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

by

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INTRODUCTION

Since the discovery of physiologic specialization in parasitic fungi, considerable research has been completed in an effort to solve some of the problems associated with this phenomenon.

The physiologic races of Puccinia rubigo-vera tritici, the fungus causing leaf rust of wheat, are of major economic importance. This fungus is prevalent in all wheat growing areas of the world and continues to be of vital interest to pathologists, mycologists, and wheat breeders everywhere.

The leaf rust of wheat fungus does not complete its life cycle in this country because of the absence of its alternate hosts, particularly Thalictrum flavum and T. delavayi of Europe, Asia Minor and Western Asia and the eastern Asiatic Isopyrum fumarioides. The fungus is perpetuated by uredospores growing on wheat plants in a favorable environment. Thus, the conclusion has been drawn that new races are produced in this country by means other than sexual. Johnston (13) reported an aberrant form of Puccinia triticina Erikss. that probably arose through mutation. The race was continuous for type and was found to be quite different from other races prevalent in the same locale.

Certain races of rust are found in all parts of the United States, and it has been determined that no race is localized to any one area (15). Over a period of years physiologic race 9 has been encountered more often in field collections of rust than any other race. It is also the one most generally encountered in late fall and winter in the Western Mississippi

Valley. The consistent and abundant occurrence of this race has led workers to believe that it is particularly well adapted to a wide range of environmental conditions and is able to overwinter more often than other physiologic races.

In rust nurseries where artificial epiphytotics of the disease are produced every year, it has been noticed by several workers that some of the many races of the organism fail to develop in as great proportions as others or may even disappear while other races tend to grow well and dominate the race picture. Until very recent years race 9 usually has predominated at the end of the growing season in the Great Plains area. This led to the belief that competition occurs between the physiologic races to the extent that certain races are completely inhibited. Thus, potentially dangerous races were eliminated when used as constituents of inoculum in mixture with more virulent races.

This study was undertaken to throw some light on the problem of competition between physiologic races of Puccinia rubigo-vera tritici. The writer has studied 4 races of leaf rust which are of major importance in the Great Plains area. Each of the races is an important member of a separate race group. The 4 races were grown in mixed culture for several generations to determine whether each of the races would persist in equal abundance or whether competition would occur and certain ones would be inhibited while others would dominate in prevalence.

REVIEW OF LITERATURE

Mains and Jackson (18) proved the existence of 12 physiologic forms of Puccinia triticina in the United States. They

made this discovery by testing 200 wheat varieties with rust collections from all parts of the United States, and among these varieties 31 were found to be differential in reaction to one or more races. From the 31 varieties a set of 7 was chosen as differentials necessary to make race determinations. Later (19) these workers published a detailed account of their experiments using 11 differential wheat varieties. Three of the differential varieties have been dropped from use because their reactions were found to be the same as other of the varieties. The unnamed differentials later were named by Johnston and Mains (15).

Mains and Jackson (19) gave the following descriptions of the reaction types for use in identifying physiologic races of leaf rust when cultured on the differential wheat varieties:

Classes of host reaction	Types of rust infection
0-highly resistant	No uredinia formed; small flecks, chlorotic or necrotic areas more or less prevalent.
1-very resistant	Uredinia few, small, always in small necrotic spots. Also more or less necrotic areas produced without development of uredinia.
2-moderately resistant	Uredinia fairly abundant, of moderate size, always in necrotic or very chlorotic spots. Necrotic spots seldom without uredinia.
3-moderately susceptible	Uredinia fairly abundant, of moderate size. No necrosis produced, but sometimes slight chlorosis immediately surrounding the uredinia.

EXPLANATION OF PLATE X

Types of infection produced by *Puccinia rubigo-vera tritici* on varieties of wheat. From left to right the types are 0, 0, 0, 1-, 1, 2-, 2, 2+, 3, 3+, 4, X, X, X, and mixed. The latter is a mixture of two races on the same leaf.

PLATE I

Classes of host reaction	Types of rust infection
4-very susceptible	Uredinia abundant, large. No necrosis or chlorosis immediately surrounding the uredinia. Infected areas sometimes occurring as green islands surrounded in each case by a chlorotic ring.

Later, the X-type reaction as described by Stakman and Levine (25) was adopted for use in leaf rust studies. They describe the type-X reaction as follows:

> Uredinia variable, apparently including all types and degrees of infection on the same blade; no mechanical separation possible; on reinoculation small uredinia may produce large ones, and vice versa. Infection ill defined.

Johnston and Mains (15) have used the term "indeterminate" or heterogenous to designate the type-X infection and have added: Tips of leaves often have large, normal uredinia while leaf bases have minute uredinia and flecks.

The latest revision of the International Register of physiologic races of the leaf rust of wheat (17) lists 129 races.

The race population of the United States has been fairly stable over a period of years and consists mainly of a few dominant forms although many races of minor importance have been encountered each year (4).

Johnston (14) states that 7 races appear to be of prime importance west of the Mississippi river. These are races 5, 9, 15, 37, 44, 126, and 128. He adds that throughout a 20-year period, 1927 to 1946 inclusive, race 9 has been the most abundant and widely distributed race. Race 9 reached its maximum

in 1933 when it comprised 64 percent of all isolates cultured that year. Since that time it has been declining in prevalence but is still the most prevalent. Races 44 and 126 have shown a marked increase in prevalence in recent years.

Chester (4) has advanced the concept of race groups in which he recognizes as single races those groups of races the reactions of which duplicate those of others of the same group on part of the differential varieties under certain environmental conditions. Various workers have presented experimental evidence that proves the existence of such a relationship between certain of the physiologic races. The majority of the members of the groups has been shown to be quite uniform in their reaction on the "stable" differential varieties, Malakof, Webster, Loros, Mediterranean and Democrat. The environmentally induced instability has been limited to the differential varieties Carina, Brevit, and Hussar. Several workers have advocated discarding the latter three varieties. Chester states that if the reactions of Carina, Brevit, and Hussar, were deleted from the International Register, the number of races would be reduced from 129 to 44 and that further study would, no doubt, reduce the number still farther. For example, Race Group 2 includes races 2, 3, 15, 25, 34, 59, 62, 102, and 127; Group 9 includes 9, 10, 13, 19, 20, 24, 27, 29, 31, 108, and 115; Group 12 includes 12, 32, 44, 58, 61, 76, 81, 84, 85, 88, and 90; and Race Group 6 includes 6, 28, 39, 105, and 126. The other races of leaf rust have been grouped in a like manner.

Physiologic races of leaf rust are very specific in parasitism as each is rather sharply restricted or specialized to

a certain host variety or varieties. All are similar or identical morphologically and for all practical purposes are limited to one host genus, Triticum. Recently workers have become cognizant of the fact that physiologic races of fungi are genetically different, if not morphologically.

Flor, (9), Johnson and Newton (11, 12), Newton, Johnson and Brown (22), and Craigie (6, 7) have made extensive studies on the genetics of the rust fungi and have brought to light many basic facts regarding pathogenicity of the rusts.

New physiologic races of cereal rusts have been obtained by selfing or crossing known races of Puccinia graminis tritici, P. graminis avenae, and P. rubigo-vera tritici. Johnson and Newton (12) in studies on the inheritance of pathogenicity in crosses between races of P. graminis tritici determined that Mendelian inheritance was the rule. They found that the pathogenicity of the F_2 rust cultures to certain varieties of wheat appeared to be inherited as a unit and was conditioned by a single pair of factors with virulence dominant. Pathogenicity to Kanred, a differential variety, was conditioned by a single pair of factors with avirulence dominant, and pathogenicity to Vernal by two pairs of duplicate factors with avirulence dominant. On Mindum virulence was dominant with a 3:1 ratio in the F_2 generation. Each of the characters appeared to be inherited independently.

Flor (9) in studies on inheritance of pathogenicity in races of flax rust, Melampsora lini, found that avirulence invariably was dominant. As a consequence, the F_1 progeny were weaker in pathogenicity than the parent races. He stated that

some pathological characters seem to be governed by a single pair of factors, while others are governed by two or more pairs. His findings suggested that the pathologic range of each physiologic race of the pathogen is conditioned by pairs of factors that are specific for each different resistant or immune factor possessed by the host variety.

There is urgent need for more extensive investigations on the genetics of the rust fungi as additional knowledge is necessary for a more complete understanding of the course of development of epiphytotics.

Many workers have shown that physiologic races of the rust fungus are affected by modifications in their environment. The presence of other living organisms and/or physiologic races constitutes an important part of that environment. As is true of all fungi, the rusts are "consumers" and compete with other organisms for survival.

Studies of the associative influences of microorganisms, usually bacteria and fungi, have been carried out largely on artificial substrates. The character of obligate parasites, such as the rusts, makes them very difficult to study, and, as a consequence, very little is known of their reactions when grown in mixture.

Reviews on the reactions of fungi when grown in combinations in close association have been published by D'Aeth (8), Porter and Carter (24), Waksman (28), and Weindling (30).

Hoppe (10) studied the associative effects of strains of Diplodia zeae, pathogenic on maize, both in artificial culture and on the living host plant. He determined that the phenomenon

of aversion was evident when certain combinations of the strains were grown in mixture. The strains studied were found to be equally pathogenic to the host plant, but some of the strains completely inhibited the growth of others when they were mixed and used as inoculum on a susceptible host. Strain no. 73 inhibited no. 150 when the two strains were mixed and used as inoculum; 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. 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.

In studies on the inhibitory action of certain fungi to others, Porter (23) used 80 species of fungi and bacteria which he grew in pairs on corn-meal agar. The fungi employed included Penicillium glaucum, P. italicum, Rhizopus nigricans, Fusarium lini, F. culmorum, F. coeruleum, Gloeosporium piperatum, Colletotrichum nigrum, C. lindemuthianum, and Helminthosporium sativum. He determined that the inhibitions which occurred were due to the presence of some product formed by the mycelium during growth.

Cayley (2, 3) studied the aversion reaction between monospore mycelia of Diaporthe perniciosa Marchal, growing on the same substrate. She found that the mycelia of single spore isolates from the same strain would mix readily but the mycelia from spores of different strains exhibited an aversion to one another. She explained that the aversion is probably due to the secretion of a toxin by the mycelia, a character which was found

to be heritable.

Nakata (20, 21) recorded aversion between strains of Sclerotium rolfsii Sacc. collected in different localities. He stated that when aversion occurs, the inocula are of different strains; when absent, they are of the same strain. He concluded that aversion is not due to a substance formed by the action of the fungus on the medium, but by a substance which is excreted by the mycelia irrespective of the medium.

Vandendries (26) demonstrated that in Pleurotus columbinus, aversion is perfectly correlated with genetic constitution.

Vandendries and Brodie (27), working in collaboration, found that the pairing reaction of monosporous thalli is governed by two pairs of allelomorphic factors, one of which is responsible for aversion. The aversion is entirely dependent upon the genetic constitution of the mycelia. They termed the phenomena of aversion, "barrage."

Blakeslee (1), studying the reaction between colonies of Mucors, found that they exercised some inhibitory action toward one another. He found that the inhibitory action was absent and the colonies met when they were of opposite sexual tendencies.

Watson (29), in a study on the development of single and mixtures of physiologic races of Puccinia graminis tritici, found that race 34 always grew well in association with one or all of the other races studied. The uredosori of race 34 on Little Club at approximately 73° F. erupted earlier than those of the other races tested. The more rapid development of 34 was accentuated at 80° F. He stated that this is the most important factor to consider when determining the prevalence of

races in the field. He found that the races tested could maintain themselves when cultured singly on the host varieties Little Club and Soft Federation and that the amount of infection did not diminish when the temperatures varied between 73 and 89° F. However, certain races always decreased in percentage when they were cultured in mixture with certain of the other races. Race 147 was unable to maintain itself after several cultural generations in association with other races. Race 34 was able to develop well in all cases. Even though races 147 and 34 gave identical reactions on Little Club, Watson assumed that there was a difference in their virulence, which cannot be detected macroscopically, but is important when the races are associated. Further, that the final composition of any one mixture after a number of generations of culturing depends upon several factors: the amount and character of each race present in the mixture, the host variety, and the temperature and the manner in which it affects the fungus and the reaction of the host to the fungus.

MATERIALS AND METHODS

The leaf rust of wheat fungus, Puccinia rubigo-vera tritici, is an obligate parasite and must be cultured on a susceptible, living wheat plant. Therefore, all of the rust cultures used in these studies were maintained on wheat seedlings and the results obtained are based entirely on the reactions of living host plants to the parasitic physiologic races.

Four physiologic races of the leaf rust fungus were selected for study: 9, 15, 58, and 126. Race 58 is very similar to race 44 which has previously been listed as one of the principal

races in the Great Plains area. The races were obtained from stock cultures maintained at Manhattan, Kansas, and used in the program of breeding for resistance to leaf rust in hard red winter wheat. These particular races were chosen for study because of their importance and prevalence in the wheat-growing regions of the Great Plains. Also, each race is an important component of a separate race group or complex (4). The physiologic races were grown in pure stock culture on seedlings of susceptible Cheyenne wheat in well isolated sections of the plant research laboratory.

Uredospores were collected from the races and kept separate in small glass vials. These collections were stored in a refrigerator at approximately 43° F. until sufficient inoculum had been collected for use. Spores stored at this temperature retain their viability over a period of several months.

In approximately one week an adequate quantity of spores of each race was collected. From each of these physiologic race collections 2.15 mg of spores were carefully weighed out with a chain balance scale thus assuring the presence of all races in equal amounts in the inoculum. The weighed portions then were composited in a glass vial and mixed thoroughly.

Before collecting the inoculum for use in this study, each race was tested for purity. The test was accomplished by transferring spores from each race to a separate set of differential varieties. When the rust had fruited on the differentials, the infection types were noted and "read" for each of the varieties in the sets. By referring to the analytical key and table of reactions listed in the International Register (17) the race

present on the differentials was easily ascertained. If it were found that the culture was a mixture of races, it was possible to reisolate and culture the desired race by making single pustule isolations from the infected differential varieties. Spores produced on the single pustule isolates were tested on the differentials and the desired race was secured. When the identity of the races in mixture on the original set of differential varieties could be recognized, a shorter method of purification was possible. The inoculum was transferred to a wheat variety specifically susceptible to the desired race but resistant to other races. Thus, a pure culture was established and maintained. When the races were found to be pure, it was necessary to increase the amount of inoculum. The races were grown and increased on the uniformly susceptible variety Cheyenne. The four races used produced a susceptible type-4 infection on this variety. So far as could be ascertained, Cheyenne was equally susceptible to each of the 4 races used.

The 8 differential varieties used in testing the original stock cultures were: the spring-wheat varieties--Webster, C.I. No. 3780; Carina, C.I. No. 3756; Brevit, C.I. No. 3778; and Loros, C.I. No. 3779; and the four winter-wheat varieties--Malakof, C.I. No. 4898; Mediterranean, C.I. No. 3332; Hussar, C.I. No. 4843; and Democrat, C.I. No. 3384. These varieties have been described by Mains and Jackson (19), Johnston and Mains (15), and Clark, Martin, and Bell (5). The infection types produced by races of leaf rust on these differentials were originally described by Mains and Jackson (19) and later by Johnston and Mains (15).

The reactions of the differential varieties to the physiologic races used are shown in Table 1. The ranges of the infection types are given.

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

Physiologic races	Type of infection on							
	Mal. 4898	Carina 3756	Brevit 3778	Web. 3780	Loros 3779	Mud. 3332	Hus. 4843	Demo. 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 unnecessary to use all eight differential varieties in determining what races had been picked up in the single pustule isolations in the course of this study. The 5 varieties necessary for the accurate determination of races and which were used were: Malakof, Webster, Loros, Mediterranean, and Democrat. The varieties Carina, Brevit, and Hussar were omitted because they often are unstable under varying environmental conditions and with but one exception are not sharply differential in separating the 4 races.

All of the wheat plants used as hosts were inoculated while in the seedling stage. The plants were grown in 2½ inch clay flower pots. The seedlings were considered ready for inoculation 10-14 days after planting or when the primary leaves were fully developed.

The pots of differential varieties for use in race identi-

fication and those of Cheyenne used to culture inoculum from the single pustules contained from 6-8 seedling plants. Ten to twenty seedlings of Cheyenne were grown in each of the pots used to maintain the composite cultures. Usually about 15 pots of seedlings were inoculated each time the composites were transferred.

The plantings were made in an isolated section of a greenhouse where no rust was cultured and care was taken to insure that aseptic conditions were maintained in that section. Care also was taken to protect seedlings from stray spores from other races when seedlings were handled during all inoculation procedures.

Two types of moist chambers were used to provide optimum environmental conditions for germinating the spores sown on the wheat seedlings. Fundamentally, the two types were alike except for size. The comparatively large chambers were galvanized-iron cylinders 15 inches in diameter and 13 inches high. These cylinders were well seated in the soil of the floor of the greenhouse over which about an inch of clean sand was spread at the bottom of the moist chambers. Two-gallon stone jars served as the smaller moist chambers. A few inches of clean sand was placed in the bottom of each jar. Pieces of greenhouse glass served as covers for both types of moist chambers. In all cases these chambers were located so that no direct sunlight fell on them, and they were placed well away from the sources of steam heat. Throughout the course of the experiments both types of chambers gave excellent results and each seemed to be equally effective.

The technique of culturing the rust followed, with slight modifications, the technique outlined by Johnston and Mains (15).

Prior to inoculation the interiors of the moist chambers were thoroughly moistened with tap water to provide a high humidity within. About 15 pots of Cheyenne seedlings then were placed inside and the leaves were moistened with distilled water sprayed from a DeVilbiss hand atomizer. For germination the uredospores must be in contact with a free water surface, so a high humidity was maintained during the 24-hour germination period to provide sufficient condensed moisture on the seedling leaves. After the leaves were well moistened a portion of the composite of inoculum containing the four races was used to inoculate the seedlings. To accomplish this, a portion of the composite of uredospores was placed on a clean sheet of paper which was held over the wheat plants in the moist chamber. Then the spores were blown and shaken from the paper to the seedling leaves, thus, a good distribution of the inoculum was insured. The moist chamber then was covered with a pane of glass. This set of seedlings was designated Composite Culture I, Generation I.

At the end of a 24-hour period the inoculated plants were removed from the moist chamber and placed on the greenhouse bench. They were placed in isolation cages in a comparatively well-isolated section of a greenhouse. This reduced the hazard of contamination of the culture by air-borne spores and kept mixtures at a minimum.

Infections were fully developed in 10 days. On the 11th day about 15 single pustule isolations were made at random from the seedlings in the composite culture. Actually the number of isolates varied from 12 to 18 during the course of the experiments. In a study of this type it would have been highly de-

sirable to have made many more single pustule isolations, but due to limited time, space, and materials the isolations were limited to approximately 15.

The technique of successfully making single pustule isolations was very difficult and time-consuming as the Cheyenne seedlings were heavily infected, the severity often being 100 percent by the Modified Cobb scale. Isolated pustules were selected at random from the infected leaves of the composite culture. The spores from a pustule were carefully collected on the moist spatulate tip of a flattened dissecting needle then were spread on moistened primary leaves of 6-8 Cheyenne seedlings in a single pot. Thus each pot of seedlings represented a separate isolate. The inoculated seedlings then were placed in the large moist chambers for 24 hours for the spores to germinate and infection occur.

After the 24-hour germination period the isolates were removed from the moist chambers and placed in small separate cages where there was little danger of mixture by spores carried from one isolate to another.

On the same day the single pustule isolations were made, Composite Culture I was transferred to a new set of Cheyenne seedlings which was designated Composite Culture I, Generation II. It was assumed that if no competition existed or no aversion was carried on by the associated races that the components of the composite would be transferred in equal amounts. The transfer was effected by placing approximately 15 pots of Cheyenne seedlings in a thoroughly moistened moist chamber then, after the leaves were sprayed with distilled water, infected plants from

EXPLANATION OF PLATE IX

Method of transferring inoculum from an infected seedling plant to aseptic ones. This procedure was followed when inoculating the sets of differential varieties with spores from the single pustule isolates and when transferring inoculum from one generation of a composite culture to establish another generation.

PLATE II

EXPLANATION OF PLATE III

Method of transferring the spores of a single pustule to a susceptible host variety for culturing.

PLATE III

Composite Culture I were held over the seedlings in the chamber and were agitated gently. Spores were dislodged and fell to the primary leaves of the Cheyenne seedlings. The infected leaves then were brushed gently over those in the moist chamber to insure a uniform distribution of the inoculum. The moist chamber then was covered with a pane of glass. After a 24-hour germination period, the plants were removed from the chamber and placed on a greenhouse bench in an isolation cage. This procedure was repeated for each of the generations through which Composite Cultures I and II were passed.

After a period of 8 days, infections were fully developed on the single pustule isolates made from the first generation of the composite culture. Inoculum from each of the isolates then was transferred to a set of differential varieties. A set of five differential varieties was placed in a small moist chamber, which had previously been thoroughly moistened with tap water, and the leaves were atomized with distilled water. An infected isolate was held directly over the set of differentials and was agitated gently so that spores fell and adhered to the moist leaves. Then the leaves of the infected plant were brushed gently over those in the moist chamber thus insuring a good distribution of the inoculum. The moist chamber was covered with a small piece of greenhouse glass. The procedure was repeated for each of the isolates.

The inoculated differential varieties were removed from the moist chambers at the end of the 24-hour period of germination and were placed on a greenhouse bench in such a manner that the identity of the sets was retained.

Rust on the differentials had fruited sufficiently in 12 days so that readings of infection types could be made on each variety and the physiologic races determined. Ranges of infections as presented in the International Register were used as a criterion for identification of the races isolated from each generation of the composite cultures. Single pustule isolations made from each generation of the composites gave a representative sample of the populations of races present. Composite Culture I was carried through 7 generations from which a total of 103 single pustule isolations were made and the races identified; Composite Culture II was continued through 10 generations from which 158 isolates were cultured and identified.

EXPERIMENTAL RESULTS

The extent of competition was studied by making a series of single pustule isolations from each generation of the composite cultures. Theoretically, if all the races used in the inoculum produced infections on the Cheyenne seedlings, it would be possible for each race to appear at least once in a series of 15 single pustule isolations.

The results of races isolated from the different generations of Composite Culture I are given in Tables 2 and 3. From the data presented it is evident that all four races of the mixture of inoculum caused infections on the seedlings in the first generation and all but race 58 was recovered in the second generation. In generations 3, 4, 5, 6, and 7, only races 9 and 15 were isolated.

Table 2. Results of single pustule isolations made from each generation of Composite Culture I.

Physiologic races	Number of times each race was recovered in each generation							Total no. of times isolated	% of total isolates
	I	II	III	IV	V	VI	VII		
9	2	5	6	8	4	4	7	36	34.9
15	1	9	10	7	10	8	8	53	51.5
58	2	0	0	0	0	0	0	2	1.9
126	10	2	0	0	0	0	0	12	11.7
Totals	15	16	16	15	14	12	15	103	100.0

While the data in Table 2 show that all four races appeared in the first generation it is not clear why physiologic race 126 appeared in such abundance and the other races in such meager amounts. This is especially surprising since that race was recovered in only two isolates in the second generation and was not recovered thereafter. In the second generation race 15 was recovered more often than any other race as it was in all succeeding generations except the fourth.

The disappearance of races 58 and 126 is very difficult to explain, but it is apparent that some form of competition was manifest. Obviously, growth of the two races, 58 and 126, had been completely inhibited, and races 9 and 15 had become dominant. If no inhibition had occurred, it is assumed that the infection centers present in the first generation would have yielded spores which under normal environmental conditions should have perpetuated the races in the next generation in about equal abundance on the congenial host variety.

Since the number of isolates varied from 12 to 16 in the various generations the percentage of isolates representing each

race probably gives a clearer picture of the results than the actual number of isolates. These are shown in Table 3. These

Table 3. Percent of isolates representing each of 4 physiologic races recovered in each of seven generations during studies on Composite Culture I.

Physiologic races	Percent of each race in the generation indicated						
	I	II	III	IV	V	VI	VII
9	13.3	31.3	37.5	53.3	28.6	33.3	46.6
15	6.6	56.3	62.5	46.6	71.4	66.6	53.3
58	13.3	0.0	0.0	0.0	0.0	0.0	0.0
126	66.6	12.5	0.0	0.0	0.0	0.0	0.0

data show very clearly that in Composite Culture I physiologic race 15 was the dominant race in all generations except the first and fourth. In the fourth generation race 15 was only slightly below race 9 in abundance. It should be noted that, although race 15 was the most abundant race, physiologic race 9 persisted in abundance. It comprised only 13.3 percent of the isolates in the first generation but increased to 31.3 percent in the second and was important in each generation thereafter.

When it became clear after single pustule isolations had been made for 7 generations that only races 9 and 15 remained in the composite culture, it was decided to repeat the experiment by establishing a new composite from which new single pustule isolates could be made. Inoculum was collected from pure cultures of the four races and equal amounts of each were composited. A portion of the composited spores was used to inoculate a set of Cheyenne seedlings. The inoculated set was designated Composite Culture II. This composite was carried through 10 generations,

and studies were made of the race populations in the same manner as described in the previous experiment. Data obtained from the study of each generation are presented in Tables 4 and 5.

Table 4. Results of single pustule isolations made from each generation of Composite Culture II.

Physiologic races	Number of times each race was recovered in each generation										Total no. of times isolated	% of total isolates
	I	II	III	IV	V	VI	VII	VIII	IX	X		
9	9	9	8	13	5	12	14	13	15	15	113	71.5
15	6	4	6	4	10	3	3	2	0	0	38	24.0
58	0	0	3	0	0	0	0	0	0	0	3	1.9
126	1	2	1	0	0	0	0	0	0	0	4	2.5
Totals	16	15	18	17	15	15	17	15	15	15	158	99.9

Table 4 shows that the races identified from the first generation isolates were preponderantly 9 and 15 with 126 being isolated only once. Race 58 was not picked up in the first two generations but was recovered in the third. The absence of race 58 from generations I and II caused some concern but its brief appearance in the third generation proved that it had not been lost from the composite.

Prior to this time the technique in sampling the race populations in each generation had been to select at random well isolated pustules for isolation and culture. These usually appeared near the leaf tips. It was decided that pustules of race 58 might be occurring on the more heavily infected portions of the leaves in close association with those of other races. When the single pustule isolations were made from the third and succeeding generations, only pustules occurring in close association with others were selected for isolation. It was very difficult to

obtain spores from only one pustule, but extreme care was exercised and few mixtures were obtained. In all cases the author was able to identify those races in the mixtures by careful study of the infection types produced on the differential varieties. When two races were identified from one isolation, the occurence was recorded. Hence, the data in Table 4 present information on more than 15 single pustule isolations from certain generations.

In the third generation physiologic race 58 was isolated three times. It may be worthwhile to repeat that 58 was isolated only from heavily infected portions of leaves.

Races 58 and 126 were not encountered in generations after the third, and it was assumed that they could not compete with races 9 and 15 in mixed culture.

In the fourth and succeeding generations it appeared that the population of race 9 was increasing over that of race 15 as it was isolated more often. The abundance of race 15 declined sharply after the fifth generation and was not isolated after the eighth generation. In the last two generations Composite Culture II yielded only physiologic form 9.

Table 5. Percent of isolates representing each of 4 physiologic races recovered in each of ten generations during studies on Composite Culture II.

Physiologic races	Percent of each race in the generation indicated									
	I	II	III	IV	V	VI	VII	VIII	IX	X
9	56.3	60.0	44.4	76.5	33.3	80.0	82.4	86.6	100.0	100.0
15	37.5	26.7	33.3	23.5	66.6	20.0	17.6	13.4	0.0	0.0
58	0.0	0.0	16.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
126	6.3	13.3	5.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Due to the variation in the number of isolates in the various generations through which Culture II was run, the results are more comparable when shown in terms of the percentage of isolates representing each race in each generation. Such percentages are shown in Table 5. It should be pointed out that at no time during this series of tests did physiologic race 9 fall below 33.3 percent of the isolates and that in all but two generations it comprised more than half of all isolates. On the other hand race 15, which was the dominant one in tests with Composite Culture I, ranged from one-fourth to one-third of all isolates in the first four generations, rose to 66.6 percent in the fifth, then gradually declined and finally disappeared from the inoculum in the ninth generation.

While the results obtained from the analyses of Composite Culture II roughly parallel in general those obtained with Composite Culture I, they differ considerably in detail. For example, it is clear that physiologic races 9 and 15 are dominant races as compared with races 58 and 126, which is in agreement with the results obtained with Composite Culture I. Furthermore, the data in the second tests agree with those of the first in indicating that race 58 is the least able to compete and survive of any of the 4 races. On the other hand the data from the two experiments are in contradiction on the relative competitive ability of races 9 and 15. The results obtained with Composite I indicated that race 15 was somewhat the better competitor, while the results with Composite II indicated that race 9 was by far the better. The reason for this lack of agreement is not apparent but it probably stems from the relatively low number of isolates

made in each generation. At any rate the two experiments agree to the extent that they indicate very clearly that races 9 and 15 are vigorous, dominant races able to survive in association with other races, while races 58 and 126 apparently are not able to compete and disappear from composite inoculum. Furthermore, both experiments indicated rather clearly that race 58 was the weakest competitor under the conditions of these experiments. These results throw considerable light on the disappearance of certain physiologic races when they are used in composite inoculum in rust nursery and other field experiments with the leaf rust of wheat. The failure of some important physiologic races such as 58 and 126 to perpetuate themselves is a vexing problem that has a particularly important bearing on the success of a program of breeding wheat for resistance to leaf rust.

DISCUSSION

It has been shown that when certain physiologic races of Puccinia rubigo-vera tritici were grown in mixed culture on a uniformly susceptible host variety (Cheyenne) the phenomenon of competition was exhibited. The races tested, 9, 15, 58, and 126, produced a susceptible, type-4 infection when grown singly on this variety. However, when the races were mixed and cultured together on Cheyenne, a type of competition resulted. Physiologic race 9 proved to be capable of maintaining itself and even increasing in prevalence when cultured in association with forms 15, 58, and 126. This reaction was not completely unexpected in light of the findings of various workers that race 9 has consistently been the most prevalent in certain wheat-growing re-

gions of the United States. It has been assumed that race 9 is adapted to wide-range environmental conditions and that it overwinters more readily than any other race. Hence, it is a more vigorous race than others encountered. Since the introduction of hard red winter hybrid lines of wheat selected for resistance to race 9, this form has been on the decline in prevalence. Pawnee wheat is resistant to race 9 and this variety is grown on a greater acreage in Kansas than any other single wheat variety. This factor is probably largely responsible for the decreasing prevalence of race 9 in the Great Plains area. The fact remains that it is a very aggressive race in view of the knowledge that it is still one of the most prevalent races even though its susceptible host varieties have been greatly decreased.

In this study physiologic race 15 proved to be capable of perpetuating itself in mixed culture almost as well as race 9. In the first experiment this form was isolated more often than all the other races combined. Race 15 was isolated 53 times out of a total of 103 isolates totaling 51.5 percent of all races isolated and identified. The other races combined totaled 50 isolates or 48.5 percent of all isolates. In the second part of the study race, 15 appeared to be less able to compete as it was isolated only 38 times from the 10 generations while race 9 comprised 113 of the isolates. This decrease in ability to compete with race 9 is difficult to explain. Possibly, some undetected change in the complex of environmental conditions had a direct effect either on the host variety or the parasitic fungus; therefore, it was less able to compete although variations due to the small number of isolates cannot be discounted.

It was isolated more often than race 9 in only one generation, the fifth. Johnston, Caldwell, and Compton (16) have shown that over a 6-year period race 15 was isolated in abundance second only to 9 from collections made in Uniform Rust Nurseries in various sections of the United States. This is evidence to uphold the view that physiologic race 15 is a good competitor in the field.

Chester (4) has reviewed the literature relevant to the chief factors that effect the development of Puccinia rubigo-vera tritici, but no mention is made of the associative effects of physiologic races when occurring in epiphytotic proportions. This problem has received little treatment in the study of microorganisms in general, and the author was unable to find reference to any such work dealing directly with the fungus studied.

No definite statement can be made as to exactly what caused the inhibition and complete disappearance of certain races from the composite cultures. It is very probable that physiologic races of Puccinia rubigo-vera tritici are like races of other fungi which, when cultured, produce characteristic reactions to changes of environal factors which include the presence of other races. Porter (23), Cayley (2, 3), and Nakata (20, 21), studying cases of inhibition, have suggested that the phenomena are due to the excretion by the mycelia of some substance, toxic to the associated organisms. These substances are largely hypothetical, their existence having been postulated in attempts to explain aversions. Also, the nature of the living wheat plant probably would not be conducive to the ready diffusion of such a toxin over any great distance. If such a growth-inhibiting substance

were produced by intermingling mycelia or by haustoria present in the same cell, the reactive distance would be short and the invading hyphae would be affected.

Hoppe (10), Cayley (2, 3), and Vandendries (26) hold that the aversions they studied were directly correlated with the genetic constitution of the organisms. Genetic studies of the leaf rust organism have been limited mainly to the inheritance of pathogenicity, but it is logical to assume that the characteristics of a physiologic race which makes it an aggressive competitor are correlated with its genetic constitution.

As was mentioned earlier, each of the races studied in these experiments is an important member of a separate Race Group. It has been shown that the races within these groups display a close relationship in regard to pathogenicity. Further work is necessary to determine whether the race components of a group are characteristically good or poor competitors among themselves as well as when grown in mixture with races from an opposing group or groups.

The results obtained in this study must be accepted with certain reservations. No comparative tests were conducted to test the germination of spores from the different races on the host variety Cheyenne. It was assumed that they were of equal viability as all were of approximately the same age. Another possibility is that the low number of single pustule isolations from each generation would not present a complete picture of the population of races present. Another factor of unknown weight was that all the tests were conducted on seedlings, and the severity of infections were considerably higher than would normally occur under field conditions.

All the races had the same initial start as the composite of inoculum consisted of equal amounts of spores from each. Macroscopic examinations of the pustules produced on Cheyenne seedlings served as a basis for determination of the virulence of a race. Outwardly, it appeared that all races tested were equally pathogenic on the primary leaves of Cheyenne. Information on the rate and number of spores produced by the races on this variety is completely lacking. This factor would have an important bearing on the problem if percentages of race infections had been studied; but even if one race did produce more spores more rapidly than its associated races, it would not discredit the author's working hypothesis.

It is believed that if no competition existed between the associated physiologic races, all the races present in the mixture would have been able to reproduce and maintain themselves on the uniformly susceptible host plant. The disappearance of a race from the association of races was considered to be evidence of competition between one or all of the races in the mixture. It is beyond the scope of this paper to discuss the hypotheses that have been advanced to explain the interaction of organisms growing on the same substrate and the ultimate inhibition or stimulation of a certain form or forms.

Finally it should be emphasized that the experiments herein discussed are considered as merely preliminary in nature and the results are not regarded as conclusive. Much more study of the problem of race competition is needed wherein the number of isolates can be greatly increased and various related factors can be studied in much greater detail. These studies should be

considered as the introductory ones of a series of investigations covering considerable time and exploring many phases of the problem.

SUMMARY

A technique was developed to study the competitive effects of 4 physiologic races of leaf rust, Puccinia rubigo-vera tritici, when grown in mixed culture in epiphytotic proportions.

Four important races of leaf rust were composited in equal amounts and grown together on the susceptible host variety Cheyenne.

The race populations in each generation of the composite cultures were sampled by making a series of single pustule isolations. The isolates were cultured and the races identified.

Data are presented showing that competition does exist between the physiologic races studies.

Physiologic races 9 and 15 were found to be the dominant competitors when grown in close association with races 58 and 126. The latter two races were unable to maintain themselves in mixed cultures with races 9 and 15 and were eliminated in the earliest cultural generations. The results of both experiments indicate that race 58 is the weakest competitor of the 4 races tested.

Race 15 was shown to be somewhat the best competitor in the first experiment while race 9 was able to maintain itself in almost equal abundance.

Race 9 was by far the best competitor in the second experiment as race 15 disappeared from the culture in the ninth generation.

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