SOME FACTORS INFLUENCING SURVIVAL AND DEVELOPMENT OF CERTAIN PHYSIOLOGIC RACES OF WHEAT LEAF RUST

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Bachelor of Science

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1951

Submitted to the Faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1953

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ACKNOWLEDGMENTS

The writer wishes to express his gratitude to Dr. H. C. Young, Jr. for constructive criticism during the course of study and in the preparation of the manuscript. Acknowledgment also is due Dr. Walter W. Hansen and Dr. John E. Thomas for offering helpful suggestions pertaining to the manuscript.

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INTRODUCTION

Leaf rust of wheat, caused by Puccinia rubigo-vera tritici (Eriks. & Henn.) Carl., is often regarded by farmers as a mild disease, common in most wheat growing areas, but rarely associated with severe crop losses. However, in Oklahoma the average annual loss approaches 5 per cent of the crop, and several times in the past this disease has caused a loss of 20 per cent or more in yield. The 1938 epiphytotic in Oklahoma resulted in a monetary loss to wheat growers of over $12,000,000. Heavy losses also were reported that year from Texas to Canada (4).

Leaf rust frequently is present all season, gradually increasing in intensity until the crop matures. In Oklahoma, leaf rust usually becomes noticeable on winter wheat in November. It overwinters on wheat most years and can be found sporulating during warm periods throughout the winter months. The severity of infection during the fall, however, is seldom correlated with the intensity of the rust in early spring. Rather, it is the amount of sporulation and reinfection that occurs during the late winter months that is important in determining the severity of rust that will be present the following spring. This period from December to March has been termed the "critical period" for determining the amount of rust development during the spring growing season. Chester (5,6) based

a method of predicting wheat leaf rust epiphytotics on the information concerning the inoculum potential gathered during this period and pointed out that in Oklahoma the weather from April until harvest usually is favorable for rust development. Therefore, a spring epiphytotic would require favorable weather for inoculum increase from December to April.

Physiologic races of the leaf rust fungus, practically identical morphologically, are distinguished by their pathogenicity on a set of differential host varieties. Certain minor pathogenic differences also may distinguish biotypes within established races (19). However, it is conceivable that races, and perhaps even biotypes, may differ in other properties. During the winter period when weather factors are critical or limiting there exists the possibility of differential survival ability of one physiologic race or biotype as compared to another. Even relatively minor differences in survival ability could conceivably allow the better adapted race or biotype to become more prevalent than other, perhaps more virulent, races due to an ability to infect and sporulate more freely during this critical winter period.

Two observations lend support to such a hypothesis. First, race 105 has been completely replaced by biotypes 105A and 105B in Oklahoma (21); and second, race 9 continues to constitute about 15 per cent of the race isolations from Oklahoma collections, in spite of the fact that there are at least three varieties widely grown in the state which are resistant to it.

The purpose of this investigation was to study the effect of certain weather factors, which might influence survival during the

winter months, on individual races of leaf rust and to more critically examine the combined effect of these factors on the field development of rust.

Races of leaf rust which are unable to survive during the winter period may cause some infections when spores are blown in from other regions. Those infections, however, are usually too late to have any appreciable effect on yield. Such is the case with wheat stem rust in Oklahoma, and losses to this disease are seldom encountered.

LITERATURE REVIEW

Since the discovery of physiologic specialization in Puccinia rubigo-vera tritici (18), much work has been done to add to our knowledge of the properties of the fungus. Mains and Jackson (18), as early as 1926, suggested that physiologic races of leaf rust might differ in the range of temperatures through which their spores would germinate, in the ability to develop within the host under various climatic conditions, and in the ability to survive the winter within the host tissue. If this were true, these factors might exert considerable influence on the relative prevalence of various races.

Survival ability may be influenced by many things, one of the most conspicuous being the rapidity of urediospore germination. Temperature and moisture are probably the two most important weather factors which affect spore germination. Johnson (14) found that the cardinal temperatures for germination of urediospores of leaf rust are 2° C. minimum and 31° C. maximum. The optimum temperature, based on vigor of the germ tubes, was 12-17° C. These findings were in accord with Mains and Jackson (18) who found that there was little germination at 5° C. or less, or at 30° C. or above. They found that the temperature most favorable for urediospore germination was from 16 to 20° C.

Relative humidity of near 100 per cent, or free water, has been found to be necessary for urediospore germination. Clayton (9),

studying the urediospore germination of Puccinia coronata, P. graminis tritici, and P. graminis avenae, found that the mean per cent of germination on glassware was high in free water, somewhat lower at a relative humidity of 100 per cent, considerably lower at 99 per cent, and practically zero at 98 per cent.

Some reports of races of rusts differing in their requirements for urediospore germination have been made. Hursh (13) studied two races of P. graminis tritici differing in their parasitic behavior and found that they showed considerable difference in urediospore germination at various temperatures and hydrogen-ion concentrations. He found that the race which was more limited in host range had less tolerance of extremes of hydrogen-ion concentration and temperature than the race exhibiting a wider host range.

Cassell (2) reported on urediospore germination of stem rust of wheat at temperatures of 2, 9, 20 and 30° C. He found that, in general, 20° C. was the most favorable temperature, followed by 9, 30, and 2° C. Race 34 was found to germinate well over a wider range than the other races studied, but for this race germ tube growth was poorest at 20° C. On the basis of germination tests, he concluded that race 36 was best adapted to high temperatures but only partially tolerant to cold temperature. Race 58 ranked second in rate of spore germination at the higher temperatures but was even less adapted to cold than race 36. Race 56 performed best at the optimum of 20° C., while races 38 and 11 were the most tolerant to low temperatures.

In his studies on the survival ability of races of oats stem rust, Hingorani (11) found that the optimum temperature at which races 2, 7,

and 8 germinated was the same. However, when the temperatures were beyond the optimum range in either direction, urediospores of race 8 germinated in greater number and more rapidly than those of either race 2 or race 7. He concluded that race 8 should be able to establish and maintain itself over a wider range of temperature conditions, insofar as establishment and maintenance depend upon urediospore germination.

Hassebrauk (10) compared the germination of spores of leaf rust races XI, XIII, and XIV in water and different concentrations of sugar solutions ranging from 0.3 to 0.6 per cent. His findings enabled him to differentiate each of these races from the others by their differences in germination in these various solutions.

Another factor important in the establishment of leaf rust is the length of the incubation period required. Butler (1) found that leaf rust infection occurred in the greenhouse moist chamber within 3 to 4 hours after inoculation at 18 to 20° C.

Chester and Jamison (8) found that race 77 required one to two days longer from the time of inoculation to the production of spores than did race 13. They suggested that race 77 of leaf rust would probably be unable to compete successfully with race 13 due to this differential incubation requirement.

Johnston (15) discovered an aberrant physiologic race of P. rubigo-vera tritici differing from other races in length of incubation period and in spore color. This aberrant race appeared in the field late in the season and was found to require from 15 to 19 days to produce uredia, compared to 8 to 10 days for other normal races.

The aberrant race also required 23 days to reach full development, while the common race 9 required only 13 days. Uredia produced by the aberrant race were small compared to those produced by race 9. In addition, the spores of the aberrant race were light orange in color compared to the orange red of other races.

Cassell (3) found that races 11, 21, 34, 36, 38, 49, 56, and 59 of the stem rust of wheat produced no uredia when inoculated, incubated, and kept at 2° C., although flecks appeared in 21-30 days. Race 38, alone, survived 85 days at 2° C. and later produced pustules when the plants were moved into the greenhouse. At slightly higher temperatures, however, race 56 thrived much better than race 38. In comparative tests between races 36 and 56 on Ceres wheat, race 36 produced the heaviest infection at low temperatures and race 56 the heaviest infection at higher temperatures.

Still another factor important in the development of leaf rust is the ability to overwinter or withstand exposure to low temperatures. Chester (5, 6, 7) pointed out that the weather of December to February influenced both directly and indirectly the survival of the rust, and that the period from February through March influenced reproduction. The build-up of inoculum takes place logarithmically (6); hence, the amount of overwintering rust plays an important part in the early spring increase of inoculum.

Johnston and Mains (16) made collections of leaf rust in the western plains area during the period from 1926 to 1931. They found that over the entire period race 9 was the predominant race. The reason for the dominance of race 9 is not definitely known, but it is

reasonable to assume that the race is well adapted to the area. They found that race 9 was the one race that most frequently overwintered in Kansas, Oklahoma, and Texas. It was the most frequently encountered race in collections made in late fall, winter, and early spring in this area. The investigators suggested on the basis of their data for the 5-year period that race 9 overwintered more abundantly than other races.

Huffman and Johnston (12) compiled data from leaf rust collections made during the period from 1940 to 1951. The collections were made in fields in all but 21 of the 105 counties of Kansas. They found that four major rust race groups comprised 88 per cent of the total isolates for the 12-year period, the order of abundance being race groups: 9, 126, 5, and 15. Certain races were found to be more abundant in one part of the state than others. Intrastate distribution of the four race groups was based on a division of the state into 6 more or less equal areas. Race 5 was equally prevalent in all of the sections except the northeast. Race 9 was found to be most prevalent in the western sections and least prevalent in the east. Race 15 was most prevalent in the northwest and in the southeast, while race 126 was most prevalent in both of the eastern sections. The prevalence of race 9 in the western sections where winter weather conditions are more rigorous would support the hypothesis that race 9 is better able to withstand extremes of weather than other races. They also found that race 9 was more abundant in collections made from September 1 to December 31 than in the spring, while races 5, 15, and 126 were more abundant in the spring collections than in the fall.

Races 105 and 105A, both of which may be considered in Johnston's race group 126, and race 9 were used in this study of various factors influencing spore germination, incubation, and overwintering ability.

MATERIALS AND METHODS

Races 9 and 105 were obtained in 1950 from C. O. Johnston, who had made the collections and identifications. They have been maintained in a pure condition at Oklahoma A. and M. College since that time. The biotype of race 105 designated as 105A was isolated in 1951 from rust collections made in Oklahoma. It should be mentioned here that race 105 and biotype 105A are indistinguishable on the accepted set of standard differentials in Oklahoma. Race 105 and biotype 105A are separated on the basis of their reaction on the variety Westar, C.I. 12110 (21).

Race 9 was selected because it has been one of the more prevalent races present in Oklahoma over the past several years, and because of the many indications that it possesses more overwintering ability than many of the other races. The biotype 105A was selected because of its importance on some of the newer wheat varieties in Oklahoma, and because it seems to have completely replaced the typical race 105 in collections made in Oklahoma. Race 105 was used for comparison with 105A for this reason. For the sake of continuity, biotype 105A will be designated simply as "race 105A" for the remainder of this discussion.

Spores of these races for all of the tests were produced on the wheat variety Malakof, C.I. 4898, which is equally susceptible in the seedling stage to all three races. Spores used in each of the tests were of approximately the same age.

Wheat seedlings were inoculated 10 days after planting in all cases. The primary leaves of the seedlings were stripped between the moistened thumb and forefinger before inoculation. The inoculations were made by distributing the spores over the surface of a beaker of water. The pots were then inverted and the leaves dipped into the beaker. As the leaves were withdrawn the spores adhered to the leaf surface. After dipping, the plants were placed in a galvanized iron moist chamber with a glass top similar to the description given by Stakman et.al. (19). The plants and the inside of the chambers were sprayed with water before the top was replaced. A cover was placed over the glass to prevent sunlight from affecting the temperature of the chamber. Unless otherwise stated, the plants remained in the chambers for 24 hours before being removed and placed in open topped muslin isolation cages on the greenhouse bench.

Twelve to fourteen days after inoculation the pustules were fully developed. Spores were then harvested by holding the pot horizontally above a sheet of white bond paper and gently tapping the leaves with a small metal rod. The spores were shaken into a test tube for storage under refrigeration.

The urediospore germination tests were made on water agar (17). Approximately 10cc. of a one per cent distilled water agar was poured into each Syracuse dish and allowed to harden. The dishes were stacked to prevent evaporation and placed at the desired temperature for at least 16 to 24 hours before the spores were sown on the surface of the agar.

Dry urediospores were puffed onto the agar by means of a small aluminum cyclone separator similar to that described by Tervet et.al. (20). A bulb from a No. 15 DeVilbiss atomizer was used as the air source. The dishes were then replaced in the temperature chambers in stacks and withdrawn for germination counts at the desired time. The germination counts were made at low power magnification on areas of the dish containing approximately 50-75 spores per microscope field. Three hundred spores were counted for each race at each temperature and time period. Tests for general urediospore viability were made at 20° C. for 24 hours.

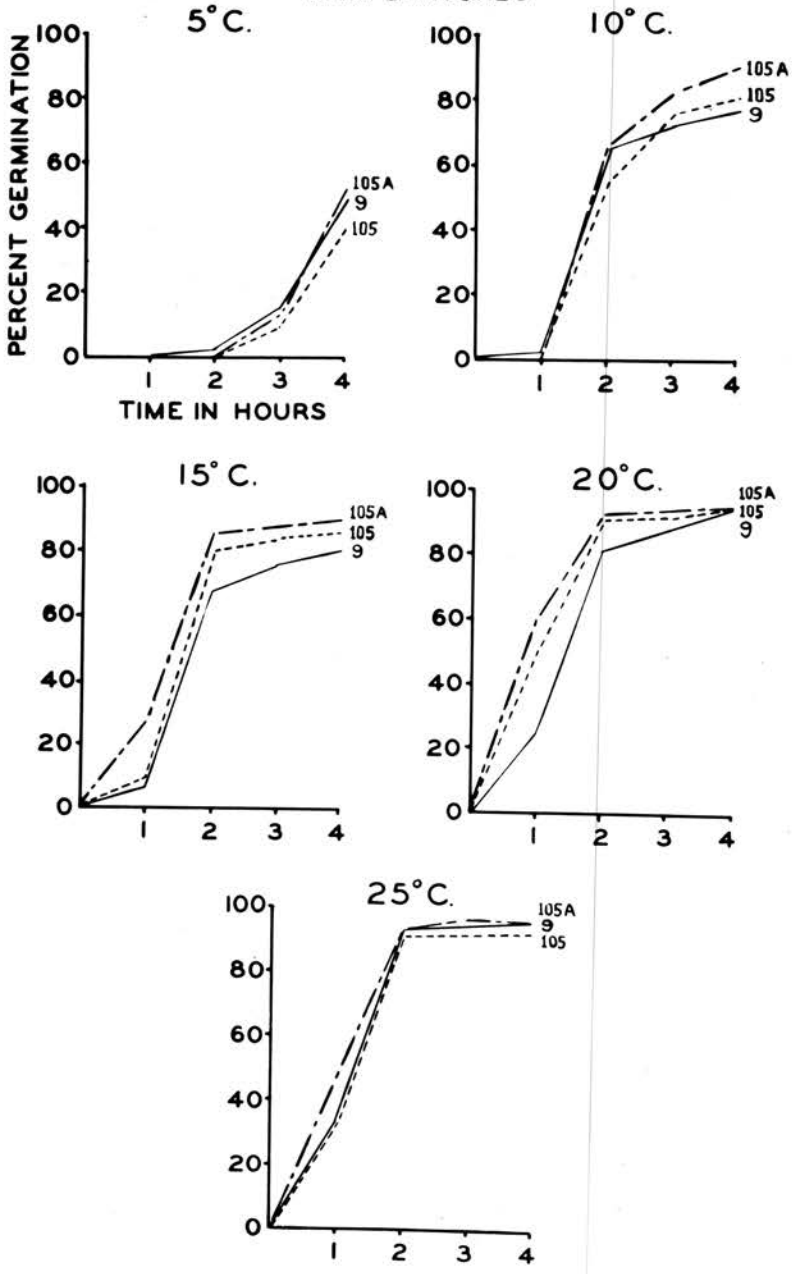
Race identifications from field collections were made in the manner described by Stakman et.al. (19). The differential varieties were: Democrat, C.I. 3384, Malakof, C.I. 4898, Webster, C.I. 3780, Loros, C.I. 3779, Westar, C.I. 12110, Westar Selection, C.I. 13090, and Brevit, C.I. 3778.

RESULTS

Germination of urediospores. The spores used in these experiments were all harvested 12 days after inoculation. However, some spore lots were stored 4 to 5 days longer than others. Spores were germinated at temperatures of 5, 10, 15, 20, and 25° C. Refrigerators were used to maintain the 5 and 10° C. temperatures, and electric incubators placed inside cold storage rooms maintained the 15, 20, and 25° C. temperatures. In no case did the temperature fluctuations exceed one degree plus or minus. The per cent of germinated spores was counted at the end of 1, 2, 3, and 4 hours. Three replicates of 300 spores, or a total of 900 spores, were counted for each race at each temperature and time period. A spore was considered germinated when germ tube growth could be noted. The results are indicated in Figure 1.

At 5° C. race 9 had started germination at the end of 2 hours, while with races 105 and 105A no germination was observed until the end of 3 hours. After 4 hours races 9 and 105A had both germinated in greater percentage than race 105. At 10° C. race 9 again started germination first. A small per cent of the spores of race 9 had germinated after one hour, but no germination of races 105 and 105A was observed until the end of 2 hours. At the end of 4 hours race 105A had germinated better than either of the other two races. At 15° C. race 105A germinated most rapidly and attained a higher maximum germination

FIGURE I. THE RATE OF GERMINATION OF UREDIOSPORES OF LEAF RUST RACES 9, 105, AND 105A AT VARIOUS TEMPERATURES



at the end of 4 hours than the other two races. At 20° C. races 105 and 105A both germinated more rapidly than race 9, although all three races attained the same maximum germination at the end of 4 hours. At 25° C. there were no differences between races. Twenty degrees was the optimum temperature for rapidity of germination of races 105 and 105A, but race 9 germinated more rapidly at 25° C. than at 20° C.

Additional tests were made at 3 and 5° C. At 3° C. race 9 had started germination after 5 hours. At the end of 24 hours, 2 to 3 per cent of the spores of race 9 had germinated, but no germination was noted in races 105 and 105A. At 5° C., as in the preceding tests at the same temperature, race 9 started germination before the other two races, but at the 24 hour period race 105A was slightly higher in total germination. Again race 105 had the smallest total germination at this temperature. Maximum germination of all these races was reached in approximately 5 hours at 5° C. After 5 hours the only difference observed was in length of the germ tubes.

The results of these tests indicate that at temperatures beyond the optimum range, urediospores of race 9 germinate faster and better than those of races 105 and 105A. This is especially true at temperatures approaching freezing. It was significant that although race 9 started to germinate quicker at low temperatures than the other races tested, it required a higher maximum for rapidity of germination. Such a phenomenon may explain why race 9 propagates more freely in the fall and early winter but is surpassed by other races in the spring.

Exposure of urediospores to sub-freezing temperatures. Spores of races 9, 105, and 105A were exposed to temperatures of minus 18° C. for 24, 48, and 72 hours by spreading them on paper in a covered Petri dish. At the end of the exposure periods the viability of the spores was determined by incubation for 24 hours at 20° C. Unexposed spores also were incubated as a check on the loss of viability due to exposure to the sub-freezing temperatures. This experiment was duplicated. The results are given in Table 1.

Race 9 retained greater viability over the entire period of exposure than either race 105 or 105A. Race 105A retained slightly greater viability than race 105 after 24 hours of exposure, but these two races were approximately equal in viability after 48 and 72 hours of exposure.

Table 1. The viability of urediospores following exposure to sub-freezing temperatures (-18° C.)

Race	Average Per Cent Germination for 2 Tests			
	Check	Length of Exposure in Hours		
		24	48	72
9	89	23	11	12
105	86	10	7	4
105A	88	16	6	6

Length of incubation period. The wheat variety Malakof was used throughout these tests because it appeared to be equally susceptible to races 9, 105, and 105A. Ten days after planting, the seedlings were inoculated as previously described and placed in moist chambers. At the end of 2, 4, 6, 10, 16, and 24 hours, two pots of each race were

removed from the incubators and placed on the greenhouse bench. Upon removal from the incubator, each pot was lightly shaken to remove as much water as possible to facilitate rapid drying of the leaves. The temperature was maintained at approximately 20° C. during the 24 hours immediately following inoculation. Temperatures ranged from 16 to 25° C., averaging approximately 20° C., during the 12 day period after inoculation.

Four days after inoculation, flecks were visible on all plants except those removed after two hours of incubation (Table 2). After 5 days, flecks were visible on all plants. Twelve days after inoculation counts of the number of pustules were made on the plants removed after 2 and 4 hours of incubation. So many pustules were produced on plants incubated over 4 hours that pustule counts were of no value.

Following 2 hours of incubation, race 9 produced 3 to 4 times as many pustules as the other races. After incubation for 4 hours, both races 9 and 105A produced over 5 times as many pustules as race 105.

Table 2. Relative intensity of infection with leaf rust races 9, 105, and 105A following various lengths of the incubation period.

Race	Incubation period in hours	Days between inoculation and appearance of flecking	Average number of pustules per leaf 12 days after inoculation
9	2	5	1.0
	4	4	17.0
	6	4	∞
	10	4	∞
	16	4	∞
	24	4	∞
105	2	5	0.2
	4	4	3.1
	6	4	∞
	10	4	∞
	16	4	∞
	24	4	∞
105A	2	5	0.3
	4	4	16.9
	6	4	∞
	10	4	∞
	16	4	∞
	24	4	∞

In a second series of tests the period of incubation in the moist chamber was reduced to a minimum of one hour and a maximum of three hours, with 15 minute intervals between the time the inoculated plants were removed. These results are shown in Table 3.

No infection took place with incubation periods of less than 2 3/4 hours. After 2 3/4 and 3 hours of incubation a few pustules were produced by races 9 and 105A but none by race 105. The incubation temperature for this test was 21° C., while greenhouse temperatures

averaged 25° C. in the daytime and 20° C. at night. Higher average temperatures during and after incubation probably account for the somewhat longer minimum period of incubation in this experiment.

Table 3. Infection produced by leaf rust races 9, 105, and 105A after incubation for short periods.

Race	Incubation period in hours	Total number of pustules 12 days after inoculation
9	2 1/2	0
	2 3/4	2
	3	3
105	2 1/2	0
	2 3/4	0
	3	0
105A	2 1/2	0
	2 3/4	2
	3	3

A third series of tests on the effect of variable incubation periods on infection was made at controlled temperatures. Three refrigerated rooms were used where the temperatures were 4±1° C., 6±2° C., and 11±1° C. Both the plants and the moist chambers were placed in the refrigerated rooms 24 hours before inoculation. Following inoculation, plants were removed after 6, 9, 12, and 24 hours of incubation, placed in the greenhouse, and observed daily for symptoms. The temperature in the greenhouse averaged approximately 20° C. during the following 10 day period.

Twelve hours incubation at 4° C. was sufficient to produce infection by races 9 and 105A, but no infection was produced by race 105 until after 24 hours of incubation (Table 4). At 6° C. infection was obtained with all three races following 12 hours of incubation; however, there were noticeably less pustules produced on plants inoculated with race 105. All three races produced infection following 6 hours of incubation at 11° C.

Table 4. Infection produced by races 9, 105, and 105A following incubation at different temperatures.

Hours of incubation period	Severity of infection								
	4±1° C.			6±2° C.			11±1° C.		
	9	105	105A	9	105	105A	9	105	105A
6	0[1]	0	0	0	0	0	+++	++	+++
9	0	0	0	0	0	0	++++	++++	++++
12	+	0	+	++	+	++	++++	++++	++++
24	+++	++	+++	+++	++	+++	++++	++++	++++

1 - 0 = No infection
+ = Trace to 1% on the Cobb scale
++ = 5 to 10% "
+++ = 20 to 30% "
++++ = 40 to 50% "

Relationship of certain weather factors to leaf rust severity. During October, November, and December, 1951, and January, February, and March, 1952, rust pustule counts and collections were made periodically on the wheat variety Cheyenne, C.I. 8885. Cheyenne is known to be susceptible to all of the races of leaf rust presently found in Oklahoma. One thousand tillers were examined each time a count was

made, and the total number of pustules observed was recorded. Weather data were obtained from recording instruments adjacent to the plots where the rust pustule counts were made. Temperature, relative humidity, and rainfall for the six month period were plotted together with the rust pustule counts. The resulting graph (Figure 2) was then examined to determine if the variation in rust intensity, as indicated by the pustule counts, could be correlated with any of these weather factors or particular combinations of factors.

It was found that a correlation between certain weather conditions and an increase in the number of pustules depended on: (1) the amount of inoculum present, and (2) on the occurrence of periods during which the relative humidity remained at 100 per cent, and preferably when free water also was present. The length of such wetting periods which was required to increase the number of pustules depended on the average temperature during and immediately following the wetting periods.

Referring to Figure 2, the first increase in the number of rust pustules occurred between November 9 and 28. Prior to that time there were four periods when spore germination and penetration could have occurred (October 22, 23, 26, and 28). The appearance of the pustules was undoubtedly somewhat delayed by the freezing temperatures which were encountered during the first week of November. Between November 28 and December 12, the number of pustules continued to increase. The wetting periods of November 11 and 15, and possibly November 24, must have contributed to this increase. The number of rust pustules gradually declined from December 12 to January 9. There were no satisfactory wetting periods between November 24 and January 20, and this,

coupled with the cold and freezing temperatures during late December which undoubtedly destroyed some leaf tissue, contributed to the decline in pustule count. Little change in rust development was noted from January 9 to February 1, and again no satisfactory wetting periods occurred. Between February 1 and February 11, however, a sharp increase in the number of pustules was noted. Only one or possibly two wetting periods were encountered which could have contributed to this increase, one on January 20, and possibly one on January 15. It will be noted, however, that the level of infection was fairly high before the abrupt rise started. Between February 9 and March 3, little change was observed in rust development, the wetting periods of early February being too short or too cool for satisfactory infection. A small increase in pustule count on March 13 can be attributed to the wetting periods of late February, probably on the 24th. and again March 1. The temperatures were rather low, however, so that the increase in pustule count was small considering the level of infection. The decline in number of pustules on March 23 undoubtedly was caused by the freezing temperatures about March 15 and by the killing of the older leaves by the rust. The sharp rise in pustule count on March 31 can again be traced to extended wetting periods on March 9 and 17.

Although it is realized that such studies must be made over a period of years before the results can be regarded as conclusive, it is evident from the data presented that the development of rust during the fall and winter period in Oklahoma depends on a number of factors, the principal one being the occurrence of wetting periods satisfactory for spore germination and penetration. The overwintering of rust as mycelium in infected leaves was of minor importance

during 1951-1952. These studies have indicated the necessity of more investigation into the problem of what constitutes a satisfactory wetting period.

Identification of races from field collections. Race identifications were made from collections taken at approximately two week intervals during the period from October 20, 1951, to June 6, 1952, to determine the races of rust present at different times during the season. Collections were made from the wheat variety Cheyenne. The following races were identified:

Race 15	27 collections	(45 per cent)
Race 5	19 collections	(31 per cent)
Race 9	12 collections	(20 per cent)
Race 21	1 collection	(2 per cent)
Race 126	1 collection	(2 per cent)

No seasonal predominance of any one race was indicated in these studies.

It was noteworthy that race 105A was not isolated at any time during the period even though Cheyenne is known to be completely susceptible. Collections of leaf rust from the entire state in May, 1952, yielded approximately 20 per cent race 105A with isolations from all varieties considered, or approximately 6 per cent if the varieties Westar and C.I. 12517, which are susceptible only to this race, are excluded. Race 105A was obtained from all varieties except Cheyenne and Pawnee in those studies. There seems to be evidence here of a varietal preference for certain races even among completely susceptible varieties and in the absence of competition for space.[1]

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

DISCUSSION

The effect of a limited number of factors concerned with the survival and development of wheat leaf rust was studied. The relationship of temperature and time to urediospore germination and to the required length of the incubation period was studied using three races for comparison. In addition, the effect of certain weather factors on fall and winter survival and development in the field was studied.

The germination tests indicated that there are physiological differences between races 9, 105, and 105A. It was found that race 9 started to germinate more rapidly at cooler temperatures than either race 105 or 105A. Although race 9 germinated more quickly at 5° C. and 10° C., it was found to have a higher optimum temperature for rapidity of germination than either of the other two races studied. Race 9 also was able to withstand exposure to freezing temperatures better than races 105 and 105A. All of these factors would tend to aid in the development of race 9, particularly in the more western sections of the great plains area. This would be in agreement with Huffman and Johnston (12) who found that race 9 appeared to be more abundant during the fall and winter period from September to the end of December.

The results of the tests on incubation requirements indicated that, at temperatures near the optimum, the minimum incubation period required for infection is from two to three hours. This is somewhat shorter than the 3 to 4 hour period reported by Butler (1). It was also noted that

there were slightly different incubation period requirements for races 9, 105, and 105A. In all cases race 105 required a longer period. Such a condition might conceivably be one explanation why biotype 105A has replaced the typical race 105 in Oklahoma.

Comparing the data acquired from both the incubation and germination tests, one question remains unanswered. At temperatures near the optimum, race 9 germinated more slowly than races 105 and 105A, yet it produced heavier infection in a shorter time at these temperatures in the incubation tests than the other races.

Perhaps the most significant result of these studies was the ability to correlate rather closely the weather data over an extended period with the development of leaf rust severity. It appeared that the occurrence of favorable wetting periods (periods of time during which the relative humidity was 100 per cent and when free water was present) are more important in rust development than overwintering of the fungus as mycelium in plant leaves. This phase of the problem needs further study since the period covered in this investigation included only one season. Some method of measuring the time during which dew or free water is present is needed since it appeared that free water was of prime importance for germination and infection. Hygrometer readings of 100 per cent do not always reflect the presence or absence of free water.

The attempt to determine a seasonal prevalence of certain races was not successful; however, it is believed that in this case the number of identifications was too small to indicate a trend. Such studies also would need to be made over a period of years to compensate for the variations in weather conditions.

SUMMARY

1. Some physiological differences between leaf rust races 9, 105, and 105A were demonstrated.

2. Urediospores of race 9 germinated more rapidly at cool temperatures than spores of races 105 and 105A, but race 9 was found to require a higher optimum temperature for rapidity of germination than the other two races tested.

3. Spores of race 9 retained greater viability after exposure to minus 18° C. than races 105 and 105A. Race 105 retained less viability than race 105A after 24 hours exposure, but the viability of these two races was about equal after 48 and 72 hours of exposure.

4. The minimum incubation period required for infection by races 9, 105, and 105A is two to three hours at 20° C., however, races 9 and 105A produced infection with a shorter incubation period, at both optiumum and cool temperatures, than race 105.

5. From a correlation of certain weather data with rust development it was found that any increase in the number of rust pustules was dependent on the amount of inoculum present and on the occurrence of favorable wetting periods. The length of wetting period required depended on the average temperature during and immediately following the period.

6. No seasonal prevalence of a particular race was noted. Races 105 and 105A were not isolated from field collections taken from the

variety Cheyenne in spite of the fact that race 105A was common on other varieties during the year. In the greenhouse Cheyenne is completely susceptible to both of these races.

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THESIS TITLE: SOME FACTORS INFLUENCING SURVIVAL AND DEVELOPMENT OF CERTAIN PHYSIOLOGIC RACES OF WHEAT LEAF RUST.

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The content and form have been checked and approved by the author and thesis adviser. The Graduate School office assumes no responsibility for errors either in form or content. The copies are sent to the bindery just as they are approved by the author and faculty adviser.

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