STUDIES OF COMPETITIVE ABILITY IN CERTAIN RACES OF WHEAT LEAF RUST

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INTRODUCTION

Leaf rust, caused by Puccinia rubigo-vera tritici Erikk. and Henn. (Carl.), is the most important disease of wheat in Oklahoma. In this area it first becomes noticeable on fall sown wheat in October and November. During the period from October to early December, when temperature and moisture conditions are favorable, it occasionally has become sufficiently severe to predispose wheat to winter killing. Winter survival and early spring build-up of rust inoculum depends upon the number of satisfactory wetting periods from December through March (6). When conditions during these months are favorable for repeated infection epidemics develop which have been known to cause 20 to 30 percent loss in the grain crop (5).

The use of resistant varieties obviously is the most feasible method of control of diseases such as wheat leaf rust. However, in the development of resistant varieties, a thorough knowledge of the predominant physiologic races of the parasite and their behavior play an important role. Many different races of leaf rust are known to exist. However, in a given area, the leaf rust population usually consists of 10 to 15 races, of which perhaps only 3 or 4 races predominate.

The ability of any particular race to survive in a population depends upon many factors. One of the most important, certainly, is the presence of a susceptible variety. Regardless of other factors,

a race which is not virulent on one or more of the commonly grown varieties will soon decrease in population.

Other factors which are known to influence the survival ability of a race are: (1) the capacity and extent of spore germination, (2) the length of the wetting period required for penetration, (3) the length of the incubation period, (4) the reaction to extremes of temperature and (5) competitive ability. This latter factor, that of competitive ability, was the primary object of this investigation. No attempt was made here to determine any of the factors which might be included in the general term of "competitive ability". Rather, the object was simply to determine which race of a mixture would survive or predominate after several uredial generations on a given variety.

During the course of these studies, many limiting problems arose which required solution. First of these was a lack of uniform and consistent spore germination. Studies of the action of self inhibitors, age of the spores, and pustule crowding upon germination were made in an attempt to solve this problem.

Difficulty also was encountered in using the standard leaf rust differential varieties to determine the percentage of any given race in a mixture. This necessitated a search for supplemental differential varieties and how they might best be used.

Perhaps the most perplexing problem was the inability to produce infection at low temperatures. It already has been pointed out that the buildup of inoculum in Oklahoma occurs at the time when wetting periods are likely to be accompanied by low temperatures. Consequently, it was decided to make these studies of competitive ability at temperatures which are most likely to be encountered in nature.

Laboratory studies indicated that the spores of the races used germinated adequately at temperatures as low as 35° F., but often when these same spores were inoculated on wheat leaves at this temperature, no infection resulted. In an attempt to find out why infection did not take place a study was made of the action of stomata in relation to infection at minimal temperatures.

REVIEW OF LITERATURE

Competition Among Races of Fungi

Studies of competition between or among races have been made with many fungi including Diplodia zeae (13), Ustilago hordei (26), Puccinia graminis tritici (15, 17, 18, 27), and Puccinia rubigo-vera tritici (14).

In all cases certain races had better survival ability than others and soon tended to become dominant.

Hoppe (13) injected a misture of three equally pathogenic strains of Diplodia zeae in corn ears when they were in the milk stage of development. The inoculated ears were harvested after six weeks and were analysed for the composition of the strains. From the original mixture of three strains, only one survived.

Tapke (26) inoculated a mixture of races 3 and 6 of Ustilago hordei on Odessa barley and 25 smutted kernels were analysed for race composition. Pure cultures of race 3 were isolated from 15 kernels, a pure culture of race 6 from only one kernel and races 3 and 6 together were isolated from 7 kernels. A new race was isolated from 2 kernels.

Johnston and Newton (15) noted that race 56 always survived best in a mixture of four races of stem rust on Ceres wheat.

Watson (26) cultured various mixtures of up to 4 races of stem rust on several wheat varieties for several generations. He concluded from his work that some races develop well in mixtures while others do not. He states,

"the final composition of any one race mixture after a number of

generations of culturing is dependent on certain factors. First, it depends upon the amount and character of each race present in the mixture. Secondly it depends upon the variety, and thirdly, the temperature insofar as it effects the fungus and the way the variety reacts to the fungus."

Loegering (17) cultured a mixture of wheat stem rust races 17 and 19 for 6 or 7 generations on Mindum durum, Little Club and Fulcaster wheats, and a mixture of races 17 and 56 was cultured for the same number of generations on Ceres, Little Club and Fulcaster wheats. In all cases race 17 gave the best survival, but the rapidity with which this race dominated the mixture was dependent upon the variety. The poorest survival of race 17 when mixed with race 19 was on Mindum durum, but when mixed with race 56, the poorest survival of race 17 was on the variety Ceres.

In a later paper Loegering (18) reported that in a mixture of races 17 and 56 on Ceres, Little Club and Fulcaster wheats, race 17 became dominant on Little Club and Fulcaster by a wide margin but dominated only slightly on Ceres. In the same manner, a mixture of races 17 and 19 was grown for 6 generations on Mindum durum, Little Club and Fulcaster wheats. On Little Club and Fulcaster, race 17 predominated after a few generations, while on Mindum durum it predominated only slightly after 6 generations.

Irish (14) cultured a mixture of wheat leaf rust races 9, 15, 58, and 126 on the variety Cheyenne. Races 58 and 126 were eliminated in the early generations and race 15 disappeared after 9 generations, leaving only race 9.

Spore Germination

Doran (7) has shown that fresh spores of Puccinia malvacearum

germinate over a wider range of environmental conditions than the older spores. As the spores age, the viability drops sharply to a certain level, and further reduction in viability is gradual.

Epperly (8) compared the germination of 3 races of wheat leaf rust urediospores and found that race 9 started germination more rapidly than races 105 or 105A at a temperature of 5°C.

Straib (25) found that the urediospores of Puccinia glumarum produced at 20 to 25°C. had a greater rate of germination than those produced at 8 to 12°C.

Allen (1) discovered the presence of a self-inhibitor which was produced by the spores of Puccinia graminis tritici when suspended in water. This substance was soluble in water and when presoaked spores were transferred to fresh water, germination was no longer inhibited.

McCallan and Wilcoxon (20) and Younder (29) have emphasized the importance of statistics for the interpretation of spore germination data. The former has listed various factors that may cause differences in germination between different replications, including deviations in source and age of spores, and density of spore suspensions.

Stomatal Movement

Pool and McKay (22) found that the germ tubes of Cercospora beticola could not penetrate sugar beet leaves when stomata were closed.

A similar conclusion was reached by Hart (9) using stem rust of wheat. Artificial light, excessive moisture and variation of temperature did not prolong the period of stomatal opening in her studies. Direct sunlight was found to be the most important stimulus. Her studies indicated that the stomata of susceptible varieties remained

open for a longer time than those of resistant varieties.

Hart and Forbes (10) have shown that darkness at the time of inoculation and throughout the early stages of infection, reduced the severity and prevalence of infection by Puccinia graminis tritici. However, no appreciable difference was found in the severity and prevalence of infection by Puccinia triticina, when inoculations were made in natural light or darkness and the same conditions were continued throughout the early stages of infection.

Radulescu (23) also confirmed that stomatal opening was primarily influenced by natural day light and only slightly affected by other factors. The rate and degree of stomatal opening were found to be different in different varieties and was an heritable character dependent upon multiple factors.

Loftfield (19) and Hart (9) found that the stomatal movement followed a definite rhythm. The stomata opened gradually after sun rise, remained open for varying lengths of time, closed gradually in the afternoon and remained closed at night.

Carroll and Welton (4) stated that in certain grasses maximum stomatal opening was found between 9 and 10 A. M., but that in every case the stomata were completely closed at night.

Heath (11) and Heath and Milthorpe (12) considered several environmental factors in relation to stomatal movement. In their studies they concluded that when all other factors remained constant, increased artificial light caused considerable stomatal opening.

Caldwell and Stone (2) stripped the epidermis of wheat leaves infected with leaf rust and found that the guard cells press each other very tightly, leaving no space for the appresoria to penetrate except

by applying pressure.

Caldwell and Stone (3) later found that the opening of stomata was unnecessary for infection of wheat leaf rust. Open stomata were observed to close when appresoria of the germinated spores came in contact with them. The presence of appresoria of Uromyces trifolli caused the open stomata of wheat to close.

MATERIALS AND METHODS

Three races of wheat leaf rust were chosen for use in this study of competitive ability. One, race 9, was for many years the most prevalent race in the Southern Great Plains. At the present time, however, it has almost disappeared from the rust population in that area. Another, race 15, is now the most prevalent race in Oklahoma.[1] The third selection was race 105A, which is capable of attacking the newer varieties Westar and Concho, and may increase in prevalence as these high yielding varieties increase in acreage.

Races 9 and 15 were originally obtained from C. O. Johnston, U.S.D.A., Manhattan, Kansas, in 1950. They have been cultured and repurified each year at the Oklahoma Agricultural Experiment Station. Race 105A was first collected and identified at the Oklahoma Agricultural Experiment Station in 1951 (30). It also has been cultured and repurified each year.

These races were cultured on two varieties in this study. Triumph C.I. 12132[2] and Comanche C.I. 11673 were chosen because they are completely susceptible in the seedling stage to the races used

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[1]Unpublished data, Department of Botany and Plant Pathology, Oklahoma A. & M. College, Stillwater, Oklahoma.

[2]C.I. refers to the accession number assigned by the Cereals Section of the Field Crops Research Branch, U.S.D.A.

and are two of the more widely grown varieties in Oklahoma.

Throughout this investigation plants were prepared for inoculation by stripping the primary leaf of 10 day old seedlings between the moistened thumb and fore-finger. The plants were then dipped into a beaker of water which had 4 milligrams of rust spores dispersed over the surface. This small portion of spores was measured with a microscopp. Following inoculation the plants were placed in moist chambers similar to those described by Stakman et al. (24).

Only mature rust spores were used. Spores were harvested usually on the tenth day following inoculation, and only the spores which detached themselves readily when the infected leaves were gently tapped were taken. Whenever it became necessary the spores were stored in test tubes in a refrigerator at 3 to 4°C.

Spore germination was measured by distributing the test spores over the surface of 2 percent water agar contained in Syracuse dishes. After incubation at room temperature (approximately 70 to 75°F.) for 8 hours 300 spores were counted in 6 groups of 50 in each group.

When race mixtures were prepared each race was added to the mixture in proportion to the percentage of germination of the spores in the particular lot of that race. Spores were harvested from the test varieties after each generation and a sample was reinoculated on the test varieties and another sample was inoculated on the differential varieties. The differentials used were Democrat C.I. 3384, which is resistant to race 9 and susceptible to races 15 and 105A, and Loros C.I. 3779, which is resistant to race 15 and susceptible to races 9 and 105A. In the beginning the variety Newthatch C.I. 12318 also was used as a differential. It is resistant to race 105A and

susceptible to races 9 and 15. Considerable difficulty was encountered when these differentials were used and will be discussed in more detail later.

The functioning of the stomata on the primary leaves was determined by making collodion impressions (28). Two samples of leaves were taken from the test plants and two procedures were followed. One sample was immersed in absolute alcohol and later dried before applying commercial collodion solution. The collodion was applied immediately to the other sample without any previous treatment. At least three leaves were used with each treatment. The collodion film was removed easily after about 15 minutes. Longer exposures caused the films to be fragile when they were removed. True impressions of the stomata were received with both procedures. Microscopic examination showed that the use of alcohol to kill the tissue prior to the application of the collodion film was not necessary to secure a true impression of the stomatal function. In this study only those stomata were considered open which had a distinct oblong opening between the guard cells. Twenty stomata on each leaf were counted, as far as possible, within one inch from the tip.

EXPERIMENTAL RESULTS

Age of Urediospores in Relation to Germination

The intial studies with race mixtures were not successful due to the inadequate and inconsistent spore germination. Therefore, certain tests were made to determine some of the factors which might be limiting. First, germination tests of fresh and stored spores were made (Table I).

TABLE I

THE INFLUENCE OF AGE OF SPORES UPON THE PERCENT OF GERMINATION IN THREE RACES OF LEAF RUST.

Age of the spores*	Average percent of germination of races:		
	9	15	105A
90 days	42.0	6.3	12.3
1 day	94.6	93.3	92.3

*Length of time from spore harvest to the time of testing. Spores were stored in test tubes at 35 to 40 F.

It was found that only freshly harvested spores could satisfactorily be used in studies of this nature.

Effect of a Water Presoak Upon Urediospore Germination

In addition, tests were made to determine if a self-inhibitor

was involved in the germination of the urediospores of leaf rust. These experiments were made in the summer months when fresh urediospores were not available. The germination of races 15 and 105A were too low to be of any value, but the results with race 9 did yield some information (Table II). Spores of race 9 which were 90 to 93 days old were

TABLE II

THE EFFECT OF SOAKING SPORES OF LEAF RUST RACE 9 IN WATER ON THE PERCENT OF GERMINATION.

Replication	Percent germination following water soaking for: 0 hrs.	4 hrs.	8 hrs.	12 hrs.	24 hrs.
I	41.7	31.0	14.7	6.7	4.7
II	42.3	41.7	42.0	12.7	9.3
Average	42.0	36.4	28.4	9.7	7.0

divided into lots of 4 milligrams. Each lot was dispersed over the surface of a 600 ml. beaker of distilled water. After 4, 8, 12 and 24 hours of soaking on the surface of the water the spores were stirred to disperse them evenly and three loops of water and spores from the surface were removed to the surface of water agar in Syracuse dishes. These samples were held at 60°F. for 8 hours and then were counted for germination. Spore germination decreased the longer the spores reamined on the water. These results, similar to those of Allen (1), indicate that a water soluble inhibitor may be present in leaf rust urediospores. Certainly there is a decided drop in spore germination following a presoaking period. Whether this is caused by a self-inhibitor or some other

phenomenon, the implications from this study are that satisfactory germination can be obtained if the spores are not held in water too long before inoculations are made.

Relation of Conditions during Urediospore Production to Germination

During the course of studies made in the spring of 1955 it was observed that spores from heavily rusted plants did not germinate as well as spores from plants only lightly infected. Also, Straib (25) indicated that the temperature at which spores are produced will influence their germination. Therefore in the fall when the greenhouse temperatures became low enough to start rust studies an experiment was devised to analyse these points.

Plants of Triumph and Comanche were inoculated with fresh spores of leaf rust race 9 using varying amounts of inoculum to produce a range of severity. One group of plants was held at 40°F. until spore maturity 22 days after inoculation, while another group of plants was held at 60°F. until spore maturity 10 days after inoculation. Leaves with the desired severity of infection were selected and the spores were harvested in the usual manner. The spores obtained were subjected to germination tests and the results are given in Tables III and IV. It was readily observed that spores produced at 40°F. were much less viable than those produced at 60°F. Also, as the severity of infection increased, the resulting spore germination decreased. For instance, when the severity of infection at 60°F., as measured by modified Cobb's Scale (21), was 5 percent, the spore germination averaged 94 percent, but when the severity of infection was increased to 40 percent, the spore germination decreased to about 55 percent. It

TABLE III

THE GERMINATION OF SPORES OF LEAF RUST RACE 9 PRODUCED AT 40° F. AND AT VARYING SEVERITIES OF INFECTION.

Percent of severity by Modified Cobb's Scale*	Variety	Percent of spore germination Replication I	II	III	Average
5	Triumph	50.3	50.3	58.7	53.1
10	"	40.0	34.0	36.3	36.7
25	"	36.3	22.7	40.3	33.0
40	"	18.3	26.7	20.0	21.6
5	Comanche	48.7	54.3	44.7	49.2
10	"	30.7	40.3	38.0	36.3
25	"	38.0	26.0	22.7	28.9
40	"	20.7	30.0	14.0	21.5

*As presented by Melchers and Parker (20).

TABLE IV

THE GERMINATION OF SPORES OF LEAF RUST RACE 9 PRODUCED AT 60° F. AND AT VARYING SEVERITIES OF INFECTION.

Percent of severity by Modified Cobb's Scale*	Variety	Percent of spore germination Replication I	II	III	Average
5	Triumph	94.3	98.0	90.7	94.3
10	"	79.3	87.7	83.0	83.3
25	"	67.3	68.0	65.0	66.8
40	"	51.3	59.3	56.7	55.7
5	Comanche	96.7	94.7	92.3	94.5
10	"	83.7	83.3	80.7	82.5
25	"	70.0	72.3	75.3	72.5
40	"	47.0	62.0	59.0	56.0

*As presented by Melchers and Parker (20).

was noted during this study that it was impossible to obtain infection of over 65 percent severity with the methods used. Henceforth, the spores which were to be used for further inoculations were harvested from plants grown at 60°F. or above following the wetting period, and from leaves with relatively low severity.

Differential Varieties

Following the experiments on spore germination another attempt was made to study competitive ability, utilizing the information obtained. However, still further difficulties were encountered. It soon became evident that the differentials in use were difficult to read. This was particularly true with the variety Newthatch, where it was necessary to separate the 4 type pustules produced by races 9 and 15 from the 2 to 2+ type pustules produced by race 105A. If the rust severity was high or the pustules were grouped closely together this separation became almost impossible. Consequently, it was decided to drop race 105A from the competition studies, and to rely upon the counts of 4 type pustules on Democrat for the presence of race 15 and the 4 type pustules on Loros for the presence of race 9.

Relation of Stomatal Movement to Infection

Using the information derived from the various spore germination tests it was possible to get satisfactory infections at optimum temperatures. However, it was the intention from the start to make the competition experiments at low temperatures. Even with the information gained in the previous studies when temperatures of 40°F. or below were used during the wetting period infection was erratic. This was

particularly true when minimum lengths of wetting were used. Spores sprayed on water agar at the time of inoculation and held at the same temperature germinated very well. Consequently it was decided to examine the function of stomata at these low temperatures.

The first test was made in January when the natural day length was about 10 hours (16). This day length was extended to 12 hours by artificial fluorescent light. After the plants had grown in the greenhouse at 65°F. for 10 days they were moved to a moist chamber in a room held at 40°F. Artificial fluorescent light was supplied in the cold room for a period of 12 hours before any stomatal impressions were made. No natural light penetrated cold room.

The number of open stomata were counted at intervals of one hour during a 10-hour period. These data are given in Table V.

It was found that almost all of the stomata were closed at 8:30 A. M. when the observations were started and remained closed until about 3:30 P. M. Most of the stomata opened sometime between 2:30 and 3:30 P. M. and remained open until sometime after 5:30 P. M. At 6:30 P. M. 97 percent of the stomata were again closed.

This same test was repeated except that the plants were not placed in the cold room until approximately 30 minutes prior to making the first stomatal impressions. During this test the fluorescent lights were used in the cold room. A third test was made in which the artificial fluorescent light was turned out and the plants remained in darkness during the time they were in the cold room. In neither test was there any change in the functioning time of the stomata.

An experiment was then designed to study the relation of stomatal movement to rust infection. Ten day old seedlings of Triumph and

TABLE V

THE ACTION OF STOMATA ON THE TIPS
OF THE LEAVES OF WHEAT
IN JANUARY, 1956

Time of observation*	Percent of stomata open in: Triumph	Comanche
8:30 A.M.	4	2
9:30	6	0
10:30	1	0
11:30	5	9
12:30 P.M.	10	11
1:30	5	1
2:30	7	5
3:30	67	37
4:30	84	73
5:30	23	24
6:30	3	7

*Plants were held at 40° F. and supplied with only artificial fluorescent light for a period of 12 hours before the first observations were made.

Comanche were placed in moist chambers at 40°F. at 9:30 A. M., 11:30 A. M., 1:30 P. M. and 3:30 P. M. and inoculated with races 9, 15 and 105A immediately. All the plants were removed to the greenhouse bench at 6:00 P. M. The results of this test are given in Table VI. In general, the highest infection resulted from the inoculations made at 11:30 A. M. Plants inoculated at that time had a wetting period of 6½ hours compared with 8½ hours for those plants inoculated at 9:30 A. M. Epperley (8) found that spores of leaf rust germinated most actively in 3½ to 4 hours at this temperature, which would indicate that it is necessary for the stomata to be open during the period of active germination to produce maximum infection. A small percent of the stomata were open at 12:30 P. M. and may account for the infections obtained from the inoculation at 9:30 A. M.

This entire series of experiments on stomatal activity was repeated in March, 1956, when the natural day-length was about 2 hours longer (16). Table VII contains the data obtained on stomatal openings at that time. It is important to note the period during which stomata were open had changed. In January they were open from 3:30 to 6:30 P. M. in both varieties. In March, however, they were open from 1:30 to 4:30 P. M. in Triumph and from 12:30 to 3:30 P. M. in Comanche. That was an advance of two hours in Triumph and 3 hours in Comanche. Therefore, when the infection experiments were made in March the inoculations were started 2 hours earlier on Triumph and 3 hours earlier on Comanche. These data are presented in Table VIII. This time the earliest (7:30 A. M. in Triumph and 6:30 A. M. in Comanche) inoculation gave the highest infection, which would indicate a closer correlation with the length

TABLE VI

THE SEVERITY OF RUST INFECTION FOLLOWING INOCULATION AT VARIOUS TIMES OF THE DAY DURING JANUARY, 1956

Variety	Time of inoculation	Length of wetting period*	Average number of pustules per 10 leaves inoculated with Race:		
			9	15	105A
Triumph	9:30 A.M.	8 1/2 hours	44	31	8
"	11:30	6 1/2 "	110	124	91
"	1:30 P.M.	4 1/2 "	32	19	3
"	3:30	2 1/2 "	0	0	0
Comanche	9:30 A.M.	8 1/2 "	42	29	28
"	11:30	6 1/2 "	23	75	42
"	1:30 P.M.	4 1/2 "	16	14	9
"	3:30	2 1/2 "	0	0	0

*At 40° F. Plants were removed from the incubators at 6:00 P.M.

TABLE VII

THE ACTION OF STOMATA ON THE TIPS
OF THE LEAVES OF WHEAT
IN MARCH, 1956

Time of observation*	Percent of stomata open in:	
	Triumph	Comanche
10:30 A.M.	3	1
11:30	3	1
12:30 P.M.	13	45
1:30	34	31
2:30	40	42
3:30	45	9
4:30	3	0

*Plants were held at 40° F. and supplied with only artificial fluorescent light for a period of 12 hours before the first observations were made.

TABLE VIII

THE SEVERITY OF RUST INFECTION FOLLOWING INOCULATION AT VARIOUS TIMES OF THE DAY DURING MARCH, 1956

Variety	Time of inoculation	Length of wetting period*	Average number of pustules per 10 leaves inoculated with Race:		
			9	15	105A
Triumph	7:30 A.M.	8 1/2 hours	225	147	240
"	9:30	6 1/2 "	178	97	121
"	11:30	4 1/2 "	5	1	2
"	1:30 P.M.	2 1/2 "	0	0	0
Comanche	6:30 A.M.	8 1/2 "	112	257	230
"	8:30	6 1/2 "	96	216	182
"	10:30	4 1/2 "	86	0	3
"	12:30 P.M.	2 1/2 "	0	0	0

*At 40° F. Plants of Comanche were removed from the incubator at 3:00 P.M. and plants of Triumph at 4:00 P.M.

of the wetting period than with stomatal opening. However, the inoculations on Triumph at 11:30 A. M. which were most effective in producing infection in January did not produce any infections in March even with a 4½ hour wetting period. Infections have been produced at this temperature (40°F.) when the wetting period was as short as 3 hours. Similarly, with one exception, the 10:30 A. M. inoculations in March were ineffective on Comanche. The exception was with race 9, which has been shown to germinate more rapidly than other races at low temperatures (8).

Race Competition

Following these studies on the function of stomata a third attempt was made to study race competition. This time only fresh spores of race 9 and 15 from moderately infected leaves were used. Since the periodic movement of the stomata was observed to be different at different times of the year, the plants in these tests were given a wetting period of 24 hours to be sure that the stomata were open during the time that free water was present. When inoculations were made on the test varieties the plants were held at 40°F. for 12 hours prior to inoculation and during the wetting period following inoculation. Separate series of inoculations were made on the two wheat varieties Triumph and Comanche. The race mixture was made so as to have 50 percent viable spores of race 9 and 50 percent viable spores of race 15 at the start. After each generation a sample of the harvested spores was inoculated on the differential varieties Democrat and Loros and the susceptible pustules on each variety were counted. Inoculations on the differential varieties were made at 65°F. throughout the incubation period. Following the first generation (Table IX) the two races remained in equal proportion on the

variety Triumph, but on Comanche race 9 had already started to dominate the mixture. Race 9 became progressively dominant on both varieties and by the end of the fourth generation this race composed over 90 percent of the mixture, no matter which variety was used.

TABLE IX

THE RELATIVE SURVIVAL OF WHEAT LEAF RUST RACES 9 AND 15 AFTER 4 UREDIAL GENERATIONS.

Generation	Test Variety	Average percent of race 9	Average percent of race 15
1	Triumph	50.8	49.2
	Comanche	63.6	36.4
2	Triumph	85.8	14.2
	Comanche	80.6	19.4
3	Triumph	85.8	14.2
	Comanche	90.4	9.6
4	Triumph	92.8	7.2
	Comanche	90.7	9.3

DISCUSSION

The various problems encountered in this study tended to restrict the scope of investigations upon competitive ability. It was particularly unfortunate that race 105A was eliminated from the study because of the potential importance of this race. However, many important points were found which will certainly be helpful in any future studies on race competition, particularly if minimal temperatures are used. For instance, when race mixtures are made it is essential that the percent of viable spores of each component race be known. It was found in the work reported here that leaf rust urediospore viability not only decreased with age but also became erratic so that it was almost impossible to obtain an average germination percentage for a given lot of stored spores. Consistent spore germination could only be obtained with freshly harvested spores.

Three other facts relative to wheat leaf rust urediospore germination were found. First, preliminary tests indicated that a self-inhibitor was present in the urediospores of leaf rust similar to that found by Allen (1) in wheat stem rust urediospores. Infection of plants held at temperatures where germination is considerably retarded may be influenced by such an inhibitor. Secondly, it was found that spores produced on leaves which had a large number of pustules did not germinate as well as those produced on leaves where the number of pustules was smaller. This fact is important where maximum spore germination is desired, and also may be of considerable importance in epidemiological investigations.

Thirdly, it was found that a much smaller percent of the urediospores of wheat leaf rust produced at 40°F. were viable than those produced at 60°F. This same phenomenon was reported by Straib (2) for the urediospores of Puccinia glumarum. Here again, is a fact which must be considered where maximum spore germination is desired as well as in studies of epidemiology.

The function of stomata in wheat leaf rust infections was studied. Hart and Forbes (10) and Caldwell and Stone (3) have reported that stomatal function played no part in wheat leaf rust infections. Their studies were all made at temperatures between 64 and 77°F. The investigations reported here deal with wetting periods made at 40°F., and at that temperature it appeared that infection by wheat leaf rust urediospores was definitely impeded by closed stomata. Histological examinations were not made, but abundant infection resulted only if the inoculations were made at such a time that the spores were actively germinating and the stomata were open. It is possible that at this low temperature the metabolic activity of the germinating spore is slowed to such an extent that it is unable to penetrate the closed guard cells.

When the problems of spore germination and penetration were at least partially solved one investigation of race competition was completed. An equal mixture of races 9 and 15 was carried through 4 uredial generations on the hard red winter wheat varieties Triumph and Comanche. Race 9 dominated this mixture after the first generation on Comanche and after the second generation on Triumph. It is interesting to note that these data are supported by isolations from field collections. Race 9 has been more frequently isolated from Comanche than from Triumph at any given location and in any given year.[3]

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[3] Unpublished data, Department of Botany and Plant Pathology, Oklahoma A. & M. College, Stillwater, Oklahoma.

It was not surprising that race 9 eventually became the dominant race on both varieties. Epperly (8) showed that urediospores of race 9 had the ability to germinate more rapidly than certain other races at low temperatures. Similarly, race 9 dominated the mixture of races reported by Irish (14). These data all lend support to the fact that race 9 is a very competitive race which is capable of dominating race mixtures on at least three universally susceptible hard red winter wheat varieties both at optimum and minimum temperature conditions. The data also indicate why race 9 persisted for so many years in the Central and Southern Great Plains.

SUMMARY

1. Wheat leaf urediospores stored at 35 to 40°F. longer than 90 days germinated erratically. Consistently high germination was obtained only from freshly harvested spores.

2. Soaking wheat leaf rust spores in water reduced the germination and indicated the presence of a self-inhibitor similar to that found in wheat stem rust urediospores.

3. Urediospores harvested from heavily infected leaves were less viable than those harvested from lightly infected leaves. Also, spores produced at 40°F. were less viable than those produced at 60°F.

4. More accurate determinations of a race mixture could be made when differential varieties susceptible to only one component were used. Counts of susceptible pustules could be made more accurately than counts of resistant pustules when the rust infection of the differential variety was high.

5. The stomata of the varieties Triumph and Comanche grown in the greenhouse were open from approximately 3:30 P. M. to 6:30 P. M. during January when the natural day length was about 10 hours. In March, when the natural day length was about 12 hours the stomata of the variety Triumph were open from approximately 1:30 P. M. to 4:30 P. M. and those of the variety Comanche were open from approximately 12:30 P. M. to 3:30 P. M.

6. Twelve hours in the moist chamber at 40°F., with or without artificial fluorescent light, did not influence the rhythmic action of the stomata.

7. At 40°F. maximum wheat leaf rust infection did not occur unless inoculation was timed so that the spores were actively germinating while the stomata were open.

8. Wheat leaf rust races 9 and 15 were mixed in equal proportions and carried through 4 uredial generations on the varieties Triumph and Comanche. After the 4 uredial generations, race 9 composed over 90 percent of the mixture on both varieties. Race 9 became dominant on Comanche after the first generation and on Triumph after the second generation. These studies were made using a 24-hour wetting period at 40°F. followed by incubation at 65°F.

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