same year two Kansas workers, Hitchcock and Carleton (18), working with both wheat and oats concluded that ".. the rusts of various cereals are probably physiological species."

From the time of Eriksson's (15) discovery of specialization in cereal rusts until 1917, his formae speciales were regarded as the ultimate units of specialization. In 1917, Stakman and Piemeisel (46) published the results of their work with a wheat stem rust, which they had originally named Puccinia graminis tritici-compacti. They found that this strain, which gave strong infections on Tricitum compactum, differed from other wheat stem rust that they had studied. This rust produced only weak infections on the hard spring wheats and the hard winter wheat (27, 45).

Levine and Stakman (29), Melchers and Parker (32), Newton (35), and Stakman et al, (43) noted still other strains that could be differentiated by the reaction of various wheat varieties. By this time it was evident that the variety Tritici was specialized into physiologically or pathogenically different strains, and that these strains could be distinguished by differences in the type of infection they produced on various varieties of the genus *Triticum*. The methods used were much the same as those used by Eriksson (15) in determining the different reactions of genera or species of the family Gramineae for the distinguishing of his *formae speciales*. By 1922 Stakman and Levine (45) had described 37 of these biologic forms of wheat stem rust. The term "biologic form" was later replaced by "physiologic form," and still later by "physiologic race," which is the accepted designation today. Work with physiologic races of wheat stem rust was later extended to various other cereal rusts, and, as a result, all of them are now known to be composed of a number of physiologic races.

Physiologic races differ from each other pathogenically, and, as a physiological basis must be postulated for such differences, the term "physiologic race" seems appropriate. When the host is entirely congenial to the race in question, large uredia are produced with little or no chlorosis. If the host is not entirely congenial, the sori are smaller and an area of sharp chlorosis usually surrounds the uredial pustules. On an uncongenial host the rust will produce small uredia surrounded by necrotic areas, or no uredia, but only small necrotic or chlorotic areas, which may often by very indistinct.

The various symptoms produced by the rust on the various varieties of wheat are known as "infection types." Mains and Jackson (31) described the infection types now used in identifying physiologic races of leaf rust when cultured on the [illegible] differential wheat varieties. The scale used ranges from 0, which is resistant, to 4, which is complete susceptibility.

The X-type of reaction, as described by Stakman and Levine (45), was added later, and adapted for use in leaf rust studies. The description of the X-type of reaction has been modified by Johnston and Mains (26), and they have expressed it as being an "indeterminate" or heterogenous type of infection.

The first problem to confront the original investigators of physiologic specialization was the discovering of the particular host varieties most suitable for distinguishing the various physiologic races. A good differential host must be homozygous, and stable in reaction to rust under fluctuating environmental conditions. In 1926 Mains and Jackson (31) reported on 12 physiologic forms or races of <u>Puccinia</u> <u>triticina</u> as determined by the infection on eleven selected differential strains of wheat. They (30) determined this by testing 200 wheat varieties with rust collections from all parts of the United States. Among these varieties they found 31 that were differential in reaction to one or more races. From the 31 varieties that gave differential reaction, a set of seven was chosen as necessary for race determinations, and later (31) the number was increased to eleven. At the present time the number consists of eight as described by Johnston and Mains (26). The set of eight consists of the winter wheat varieties Malakoff, C.I. 4898; Mediterranean, C.I. 3332; Hussar, C.I. 4843; and Democrat, C.I. 3384, and the four spring varieties, Carina, C.I. 3756; Webster, C.I. 3780; Brevit, C.I. 3778; and Loros, C.I. 3779. These varieties were described pathologically by Johnston and Mains (26), and agronomically by Clark, Martin, and Ball (7).

Since 1926 new races have been described in nearly all regions where wheat is of economic importance, and today there are 129 described races of leaf rust of wheat which have been isolated and identified.

Johnson and Newton (21) have pointed out that the economic importance of physiologic specialization lies in the fact that the existence of many physiologic races in a rust confers on it a pathogenic versatility whereby the rust may attack a wide range of host varieties, even if each individual race possess only a narrow host range.

Sappin-Trouffy (42) studied the behavior of the nuclei in many rust species. He established that the pycniospores and the hyphae from which they develop are uninucleate, but that the aeciospores, urediospores, and the mycelia arising from them are binucleate. The binucleate condition is found in the immature teliospores, but in the mature teliospores a fusion of the two nuclei takes place. When the teliospore germinates four nuclei are produced, this being the result of two successive nuclear divisions of which one is reduction division. These nuclei move into the developing basidiospores, which, when first produced, are uninucleate. Once the alternate host is infected by the basidiospores there soon follows the development of the pycnia, beneath which are formed masses of uninucleate mycelium called protoaecia.

Blackman (1) and Christman (6) regarded the transition from the uninucleate to the binucleate condition as one involving a sexual fertilization. The process involved was not known until Craigie (9, 11) discovered that certain rusts were heterothallic, and that the pycnia performed a definite function (10). Prior to that time the pycnia had been regarded as functionless. Craigie (12) showed that when pycniospores of Puccinia graminis and P. helianthi Schw. were transferred from one pycnial pustule to a number of other pustules, aecia were formed beneath about half of the pustules receiving the transferred pycniospores. He also showed that if no transfers were made no aecia were formed. From this work Craigie deduced that the pycniospores are the bearers of one of each pair of the nuclei that become associated in the young aecium. He also classified the pycnia into two different sexual or intersterility groups. These groups he designated as plus and minus rather than male and female. Johnson and Newton (21) pointed out that the thalli are hermaphroditic but self-sterile. After this discovery it was possible to cross physiologic races.

Since the alternate hosts for leaf rust do not occur in the United States new races are believed to arise from mutation. At one time the Bridging Host theory of Marshall Ward was given some consideration. This hypothesis stated that " ....., .. a race of rust that can infect one host variety, but not a second, may gain the ability to infect the second if it is first passed through an intermediate variety," according to Chester (5). It was later proved that such changes seldom occurred so the Bridging Host theory is no longer regarded as the correct solution in the problem of race origin. Ward probably was dealing with cultures that were not pure, or with mixed cultures. While there is no proof that bridging hosts play any part in the origin of races, they do play a very important part in the changes in the composition of race populations.

Since neither hybridization nor bridging seems to account for the origin of new physiologic races of the leaf rust of wheat in the United States, other explanations must be explored. Levine (28) states that, since in the United States the alternate host is absent, hybridization evidently is not the cause for new races, but that mutation seems to be the most logical explanation to date.

There are records of what appear to be mutations, and these involve both uredial color and pathogenicity. Johnston (22) described a field collection of leaf rust (race 27) different in pathogenicity from other known races of leaf rust. It was characterized by a long incubation period, and by uredia lighter in color than those of other races.

Among the cereal rusts many such mutations have been reported, and as such they cannot be considered to be rare phenomena. Although mutations are not rare occurrences, neither is it justifiable to conclude that such mutations occur at random in the various strains of a rust. Stakman et al (44) noted a condition of instability in certain strains which lead to the production of several different mutations. Cotter (8), Gassner and Straib (17), Newton and Johnson (36, 37) and D'Oliveira (14) have noted the recurrence of the same mutations in the various rusts under their respective observations. Although the great majority of rust strains which have been kept in culture over periods of considerable time have given no evidence of mutability, there is ample evidence that mutation is a factor of great importance.

Over a period of years the race population of the leaf rust of wheat for the United States has been relatively stable, with a few dominant races, and many of minor importance occurring each year. The latest revision of the International Register of physiologic races of the leaf rust of wheat (24) lists 129 races, but the number is now known to be over 130.

According to Johnston (22) seven races appear to be of prime importance in the area west of the Mississippi River. These being races 5, 9, 15, 37, 44, 126, and 128. He also reports for the twenty year period from 1927 to 1946 that race 9 has been the most abundant and widely distributed of all of the races. In 1933 race 9 comprised 64 percent of all isolations made for that year. Races 44 and 126 have shown marked increases in late years, while race 9 has been declining in prevalence. In 1949 Johnston and Levine (25) observed that physiological races 9, 15, 58, and 126 were among the first six races most commonly collected in 29 states east of the Rocky Mountains. Of these six races, race 5 was the one isolated most often, and race 105 was in fourth place.

The various races of leaf rust of wheat have been grouped into race groups. According to Chester (5) these race groups should be recognized as single races, and as such they would include those races in which the reactions duplicate those of others of the same group on part of the differential varieties when cultured under certain environmental conditions. When cultured on the differentials the majority of these groups have been found to be quite uniform in their reaction on those varieties which have been designated as "stable." These so called stable varieties are; Malakoff, Webster, Loros, Mediterranean, and Democrat. Carina, Brevit, and Hussar are known to be unstable under different environmental conditions. There is some tendency by several workers to drop these three unstable varieties from the differential. Chester (5) is of the opinion that if these three were omitted then the number of races would be reduced from their present number to about 44, and that further study would, no doubt, reduce that number still more.

Many workers have shown that the presence of other living organisms or other physiologic races, as well as varying environmental conditions, sometimes affects the abundance and distribution of physiologic races of the rust fungi. It is well known that a change in temperature will increase or decrease the amount of rust produced. In like manner, if a moderately resistant plant is heavily infected with smut or mildew, that plant sometimes becomes more susceptible to the rust.

D'Aeth (13), Porter and Carter (41), Waksman (49), and Weindling (51) have reported on the reactions of fungi when grown in combinations, and in close association, but such work was carried on largely on artificial substrates. The rusts, like other obligate parasites, do not lend themselves to such types of investigation. As a result of their habit of growth very little is known of their reactions when grown in mixtures, either with other organisms or of different races of the same organism.

Using both artificial culture and the living host plant, Hoppe (19) studied the associative effects of strains of <u>Diplodia</u>

zeae, which is pathogenic on maize. He determined that a condition of aversion existed when certain combinations of the strains were grown in mixture. The strains studied were found to be equally pathogenic to the host plant, but when grown as mixtures on a susceptible host some strains completely inhibited the growth of other strains. Strain no. 73 inhibited no. 150 when the two strains were mixed and used as inoculum, and strain no. 26 inhibited both 73 and 150 in all but one of 29 ear inoculations made with a mixture of the three strains. He stated that the phenomenon of mutual aversion appears to be dependent upon genetic differences in the strains, and, also, that careful physiologic studies of the strains might show differences in rates of spore germination and subsequent growth which could be correlated with their so-called inhibitory powers.

Porter (40), Cayley (3, 4), Nakata (33, 34), Vandendries (47), Vandendries and Brodie (48), Blakeslee (2), and Watson (50), as well as others have reported that aversion between strains of various fungi, such as Mucors exists.

During the past few years two very important discoveries in the field of leaf rust study have contributed much to the knowledge in that field. Johnson and Newton (21) state that it has been proven that the rusts are made up of numerous parasitically different strains, a condition known as physiologic specialization, and that heterothallism exists in the rusts.

Several workers have noticed, in their rust nurseries and under conditions of artificial epiphytotics, that certain races have constantly, year after year, developed in greater proportions than have others, and that certain races have completely disappeared by the end of the growing season. The action of these various races of wheat leaf rust have led to the belief that a condition, to be known as "competition," exists between the physiologic races. This competition is so great in some cases that certain races are completely removed from the local rust picture. According to Peturson (39), this is of importance in the field of plant breeding because the introduction of a variety of wheat, resistant to the common races, will result in reduced competition. This will afford a greater opportunity for the development and spread of virulent physiologic races that have in the past been suppressed.

MATERIALS AND METHODS

Puccinia rubigo-vera tritici, the leaf rust of wheat, is an obligate parasite, and as such it must be cultured on susceptible, living host material. The rust cultures used in these studies were maintained on wheat seedlings, and the results obtained are based entirely on the reactions of the living hosts to the parasitic physiologic races.

It has been stated that other investigators had found that many of the physiologic races of rust used in field inoculations were not recovered in isolations made from resulting infections. In order to throw more light on this phenomenon in Kansas, collections of leaf rust on wheat were made by the author in the rust nursery at Manhattan, Kansas, in the spring of 1949. The collections were made early in May when well isolated infections were beginning to appear on upper leaves of susceptible checks of Trumbull and Cheyenne. These collections were analyzed, during the fall and winter of that year, for their physiologic races. Urediospores were taken from well isolated single uredia and used to establish cultures. Forty-seven such collections were analyzed. This work was conducted in the greenhouses at Manhattan, Kansas.

In another study four races of major importance in the hard red winter wheat area were used. Each race used was of a different race group, and the races used are known as physiologic races 9, 15, 58, and 126. The four races were grown in composites of two races per composite, with all possible combinations of two races. Thus there was a total of six composites, each representing a different combination of two races. The problem involved was to determine whether each race would continue to persist in equal abundance in the composite after repeated transfers, or whether there was actually an elimination or reduction in the abundance of one in favor of the other. The composites were carried through several generations in order to arrive at a definite conclusion as to the presence or absence of competition.

The races were obtained from pure stock cultures maintained in the Plant Research Laboratory, Manhattan, Kansas. These, and other races, are used in the plant breeding program at that station. The four races were selected because of their importance and prevalence in the Great Plains area of the United States. Each race is an important member of a larger race group. The races used were maintained in pure stock culture on seedlings of

Cheyenne wheat. The stock culture of each race was maintained in a well isolated section of the plant research laboratory, thus protecting its purity.

When a generation of urediospores was mature the loose spores of each race were knocked off the leaves onto sheets of paper, then collected and stored in separate small glass stoppered vials which were properly labeled. Collected spores were stored in a refrigerator at about 43°F. until sufficient inoculum had been collected for use. Spores stored at this temperature are known to remain viable over a period of several months.

When a sufficient quantity of spores of each race had been collected a germination test was made of each race, by means of hanging drops in Van Tieghem Cells. The germination was made from material that had been bottled from one to two weeks -- the period of collection. The prepared slides were then placed in a moist chamber, and examined at 2½ to 3 hours, and again at 5½ to 6 hours. Using a high power microscope, from 6 to 8 groups of 10 spores per group were counted for germination. Four such slides were made for each race, and an average of the four was taken to determine the percent of germination of each of the four races. In the total each race was represented by at least 24 counts of 10 spores per count. Race 9 averaged 76 percent germination; race 15, 68 percent; race 58, 73 percent; and race 126, 6[illegible]½ percent. These averages are within the limits of error.

Following the germination test the spores of each race were weighed on a chain balance scale to determine the weight of inoculum available for making combinations. With the four races used, six different combinations of two races per combination were possible. For each composite of two races, equal amounts of the spores of the pure cultures of each race were weighed, and placed in a glass vial, where the spores of the two races were thoroughly mixed.

Before the spores for this work were collected the source cultures were tested for purity. This was accomplished by transferring spores from each race to a separate set of differential varieties. When the rust had fruited the infection types were noted and recorded for each of the varieties in the differential. The International Register (24) was then consulted, and the race present on the differentials was determined. The ranges of the infection types and the reactions of the differential varieties to the physiologic races used are shown in Table 1.

Table 1. Reaction of differential varieties of Triticum vulgare to physiologic races of Puccinia rubigo-vera tritici.

Physiologic races	Type of infection on							
	Malakoff 4898	Carina 3756	Brevit 3778	Webster 3780	Loros 3779	Mediterranean 3332	Hussar 4843	Democrat 3384
9	4	1-2	1-2	4	4	0-1	1-2	0-1
15	0	0	0-1	0	0-1	4	0-1	4
58	0	2	4-3	1	3	4	2	4
126	4	1	2	1	4	4	2-	4

Because the races and their reaction types were known, it was not necessary to use all eight of the differentials in determining the races that were later isolated from the various composites by the single pustule method during the course of this work. Carina, Brevit, and Hussar were not used because they often are unstable under varying environmental conditions, and with but one exception are not sharply differential in separating the four races. Malakoff, Webster, Loros, Mediterranean, and Democrat were the five varieties used. Mediterranean and Democrat give identical reaction types for each of these four races, but both were retained to note possible errors or deviations.

The hard red winter wheat variety Cheyenne was used as the host for the stock cultures, the composites, and for the single pustule isolations. Earlier observations have indicated that it was equally susceptible to each of the races.

All host plants used in these studies were inoculated in the seedling stage. The seedlings were grown in fertile soil in 2½ inch flower pots, and kept in a greenhouse room free from rust cultures until ready for use. They therefore were exposed only to the various rust cultures with which they were inoculated. About 20 seedlings of Cheyenne per pot were used for stock cultures while only 5 to 7 Cheyenne seedlings per pot were used for single pustule isolates. Approximately 8 seedlings per pot were used for each differential host variety for each test.

At the time of inoculation the pots of seedling plants were placed in one of two types of moist chambers, these chambers being much alike, except for size and material. The larger ones were galvanized sheet metal cylinders, and the smaller were two gallon stone jars. Both were placed on the floor of the greenhouse under the benches, and contained clean sand floors. Both were sealed with ordinary panes of glass. The chambers were so located that direct sunlight could not enter them, and they were also located at some distance from steam lines. Both types of chambers gave equally good results in all phases of this work.

The technique of culturing the rust followed the technique for rust culture as outlined by Johnston and Mains (26). Slight modifications, to fit the circumstances, were made.

The following technique was used to inoculate seedlings for the composite cultures. Prior to inoculation the sand in the moist chambers, and the sides of the moist chambers were thoroughly moistened with tap water. Five pots of Cheyenne seedlings were placed inside, and the leaves moistened with a distilled water spray. The spores of rust will not germinate unless they are in contact with a free water surface. The humidity must remain high for at least 12 hours, and most workers maintain it for a 24 hour period. After the leaves were moistened they were dusted with a portion of the desired composite of inoculum. A portion of the inoculum was placed on a clean sheet of paper, and the spores were blown and shaken onto the wheat seedling leaves. The moist chamber was then sealed with a pane of glass, and allowed to remain sealed for 24 hours. Each such set of seedlings was given a generation and a composite number.

At the end of 24 hours the seedlings were removed from the moist chamber and placed on the greenhouse bench in isolated partially enclosed compartments located in different areas of the greenhouse. Such an isolation reduced the possibility of mixture, either among the composites or from air-borne spores.

Within ten days the infections were fully developed, and about 15 single pustule isolations were made at random from the seedlings in the composite culture. Time, space, and materials did not allow for more such isolations, but more would have been highly desirable.

Once a pustule had been selected it was carefully observed as to possible contamination from spores of pustules surrounding it. The spores of the selected pustule were carefully removed by means of a moistened spatulate tipped needle, and transferred to the moistened primary leaves of 5 to 7 Cheyenne seedlings in a single pot. Each pot then represented a single pustule isolation. Inoculated seedlings were placed in a moist chamber for 24 hours, and then removed and placed in isolated cells on the greenhouse bench.

Following the single pustule isolations the composite was transferred to a new set of Cheyenne seedlings, which then received a new generation number. This transfer followed the same pattern as described for the first generation, except that the spores were transferred directly from the old host to fresh seedlings of Cheyenne wheat. The infected seedlings were held over the uninfected seedlings, and the loose spores were gently shaken onto the moistened leaves. The newly infected leaves were then gently brushed with the leaves of the old host to insure a uniform distribution of the inoculum. Following this step the moist chamber was sealed for 24 hours, after which the seedlings were removed, and placed, in isolation, on the greenhouse bench. This procedure was followed for each succeeding generation.

At the end of 8 to 10 days infections were fully developed on the single pustule isolates. Inoculum from each of the isolates then was transferred to a set of differential varieties by means of dusting and brushing. A set of the differentials was placed in a moist chamber, which had previously been thoroughly moistened with water, and the leaves were then moistened with water from an atomizer. The infected isolate was held directly over the set of differentials and agitated gently so that the spores fell and adhered to the moist leaves. The leaves of the infected plant were then brushed gently over those in the moist chamber to insure a good distribution of the inoculum. The moist chamber was then covered with a pane of glass for 24 hours. Upon removal from the moist chamber the differentials were placed on the greenhouse bench in such a manner that the identity of each set was retained. This procedure was repeated for each of the isolates.

After a period of 10 to 12 days the rust had fruited sufficiently so that readings of the infection types could be made, and the physiologic race present determined.

EXPERIMENTAL RESULTS

Recovery of physiologic races of the leaf rust of wheat from infections in the Rust Nursery at Manhattan, Kansas, in 1949 constitutes the first part of the experimental results. The object of this work was to determine the number and percentages of recovery of the races used in creating the artificial epiphytotic. A total of 13 races had been used in the spring of 1949 to create the artificial epiphytotic. They were physiologic races 2, 5, 6, 9, 10, 15, 19, 20, 45, 58, 93, 105, and 126.

Of the 47 collections analyzed, only five of the thirteen races were recovered. The recovered races were 5, 9, 15, 45, and 126. Race 9 was recovered seventeen times, or 36.17 percent; races 45 and 5 were each recovered nine times, or 19.15 percent. Race 15 was recovered seven times, for a total of 14.9 percent, and race 126 was isolated only five times out of the forty-seven isolations, or 10.64 percent of the total isolations.

It is important to note that no race, other than those used for the infection, was isolated in this work. The spring of 1949 was considered to be most favorable for rust development, but such races as race 11, one not used in the inoculum, were not isolated.

Race 11 is a very important race in Mexico, and isolations made at Manhattan, Kansas, from Mexican collections of 1949 showed that that race was very prevalent in Mexico in 1949. The absence of this race seems to indicate that any foreign infection that might have occurred in the rust nursery did not come from the southern part of the American wheat belt. This observation indicates that most of the 1949 leaf rust infections of wheat were of a local origin -- probably from inoculum used to produce the epiphytotic. In this regard Johnston and Levine hold a similar view. From observations they made on the physiologic races of the leaf rust of wheat in the United States in 1949, they (25) report that the 1949 infections of leaf rust in the United States did not originate in Mexico.

Since the inoculum used consisted of the 13 races in equal amounts, it should be expected that they would have been recovered in about equal amounts, even from a small sampling of the field.

All collections were made from Cheyenne and Trumbull, two varieties used as checks in the rust nursery. Thus the matter of host as favoring one race to another probably was not a factor. Both varieties are assumed to be equally susceptible to the races used, although no positive test of this phase has been made.

A total of 28 observations was made on isolations from Trumbull, and 19 observations made of the collections from Cheyenne. Race 9 was recovered eight times on Trumbull, and 9 times on Cheyenne. Race 126 was found to occur twice on the Cheyenne host, and three times on the Trumbull host. Race 5 was isolated 6 times from the Cheyenne host, and 3 times from the Trumbull host. Race 15 was recovered 6 times from Trumbull, and only once from Cheyenne, but race 45 was recovered only from the Trumbull host.

From these observations it may be concluded that Cheyenne is not a favorable host for race 45. Both hosts appear to be equally susceptible to races 9 and 126, and Trumbull appears to be more susceptible to race 15 than is Cheyenne. Cheyenne may be a more susceptible host to race 5 than is Trumbull.

Competition was studied by making a series of single pustule isolations from each generation of each of the composites used. Theoretically, if there was no competition, and if each race used in the inoculum produced infections on the Cheyenne seedlings, it would have been possible to recover each race in about equal proportion in any series of single pustule isolations.

The results of the races isolated from the different generations of the six composites are given in Tables 2 through 5. The first series of tests involved analysis of the three composites

9-15, 9-58, and 58-126. The data obtained are summarized and presented in Table 2. It is evident that each race used in each of the three composites was recovered from the first through the fifth generation. In the sixth generation only race 9 was recovered from the composites 9-15 and 9-58. Race 15 and 58 were not isolated again after the fifth generation, but race 9 continued to be isolated in abundance. Although these tests indicate that races 15 and 58 had disappeared, mass tests of the composites at the end of the eighth generation proved that they still were present in trace amounts.

In the composite 58-126, both races were isolated through the sixth generation, but only race 126 was isolated in the seventh and eighth generation. Here also a test of the composite indicated that race 58 was present as a trace.

The experiments summarized in Table 2 were carried out during the fall and winter of 1949 - 1950. The work presented in Table 3 was conducted during the late winter and early spring of 1950. Time, equipment and space did not permit analysis of all composites to be conducted at the same time.

Table 2. Results of single pustule isolations made from each generation of three composite groups.

Physiologic race	Number of times each race was recovered in generation I	II	III	IV	V	VI	VII	VIII	Total no. of isolates	Percent of total isolates
				Composite 9-15						
9	9	12	9	12	14	20	15	15	106	79.85
15	7	6	6	7	2	0	0	0	28	21.64
Totals	16	18	15	19	16	20	15	15	134	
				Composite 9-58						
9	9	12	17	13	14	20	15	15	115	83.95
58	7	5	3	5	2	0	0	0	22	16.05
Totals	16	17	20	18	16	20	15	15	137	
				Composite 126-58						
126	7	9	12	13	15	17	15	15	103	76.29
58	10	9	5	3	2	3	0	0	32	23.70
Totals	17	18	17	16	17	20	15	15	135	

The results of the analysis of the third possible composite involving race 9, i.e. 9-126, are shown in Table 3. In this case race 9 was the only race isolated after the third generation. However, as in all other composites, tests of the composites on the differentials showed the presence of more than one race of rust, up to and including the last generation for each composite studied. In all cases studied, except 9-126, the mixture was easily noted, but in the case of 9-126, in the VII generation only a trace of mixture was noticeable, and that consisted of a few type four uredia on Democrat, C.I. No. 3384.

The data in Table 3 show that in all cases both races were isolated in about equal numbers in the first generation. A larger sampling probably would have produced even closer agreement. Since both races in all composites appeared in about equal numbers in the first generation it is assumed that the sources of infection would have yielded spores, which, under normal conditions, should have continued the races in the next generation in about equal abundance on the host plant.

Table 3. Results of single pustule isolations made from each generation of three composite groups.

Physiologic race	Number of times each race was recovered in generation I	II	III	IV	V	VI	VII	Total no. of isolates	Percent of total isolates
				Composite 9-126					
9	7	12	13	15	15	15	15	92	90.19
126	5	3	2	0	0	0	0	10	9.98
Totals	12	15	15	15	15	15	15	102	
				Composite 15-126					
126	9	12	10	12	13	16	14	86	80.37
15	7	4	5	3	2	0	0	21	19.62
Totals	16	16	15	15	15	16	14	107	
				Composite 15-58					
58	7	9	11	14	15	15	15	86	81.99
15	8	6	4	1	0	0	0	19	18.09
Totals	15	15	15	15	15	15	15	105	

When it became clear, after 7 or 8 generations that one race was being recovered in much greater frequency than the other the work was brought to a close.

Since the number of isolates per generation varied from 12 to 20 the percentage of isolates for each generation probably gives a clearer picture of the results than the actual number of isolates. These are shown in Tables 4 and 5.

These data show that in all composites where race 9 was one of the races involved it soon became the dominate race, and that by the end of three to five generations it was the only one isolated by the small random sampling method. In all composites where race 15 was involved it was suppressed by the other three races. This seems to indicate that race 15 is not able to compete with the other four races involved in this study.

Table 4. Percent of isolates representing races of three composites recovered in eight generations.

Physiologic races	Percent of each race recovered in generation							
	I	II	III	IV	V	VI	VII	VIII
				Composite 9-15				
9	56.25	66.66	60.00	63.15	87.50	100	100	100
15	43.75	33.33	40.00	36.84	12.50	0	0	0
				Composite 9-58				
9	56.25	70.59	85.00	75.22	87.50	100	100	100
58	43.75	29.41	15.00	27.77	12.50	0	0	0
				Composite 58-126				
126	41.17	50.00	70.58	81.25	88.23	85.0	100	100
58	58.82	50.00	29.41	18.75	11.76	15.0	0	0

Table 5. Percent of isolates representing races of three composites recovered in seven generations.

Physiologic races	Percent of each race recovered in generation						
	I	II	III	IV	V	VI	VII
				Composite 9-126			
9	58.33	80.00	86.66	100	100	100	100
126	41.66	20.00	13.33	0	0	0	0
				Composite 15-126			
126	56.25	75.00	66.66	80.00	86.66	100	100
15	43.75	25.00	33.33	20.00	13.33	0	0
				Composite 15-58			
58	46.66	60.00	73.33	93.33	100	100	100
15	53.33	40.00	26.66	6.66	0	0	0

The pustules used for sampling the race populations in each generation were selected at random on the leaves of the infected host. Not only were large, well isolated pustules chosen, but they were taken from the more heavily infected portions of the leaves, where they were in close association with those of other races. Pustules were taken from the tips, bases, and middle of the leaves, and the area of selection was noted. Regardless of the area of selection and the size of the pustules, no difference was noted in favor of one race over another. Since only small numbers of isolates were used in each generation the importance of these factors cannot be definitely evaluated.

Irish (20) noted that race 58 was isolated only from heavily infected portions of the leaves. He used the same four races as a single composite that were used in pairs in the studies reported here. The author noted no such location-race relationship where the races were used in composites of two races per composite.

In all composites where race 9 was involved that race never represented less than 56.25 percent of the total isolations in any one generation. The percentages for the other races varied with the composite under consideration.

It is clear from the data presented in Tables 2 to 5 that race 9 is dominant to the other three races, and that race 15 is unable to compete with any of the races used in this work. The composite 58-126 is somewhat different from the others. It is the only case where both races were isolated as late as the sixth generation. In composite 9-126, race 126 was not isolated after the third generation, while in composite 15-58 race 15 was not isolated after the fourth generation. In the remaining three cases only one race was isolated after the fifth generation.

These results indicate one of the possible reasons why certain physiologic races, when they are used in composite inoculum in the rust nursery, disappear before the end of the growing season. This is a problem of grave concern to the plant breeder because in a breeding program for disease resistance the development of a variety resistant to the dominant races will result in the disappearance of those races. The disappearance of such dominant races will allow those races that have been of lesser importance to develop to a point where they will become of major importance. The development of Pawnee wheat which is resistant to race 9 has resulted in a steady decrease in that race as the acreage of Pawnee has increased. On the other hand the once unimportant race 5, to which Pawnee is very susceptible, is rapidly becoming an important race in the same area (25).

DISCUSSION

In the study of the samples from the 1949 rust nursery the number investigated was too small for the results to be considered as conclusive. It seems safe to assume that race 9 is still a race common to this area, and that it is a race able to maintain itself and increase. It is not clear why only small numbers of isolates of the other races were obtained but it is suggested that it may have been due to competition or suppression among the races. There seems to be no explanation why race 15 was not isolated with about equal frequency, even in a small sample, from both Trumbull and Cheyenne, nor why race 45 was not isolated from the Cheyenne host.

Evidence has been presented that when certain physiologic races of the leaf rust were grown in a composite culture on a uniformly susceptible host competition between races apparently occurred. When grown as pure cultures on Cheyenne, the uniformly susceptible host, races 9, 15, 58, and 126 produced a susceptible type-4 reaction. When these races were cultured in composites of two races per composite a condition of competition resulted. In each of the composites where race 9 was one of the components that race was capable of maintaining itself and even increasing in prevalence while the other race involved was not recovered after several generations of single pustule isolations.

This reaction on the part of race 9 was not completely unexpected. Various workers have time and again noted that race 9 has been the most prevalent race in the Great Plains area of the United States. The introduction in recent years of new hard red winter wheat varieties derived from selections or hybrids has resulted in a decline in the prevalence of race 9. It is assumed that the intensity of race 9 in the past has been due to its wide range of environmental adaptation, and that it is capable of overwintering more readily than the other races used in this study. The present decline of race 9 in the hard red winter wheat area of the United States appears to be due in part to the introduction, and ever increasing acreage of Pawnee wheat, a variety which is resistant to that race.

In the study reported herein race 15 was able to maintain itself for only a few generations when placed in competition with any of the other three races used in the composites. This is not in agreement with the results obtained by Irish (20) who used a single composite of the same four races employed in the present studies. He reported that race 15 "---- proved to be capable of perpetuating itself in mixed culture almost as well as race 9."

Races 58 and 126 were found to fall into position between races 9 and 15 in competitive ability. Both race 58 and 126, when in competition with race 9 were suppressed. When in competition with race 15, however, they suppressed that race. When 58 and 126 were cultured in the same composite, they both were isolated for six consecutive generations although race 58 decreased in abundance after the second generation. It was not until the seventh generation that race 58 failed to appear among the isolates. This indicates that races 58 and 126 are both good competitors, with race 126 being somewhat the better.

Chester (5) has published an extensive review of the factors related to the development of leaf rust of wheat, but he makes no mention of the associative effects. Johnson and Newton (21) have reviewed the specialization, hybridization and mutation of the cereal rusts, but again the phase of competition is not covered. Peturson (39), working with stem rust, and Irish (20), working with the same material as studied by the author, both suggested the existence of competition in the material under study.

The author was unable to find a definite statement as to the exact cause of this inhibition or competition among the respective races in any of the literature examined. Cayley (3, 4), Nakata (33, 34), and Porter (40), in their studies of cases of inhibition in other organisms, have suggested that this condition is due to the excretion by one organism of some substance which is toxic to the associated organisms. They conducted their respective work, largely, on artificial substrates, and the nature of the living host probably would not be conducive to the ready diffusion of such a toxin for any great area of plant tissue.

Cayley (3, 4), Hoppe (19), and Vandendries (47) contend that the conditions of aversions found in their studies were directly correlated to the genetic constitution of the organisms. In wheat such studies of genetics of the leaf rust organism have been limited to the inheritance of pathogenicity. From such observations it is logical to assume that the characteristics of a given physiologic race, which makes it an aggressive competitor are directly correlated with its genetic constitution.

Each of the races studied in this work is an important member of a separate race group. It has been shown that competition exists between these races. It has also been shown that the races within a given group are closely related in regard to pathogenicity. Further work is necessary to determine if the races of one group are better competitors than the races of another group. Work also is needed to determine whether the races within a race group are able to compete among themselves.

The results presented in this paper are not presented as conclusive proof of racial competition. They are but one phase of a much larger problem, and as such they are only preliminary in nature. The work was not repeated, and as a result the first observations have not been confirmed. It was assumed that Cheyenne was equally susceptible to all of the races, but no absolute proof has been offered. It was determined that the spores were about equal in their ability to germinate, but no study was conducted to determine if they had the same power of entering the stomata of the host leaf. The spores were weighed out in equal amounts, but no effort was made to determine if they were of the same size for each race. Therefore it is not known that exactly the same number of spores of each race was used in each composite.

Although in the field of statistics small random samples are considered to be reliable, a larger sampling, or a total sampling probably would have given more exact information. A larger sampling might have given a better understanding of the area of the leaf which resulted in the best development of certain, if not all, of the races.

Other points to be considered are that all work was conducted under greenhouse conditions, and upon seedling plants. The greenhouse represented controlled conditions, and the degree of infections on the seedlings was considerably higher than would normally occur in the field.

It is believed that if no competition existed both races used in each composite would have existed in equal amounts for an indefinite period of time. The observations made indicated that both races did not maintain themselves in equal amounts, and that one race invariably was lost from the composite. This disappearance of one of the races was considered by the author to be evidence of a condition of competition among the races studies.

Much more study on this problem is needed, more and different races need to be used in the composites, and larger number of isolations need to be made, with several checks being used. These studies should be regarded as introductory in nature, and should be used as a basis for more work covering this and related phases of the large problem of competition among races of leaf rust of wheat.

SUMMARY

A study was made to determine the races present in the 1949 rust nursery at Manhattan, Kansas. A total of 13 races had been used to create the artificial epiphytotic that year, but only five of the races were recovered, with race 9 being recovered the greatest number of times. The check varieties Trumbull and

Cheyenne served as the source of uredia, and four of the five races were recovered from both varieties. Race 45 was recovered only from the Trumbull host. No race, other than those used for the infection, was isolated in this work. This phase of the work was conducted in an effort to determine if competition existed among races in the field.

A second study was made of the competitive effects of four physiologic races of leaf rust of wheat when grown in composites of two races per composite. A total of six different composites were used representing all possible combinations of the four races.

Each composite was carried through seven or eight generations. A series of single pustule isolations were made at random in each generation. These were cultured and analyzed for the races involved by means of a set of differential varieties of wheat.

Evidence is presented to show that competition apparently occurred among the races studied.

Physiologic race 9 was found to be the dominant race in those composites where it was one of the races involved. On the other hand it was noted that in no case was race 15 able to persist in the presence of the other three races.

Races 58 and 126 proved to be about equal in their ability to compete with other races, with 126 being somewhat the better competitor, except when both were placed in competition with race 9. In competition with race 9, race 58 remained in the composite longer than race 126.

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