EFFECT OF CERTAIN ENVIRONMENTAL CONDITIONS ON THE IDENTIFICATION OF PHYSIOLOGIC RACES OF *PUCCINIA RECONDITA TRITICI*

by

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B. S., Kansas State University, 1951

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Botany and Plant Pathology

KANSAS STATE UNIVERSITY

1960

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INTRODUCTION

Puccinia recondita tritici Rob. ex Desm., commonly called leaf rust, is one of the most abundant and widely distributed diseases of wheat, and is the cause of some loss in yield nearly every year. In some years, the losses may be quite severe (17), depending mainly on favorable environment and extensive acreage of susceptible varieties. So far, the only practical method of control is breeding for resistance. This method has been used extensively for the control of leaf rust as well as other rusts.

Breeding for resistance is complicated by the occurrence of many physiologic races of the fungus. The latest revision of the International Register of physiologic races of leaf rust of wheat (15) lists 163 described races. Of these, at least 46 have been found in Kansas since 1940 (11). However, only a few of these races occur each year in considerable abundance. If a breeding program is to be successful, these races must be known and carefully considered.

Studies on the identification of the physiologic races of leaf rust of wheat have been carried on at the Kansas Agricultural Experiment Station for many years. Considerable difficulty has been encountered in the identification of races at different times. This appeared to be due principally to the reaction of both host and organism under different environmental conditions, particularly at different temperatures. Until recently no studies had been made in Kansas on the identification of

physiologic races when infections were developed on different varieties under different environmental conditions.

The studies discussed herein deal primarily with the effects of changing temperatures on the identification of physiologic races of leaf rust of wheat.

REVIEW OF LITERATURE

Leaf rust has long been recognized as a common and widely distributed disease of wheat as pointed out by Carleton (3) in 1898. The disease was then commonly called "orange leaf rust" of wheat. Carleton noted the presence of this disease in Kansas in 1898 and observed that some injury seemed to be caused by the rust.

Stakman first showed in 1917 that Puccinia graminis tritici could be subdivided into physiologic races, which are similar or identical morphologically (22). All would attack wheat, but each race differed from the others in its capacity to attack certain wheat varieties. One year later Melchers and Parker reported on similar findings at the Kansas Station (18).

In a brief abstract in 1923, Mains and Jackson reported that they had assembled leaf rust collections from various parts of the United States and tested them on 200 wheat varieties of which thirty-one proved to be differential hosts to one or more leaf rust races (4).

Following this brief report, the same authors published a detailed account of their studies in 1926. They chose 11 wheat

varieties to use as differentials, with which they isolated 12 distinct physiologic races of leaf rusts (16).

In 1932, Johnston and Mains (13) added 25 new races to the list of known races of the fungus. These combined with eight races found by other workers increased the total to 53 physiologic races. The additional races were added as a result of experiments with eight differential varieties of wheat; four with winter habit of growth and four with spring habit. The other three varieties which had been used by Mains and Jackson were not used because it was shown that they exhibited reactions similar to some of the eight differential varieties retained and therefore were of no particular value in race determination. The varieties with a winter habit of growth used by Johnston and Mains were Malakof (C.I. 4898), Mediterranean (C.I. 3332), Hussar (C.I. 4843), and Democrat (C.I. 3384). The spring varieties were Webster (C.I. 3780), Carina (C.I. 3756), Brevit (C.I. 3778), and Loros (C.I. 3779). These varieties have been used since that time. The latest revision of the International Register of physiologic races of leaf rust of wheat (15) lists 163 races of leaf rust identified with the use of these eight varieties.

Race determinations were based on the physiologic reaction of the differential wheat varieties to the rust fungus. The reactions based on infection types varying from 0 to 4 were described by Mains and Jackson in 1926 (16) as follows:

0 - Highly resistant - No uredia formed: small flecks, chlorotic or necrotic areas more or less prevalent.

1 - Very resistant - Uredia few, small, always in small necrotic spots. Also, more or less necrotic areas produced without development of uredia.

2 - Moderately resistant - Uredia fairly abundant, of moderate size, always in necrotic or very chlorotic spots. Necrotic spots seldom without uredia.

3 - Moderately susceptible - Uredia fairly abundant, or moderate size. No necrosis is produced, but sometimes slight chlorosis immediately surrounding the uredia.

4 - Very susceptible - Uredia abundant, large. No necrosis or chlorosis immediately surrounding the uredia. Infected areas sometimes occurring as green islands surrounded in each case by a chlorotic ring.

In 1944, Stakman, Levine, and Loegering (24) described the (0) type of infection as being immune and no uredia developing; when hypersensitive flecks appeared, the use of the semicolon was designated to indicate this type of a reaction, thus, (0;).

The mesothetic or "X-type" of reaction was reported by Stakman and Levine in 1922 (23). This type of reaction was first encountered with stem rust, and the pustule types are heterogenous. This would indicate that all types and degrees of infection could occur on the same leaf blade.

In 1954, Heyne and Johnston (10) described a type of infection which they designated as the "Y" type. Here the tip of the primary leaf was fully susceptible (type 4) but the base showed more resistance (type 0;, 0-1, 1, 2). This differs from the

mesothetic type X in which the different infection types are interspersed.

Plus and minus signs are used to indicate variation within a given type, ++ and = (double minus) indicate the upper and lower limits, of each type respectively. The symbol ± indicates a variation between + and - for the type (24).

In 1930, Scheibe (20) raised the question whether the limited number of standard differential varieties gives sufficient race distinction. More differentials would increase the number of races and at the same time greatly complicate race study and the use of physiologic specialization as a control in breeding for rust resistance. Scheibe stated that it cannot be denied that two rust collections which key out to the same rust race by use of the standard differentials may be distinct genetically.

Gassner and Straib (7) in 1931 inoculated a number of commercial and differential wheats with leaf rust race 14 and incubated the plants at various temperatures. There were several cases in which the reaction was reversed, from susceptible to resistant or vice versa, with a change in incubation temperature. Thus, Malakof was susceptible at 6.0° C., and resistant at 10.9°, 16.6°, and 18.7° C. Democrat was resistant at 18.7° and susceptible at the other three temperatures.

Hassebrauk of Braunschweig, Germany (9) in 1939 found that lowering the temperature from 20° to 6° C. increased the susceptibility of Malakof and to a lesser extent that of Mediterranean,

Democrat, and Hussar, while it increased the resistance of Carina, Brevit, and, to a slight degree, Webster. Loros was unaffected by the temperature change. Hassebrauk stated that it was of great importance to maintain optimal external conditions in rust specialization studies, and in this connection the question of the substitution of more uniformly reacting varieties for the very unstable Carina, Brevit, and Hussar also demanded careful consideration.

In 1941, Newton and Johnson of Canada (19) reported results of tests on the effect of temperature and light on the reaction of varieties used for leaf rust race determination. They found that Malakof and Democrat became increasingly susceptible with lower temperatures, while Carina, Brevit, and Hussar became increasingly resistant. Webster and Mediterranean did not react consistently in either direction, and Loros showed very little change in reaction due to temperature changes. All of the differential varieties showed a more or less marked tendency to become increasingly resistant with conditions of short day and weak light. Newton and Johnson stated that in general it appeared that temperatures affected the reaction more than did light.

A study was made in 1934 by Gassner and Hassebrauk (6) on the effect of wheat nutrition on leaf rust reaction types. They grew wheat seedlings of the differential varieties in pots of sand to which were added nutrient solutions of different nitrogen phosphorus and potassium ratios and concentrations. They found

by means of drastic variations in potash and nitrogen supplies it was possible to induce far-reaching modifications in the reactions of the standard collection of differential varieties to leaf rust race 14. They concluded that, this being the case, it is questionable whether the ordinary procedure of growing the standard varieties for physiologic race determination in field or garden soil gives sufficiently uniform results. They suggest that it may rather be preferable to grow the plants on a synthetic substratum with a constant nutrient content.

In 1939, Hassebrauk (9) published a comparison of leaf rust infection types on differential wheat varieties with and without continuous coverage with bell jars. He reported that the effect of the high humidity thus induced was not as striking as those of temperature, but in general plants exhibited greater susceptibility under high humidities.

Hassebrauk also reported in 1939 (4) on the comparison of the leaf rust infection types on the primary leaves of seedlings of different ages. He found that the youngest plants, of the varieties Carina and Brevit were most resistant to races 19 and 31 but the reverse was seen with races 20 and 14.

In 1957, Basile (2) of Rome, Italy, presented a plan to group the races of leaf rust which showed a similarity in the development of infection types under certain environmental conditions. She prepared a diagnostic key which consolidated the closely related races into unified groups. Under this scheme, each of the unified groups would include those physiologic races which produce identical or similar infection types on five of the

standard differential varieties. The critical varieties were: Malakof, Webster, Loros, Mediterranean, and Democrat. The three varieties that appear to be unstable under changing environmental conditions were not used.

MATERIALS AND METHODS

The cultures used in accumulation of data for the study were taken from the stock cultures which had been identified and used by C. O. Johnston for rust testing work that is carried on by the U.S.D.A. at Manhattan, Kansas. These cultures were inoculated onto differentials at frequent intervals to check for purity and race determination. The utmost precaution was taken to see that the cultures were properly identified and pure enough so that it could be certain that the results obtained would not be caused by improper race determination or mixtures.

Seedlings of the differential varieties and all other plants to be inoculated were started in a separate section of the greenhouse where no rust was grown. The environmental conditions for this section were kept as nearly optimum for wheat seedlings as possible. Seedlings of the eight differential varieties were grown in 9½ x 5½ x 3 inch aluminum bread pans. A uniformly mixed soil was obtained by using a Royer compost mixer. The soil mixture consisted of five parts of sandy loam soil to one-half part sheep manure and one-half part peat moss. After each pan was filled with this soil mixture, eight spaced impressions were made in the soil with a marker that was made for this purpose.

The marker was made by using a piece of ply-wood which would fit inside the aluminum pans. Eight three-quarter inch in diameter wooden pegs, which were one inch in length, were attached evenly to this piece of ply-wood. Approximately eight to ten seeds of each differential variety were placed in one of the spaced impressions made in the soil. This made it possible to grow all eight varieties in one pan and still keep them separated so that infection types could be determined for each variety. All the seed of the eight differential varieties used in this study was obtained from C. O. Johnston, U.S.D.A., Manhattan, Kansas.

When the differential seedlings were ten days old, they were removed from this section for inoculation. The moist chambers used were heavy galvanized metal cylinders about 15 inches high and 15 inches in diameter. About an inch of fine sand was placed in the bottom of each chamber so that when water was added a high humidity would be maintained throughout the urediospore germination period. The sides of the chambers were lined with cotton batting which was also moistened in an attempt to maintain the highest humidity possible inside the chamber.

After the chambers were thoroughly moistened, the pans of wheat seedlings were placed in the chambers. A three inch clay pot containing approximately 30 wheat seedlings infected with the race of rust to be used was brushed over a pan containing the differential seedlings causing urediospores to fall on the leaves. Then the plants were moistened with tap water by the use of a

two gallon Hudson knapsack sprayer. By pumping this sprayer up to a rather high pressure a fine mist-like spray was obtained and the leaves were covered with small droplets of water. After the plants were thoroughly moistened, a sheet of ordinary greenhouse glass, approximately 3/16 of an inch in thickness, was placed on the top of the chamber, making certain that all edges were completely covered. With the use of the glass covers, a certain amount of light was allowed to enter the chambers.

Three sets of differentials were inoculated with an identical race at the same time and allowed to remain in the moist chambers for about 14 hours. The temperature inside these chambers was maintained between 70 and 75°F. Asuyama (1) of Japan reported in 1939 that the optimum temperature for urediospore germination of leaf rust on wheat was regarded to range from 18 to 25° C. After the 14 hour period, the inoculated sets of differentials were removed from the chambers. One set was placed in a greenhouse section maintained at an average temperature of 85° F. Another set was placed in a section in which the temperature was controlled at an average of 70° F. The remaining set was placed in a controlled temperature chamber built in one of the greenhouse sections. This chamber was cooled both by a 3/4 ton air conditioner and by an outside air intake fan. The temperature in the cooled chamber was maintained at 60° F. for this study. Thermographic records were used for all air temperatures reported. The light intensities were measured by using a Weston Illumination Meter, Model 603.

To shade the differential seedlings for the constant light intensity study, wooden frames were built which were 18 inches high and 36 inches long, and covered with cheese cloth. The frame-work was made by using one inch square wooden stakes. These cheese cloth covered frames were placed in both the high and medium temperature greenhouse sections. When the differential seedlings were put under these frames, the light intensity was nearly equal for the three temperature levels.

For the low constant temperature study, two walk-in refrigerators were used. In each refrigerator a four bulb fluoresence light was hung nine inches above the differential wheat seedlings. These lights yielded an intensity of 840 foot-candles. Each day the lights were used for an eight hour period. One of the refrigerators was held at a constant temperature of 70° F., and the other refrigerator was maintained at a constant temperature of 55° F. The differentials that were used as checks in this study were kept in a greenhouse section at a temperature of between 67 and 80° F., which is near the normal greenhouse temperatures for leaf rust studies. The method described previously for the rust inoculations of the differential seedlings was also used in this study.

After a set of differentials had been allowed to develop pustules, the type of infection on each variety was recorded and the physiologic race was determined by varietal differentiation. Adequate pustule formation for accurate readings usually developed in 10-14 days. At the low constant temperatures it took longer

for adequate pustules to develop. Unless otherwise specified, the reading for this study was made at 12 days after inoculation.

Race determinations were made by using the key supplied in the fifth revision of the International Register of physiologic races of leaf rust of wheat (15). The physiologic races were keyed by noting the infection type of differential varieties in this order: Malakof, Carina, Brevit, Webster, Loros, Mediterranean, Hussar, and Democrat. With the exception of the use of the (0) followed by a semicolon for flecking, the infection types were recorded as described by Mains and Jackson (16). The "Y" type of infection, as distinguished by Heyne and Johnston (10), was recorded as a "Y". The infection types were recorded only from the primary leaves of the wheat seedlings.

Each set of differentials had a wooden label giving the physiologic race used and the date of inoculation. The readings of the sets of differentials and the temperature levels were recorded in a notebook. The race determinations on the sets of differential at each temperature level were made from these readings.

The accumulation of data thus derived was used to determine the effect of certain environmental conditions on the identification of physiologic races of leaf rust of wheat.

EXPERIMENTAL RESULTS

It has been emphasized by many plant pathologists, working with leaf rust of wheat, that environmental conditions had a marked effect on the type of infection that certain physiologic races would produce on some differential varieties (13) (25). Mains and Jackson (16) noted that Hussar often was highly resistant to a race of leaf rust during the fall and winter and only moderately or slightly resistant to the same race in the late spring. In order to interpret accurately the results of chances in the host-parasite relationship of the wheat leaf rust fungus, various tests were made by using different controlled environmental conditions.

Infection Types at Three Different Average Temperature Levels and Different Light Intensities

Leaf rust infection types on certain differential varieties were greatly affected by different temperature levels as shown by Table 1. The results were obtained from five replications of nine physiologic races tested at three fluctuating temperatures. It was found that the infection types on the varieties Malakof, Democrat, Webster, and Mediterranean were nearly constant for all three temperature levels. The variety Loros was just as steadfast at the two lower temperature levels, but at the higher temperatures it had a tendency to increase in susceptibility. However, on the variety Loros, infection types would never increase from complete resistance to complete susceptibility.

Table 1. Infection types on differential varieties of Triticum vulgare produced by nine physiologic races of Puccinia recondita tritici at three temperature levels in five replications.

Physiologic race used	Mean temp °F.	Differential Wheat Varieties								
		Malakof	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat	Apparent race
1	85	0;	0;	0;	0;	0-2-	0-2	3	0;	53
	70	0;	0;	0;	0;	0;	0-Y	2	0;	1
	60	0;	0;	0;	0;	0;	0;	2-	0;	1
5	85	4	0;	1-2	0;	1-2	4	3-4	4	52
	70	4	0;	0-1	0;	0-1	4	2	4	5
	60	4	0;	0;	0;	0;	4	2-Y	4	5
6	85	4	2	4	2	4	4	4	4	6
	70	4	2	4	2	4	4	4	4	6
	60	4	2	2	2	4	4	4	4	28
9	85	4	4	2	4	4	0;	2-4	0;	13-19
	70	4	2	2	4	4	0;	2	0;	9
	60	4	2	2	4	4	0;	Y	0;	near 9
11	85	0;	4	4	2	3-4	1	2-4	0-1	near 131
	70	0;	2	4	2	4	1	2	0;	11
	60	0;	2	4	2	3-4	1	2-Y	0;	11
15	85	0;	0;	1-2	0;	0-2	4	4	4	2
	70	0;	0;	0-1	0;	0-1	4	2	4	15
	60	0;	0;	0;	0;	0;	4	2-Y	4	near 15
58	85	0;	4	4	2	4	4	4	4	12
	70	0;	2	4	2	4	4	3-4	4	58
	60	0;	2	4	2	4	4	2	4	58
105	85	4	2	3	2	4	4	4	4	near 6
	70	4	2	3	2	4	4	2	4	105
	60	4	2-	2	1	4	4	2-Y	4	near 126
122	85	4	4	4	4	4	4	4	4	77
	70	4	4	4	4	4	4	2	4	122
	60	4	2-4	2	4	4	4	2-Y	4	35 or 54

Carina and Brevit quite often, depending upon the physiologic race used, would change from a resistance type of reaction at lower temperatures to a totally susceptible reaction at higher temperatures. It appeared that the infection types on the variety Hussar was affected more by changing temperatures than any of the other differential varieties. Hussar increased in susceptibility at higher temperatures with eight of the nine physiologic races used. Also, the "Y"-type of reaction would often appear on Hussar when the temperature was maintained at an average of 60° F.

Using the International Register of physiologic races of leaf rust of wheat (15), an attempt was made to identify the races at each temperature level. The indication of the apparent races for the three temperature levels are shown in Table 1. Whenever the reaction on any one of the eight differential varieties appeared to differ in resistance or susceptibility, it became possible for the race to "key-out" unlike the original culture. With physiologic races 1 and 5, the variety Hussar increased in susceptibility at the higher temperature. This caused the culture of rust to be identified as race 53 and 52, respectively. However, when an original culture of race 6 was used, the variety Hussar remained susceptible at all three temperature levels. But at the lower temperature the varieties Carina and Brevit tended to increase in resistance, causing this culture to be identified as race 28.

Reactions of physiologic race 9 on the differential varieties indicated that Carina, Brevit, and Hussar increased in susceptibility at high temperatures. The "Y" type of reaction also appeared on the variety Hussar at the lower temperature. If Carina was the only variety to increase in susceptibility, the race 9 culture would appear to be race 19. However, if Carina, Brevit, and Hussar all increased in susceptibility, this culture would key-out to race 13. Because the "Y" type of reaction had never been recorded in the International Register, it became difficult to classify the race when this reaction appeared. "Near" was used as a prefix of the race number whenever the reaction on any one of the differential varieties appeared to be unusual, but not enough to change the race identification. The race 9 culture was identified in Table 1 as "Near 9" at the lower temperature because of the "Y" type reaction occurring on Hussar.

Carina and Hussar appeared to increase in susceptibility at the high temperature level when the differentials were inoculated with races 11 and 58. When this increase in susceptibility occurred, the two races seem to be more nearly races 131 and 12. If race 15 was used as the source of inoculum, Carina and Brevit remained highly resistant at all three temperatures. But with the increased susceptibility of Hussar, the original race 15 appeared to be race 2 at the highest temperature.

When races 105 and 122 were used, Carina, Brevit, and Hussar appeared to be susceptible at the high temperature level and

resistant at the low temperature level. Occasionally the "Y" type reaction appeared on Hussar at the low temperature. The original race 105 appeared to be near race 6 at the high temperature and near race 126 at the low temperature. At the high temperature race 122 keyed-out to be race 77 and at the low temperature this race appeared to be near race 35 or 54. Identification of these two races became extremely difficult because the rust reaction seemed to be greatly influenced by temperature changes.

Infection Types at Three Different Average Temperature Levels but at the Same Light Intensity

As stated earlier, the light intensity was only about half as great in the controlled low temperature chamber as it was in the two greenhouse sections. With the use of the cheese cloth shades, a study of the effect of different temperature levels on the leaf rust reactions of the differential varieties was made at nearly a constant light intensity. The results are shown in Table 2. Five of the physiologic races used in the previous study were tested. There were two replications made of the tests shown in Table 2. In all cases, the varietal reactions appeared to be similar to the interpretations shown in Table 1. Malakof, Democrat, Webster, and Mediterranean remained nearly stable in their reactions to the rusts. Meanwhile Carina, Brevit, and Hussar increased in susceptibility at the high temperature and became more resistant at the lower temperature. It was

Table 2. Effect of different temperature levels on the identification of physiologic races of *Puccinia recondita tritici* at constant light intensity.

Physiologic race	Mean temp °F.	Differential Wheat Varieties								Apparent race
		Malakof	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat	
1	85	0;	0;	2-	0;	1-	0;	3	0;	near 53
	70	0;	0;	0;	0;	0;	0;	2-	0;	1
	60	0;	0;	0;	0;	0;	0;	2-	0;	1
5	85	4	0;	1	0;	1	4	3	4	52
	70	4	0;	0;	0;	0;	4	2+	4	5
	60	4	0;	0;	0;	0;	3	2	4	5
9	85	4	4	2	4	3	0;	3	0;	near 13
	70	4	2+	2	4	4	0;	2	0;	9
	60	4	2	2	4	4	0;	Y	0;	near 9
15	85	0;	0;	2-	0;	2-	4	4	4	near 2
	70	0;	0;	0-1	0;	0-1	4	2+	4	15
	60	0;	0;	0;	0;	0;	4-	2	4	15
122	85	4	4	4	4	4	3	4-	4	near 77
	70	4	4	4	4	4	4	2+	4	122
	60	4	2++	2	4	4	4	2	4	35

observed that the chlorotic flecks appeared to be larger and the type 3 infection seemed to occur more often when the light intensity was lowered. The rate of uredial development appeared to be somewhat slower when the wheat seedlings were shaded.

Stability of Infection Types at Controlled Low and High Temperatures

Hassebrauk (9) and Newton and Johnson (19) reported the varieties Malakof and Democrat increased in susceptibility at lower temperatures. The writer had observed these two varieties to be unaffected by temperature changes. No data was presented in Tables 1 and 2 at temperatures lower than 60° F. Hassebrauk (9) reported of lowering the temperature from 20° to 6° C. Newton and Johnson (19) worked with a constant temperature of 57° F.

The writer, using two walk-in refrigerators, made a study of the reaction of leaf rust on the differential varieties at low constant temperature and light levels. The artificial lights produced a light intensity of 840 foot candles. As shown in Table 3, one refrigerator was maintained at 55° F., the other one was held at 70° F., and the differentials used as checks were kept under normal greenhouse conditions. Races 11, 15, and 58 were used for this study. Normally, Malakof was highly resistant to all three of these races. Democrat was resistant only to race 11. Each race was tested twice under the conditions produced in the walk-in refrigerators. The rate of infection development was greatly reduced in the refrigerators. Records were made after adequate pustule formation had appeared.

Table 3. Comparison of certain physiologic races of Puccinia recondita tritici grown at two constant temperature levels and a light intensity of 840 foot candles.

Physiologic race used	Date inoculated	Date read	Temp. °F.	Differential Wheat Varieties								
				Malakof	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat	Apparent race
15	Nov. 13	Nov. 25	70	0;	0;	0;	0;	0;	4	3	4	2
	Dec. 7	Dec. 18	70	0;	0;	0;	0;	0;	4	3	4	2
	Nov. 13	Dec. 4	55	1=	0;	0;	0;	0;	4	2+	4	near 15
	Dec. 7	Dec. 26	55	1=	0;	0;	0;	0;	4	4	4	2
	Nov. 13	Nov. 23	67-80*	0;	0;	0;	0;	0;	4	Y	4	near 15
	Dec. 7	Dec. 17	67-80*	0;	0;	0;	0;	0;	4	Y	4	near 15
11	Nov. 13	Nov. 25	70	0;	3	3	2+	4	Y	2-	Y	near 11
	Dec. 7	Dec. 18	70	0;	2+	3	2+	4	Y	2+	Y	near 11
	Nov. 13	Dec. 4	55	1=	3-	3-	0;	4	4	2+	3	near 11
	Dec. 7	Dec. 26	55	1=	4	3	2+	4	Y	Y	Y	near 11
	Nov. 13	Nov. 23	67-80*	0;	2+	4	1	4	0-2	2+	0-2	11
	Dec. 7	Dec. 17	67-80*	0;	2	3	2-	4	0-1	2	0-1	11
58	Nov. 30	Dec. 12	70	0;	2	4	0-1	4	4	2	4	58
	Jan. 19	Feb. 4	70	0;	2	4	0;	4	4	2+	4	58
	Nov. 30	Dec. 18	55	0;	4	4	0;	4	4	2	4	near 12
	Jan. 19	Feb. 11	55	0;	2	4	0;	4	4	2	4	58
	Nov. 30	Dec. 9	67-80*	0;	2+	4	2-	4	4	2	4	58
	Jan. 19	Jan. 29	67-80*	0;	2+	3+	2-	4	4	Y	4	near 58

* Normal greenhouse conditions.

As indicated in Table 3, an infection type of 1= was produced on Malakof at the 55° F. temperature with races 11 and 15. Type 1= meant that very minute uredia had developed. Only flecking appeared on Malakof when race 58 was used. With race 11, the infection types appearing on Democrat at the 55° F. temperature seemed to be from 3 to "Y". The "Y" type of reaction also appeared on both Democrat and Mediterranean when the temperature was held at a constant 70° F. But on the differentials that were used as checks under normal greenhouse conditions the variety Democrat remained resistant to race 11. The low light intensity in the refrigerator might have stimulated the "Y" type of reaction. Because of the poor light intensity it was impossible to place much faith in the results, but it did appear that Malakof and Democrat increased slightly in susceptibility at temperatures below 60° F.

Effect of Age of Culture on the Type of Rust Infection

Earlier observations had indicated that the type of rust infection was modified somewhat by the age of the infection. In general, infections tended to advance somewhat more toward susceptibility as they became older. However, no data were recorded to illustrate this tendency. During the course of the studies reported herein, careful studies were made on the phenomenon using physiologic races 19 and 52 in inoculations made at 10 different dates. The infection types on the eight differential varieties were recorded for each race 10 days after

Table 4. Effect of age of infection on the physiologic race identification of two cultures of *Puccinia recondita tritici*.

Date of inoculation	Original culture identified as physiologic race 52		Original culture identified as physiologic race 19	
	Race identified at 10 days	Race identified at 12 days	Race identified at 10 days	Race identified at 12 days
Oct. 27, 1959	5	52	9	19
Nov. 7, 1959	52	52	13	13
Nov. 17, 1959	5	52	9	13
Nov. 28, 1959	5	52	13	13
Dec. 9, 1959	5	5	9	9
Dec. 22, 1959	5	52	19	13
Jan. 6, 1960	5	52	19	13
Jan. 19, 1960	5	52	13	13
Feb. 1, 1960	5	52	9	13
Feb. 12, 1960	5	52	19	13

inoculation and again 12 days after inoculation. The results are shown in Table 4.

The results obtained show that race 5 gave infection types characteristic for race 5 at 10 days after inoculation with but one exception. However, at 12 days after inoculation all but one gave reactions characteristic of race 52. In this race the reason for the apparent change in races was due to the reaction of the variety Hussar. At 10 days Hussar gave an infection type characteristic of race 5, whereas at 12 days it gave reaction characteristic of race 52.

Race 19 also exhibited apparent changes in race identification in cultures of different age. In this case, however, there was less uniformity in identifications between cultures resulting from inoculations made at different dates. For example, 10-day old cultures of race 19 were identified as races 9, 13, and 19. There was more uniformity in 12-day old cultures, however. All but two were identified as race 13.

The greater instability of race 19 in cultures of the same age apparently was due to the variations in the reactions of the varieties Carina and Hussar. Thus it becomes evident that when two unstable varieties were involved there is greater instability in race identification than when only one differential was involved. Table 4 also shows that with increasing age, the race originally identified as 19 gave reactions characteristic of race 13 in eight out of ten cultures 12 days after inoculation. This was due primarily to the change in the reaction of Hussar from type 2 (resistance) to type 4 (susceptibility).

Statistical Proof of the Reliability of the Results

Using the contingency chi-square test, a measure of the environmental stability of infection types on each of the differential varieties was obtained. As shown in Table 1, five replications of each of nine physiologic races were tested at three fluctuating temperatures. This gave 15 possible chances for the standard infection types to occur on each variety for each race. By using nine physiologic races, there would be a

total of 135 possible chances for the standard infection types to occur.

Table 5 indicates the number of times the infection types were in agreement or disagreement with standard infection types for these nine known physiologic races. The contingency chi-square value for all eight of the differential varieties was significant beyond the .001 level. This indicated that differences did occur in stability among the eight varieties. The horizontal black lines in Table 5 show where significant differences occurred between these varieties. Malakof, Democrat, Webster, and Mediterranean appeared to be quite stable at the three temperature levels. Loros, with seven disagreements showed a significant difference from the other more stable varieties. Carina and Brevit could not be separated statistically, indicating that they both had about the same degree of stability. Hussar was in a class by itself, and the leaf rust reactions on this variety appeared to be controlled to a great extent by changing environmental conditions.

DISCUSSION

It is clear from the evidence presented in Table 1 that temperature must be taken into consideration in the identification of physiologic races. In some instances the same culture of rust appeared to be a different physiologic race at each of the three temperatures employed. Five of the differential varieties, Malakof, Democrat, Webster, Mediterranean, and Loros,

Table 5. A measure of stability of infection types on each of the differential varieties of Triticum vulgare infected with nine physiologic races of Puccinia recondita tritici at three fluctuating temperatures.

Wheat variety	Relationship with standard infection types of known physiologic races: Number in agreement	Number in disagreement	Degree of stability
Malakof	135	0	excellent
Democrat	135	0	excellent
Webster	134	1	very good
Mediterranean	133	2	very good
Loros	128	7	good
Carina	116	19	poor
Brevit	110	25	poor
Hussar	75	60	very poor

appear to be very unstable. The races that are separated by these three varieties apparently are quite often just a variation caused by different environmental conditions.

The results of Table 3 indicated that the varieties Malakof and Democrat increase slightly in susceptibility at low constant temperatures. In all cases, Malakof never increased more than from flecking to 1= in infection type. This was still considered a high type of resistance even though small uredia had developed. It would be safe to say that Malakof was the most stable of the differential varieties tested. With the use of race 11, Democrat seemed to increase slightly in susceptibility at the 55° F. temperature. However, at temperatures above 60° F.

Democrat appeared to be very stable for all three races. Most greenhouse leaf rust testing would be done at temperatures above 60° F., and Democrat could be considered a very satisfactory differential variety.

Possibly a standard set of environmental conditions could be set up for all stations working with and testing leaf rust. All physiologic race identifications of leaf rust would be made under the same environmental conditions. This would eliminate the variances that occur because of changing environmental conditions. However, a program of this type would be difficult to control and would require a large amount of expensive equipment. The physiologic race determination work is carried on in practically all parts of the world. It would be almost impossible to set up the same environmental conditions at all these stations.

Another approach would be to establish one central rust testing laboratory for the entire world. Both the variations caused by environment and the different interpretations made by personnel would be eliminated by this approach. If international differences could be settled this might be a very satisfactory answer to the problem.

Hassebrauk (9) stated that the question of the substitution of more uniform reacting varieties for the very unstable Carina, Brevit, and Hussar demanded careful consideration. For the past year, the U.S.D.A. has tested a group of wheat varieties which they call supplemental differentials. The group was made up of 12 different wheat varieties. These varieties were mostly

parents of commercial wheats used throughout the United States. One of the criticisms of the standard set of differentials was that they are not often used as parents for the commercial wheats, and that it could be possible for a sub-race to develop that would not have any effect on the standard set, but still cause infection on the wheats that are commercially grown. The main function for these supplemental varieties is to check for important sub-races within the races already known. However, some of these varieties may be used as substitutes for some of the varieties in the present standard set of differentials. It should be stressed that the environmental stability of any wheat variety needs to be obtained before it is used to differentiate physiologic races of rust.

The concept of race groups, which was discussed earlier, would eliminate the use of Carina, Brevit, and Hussar (2). Under this plan, each of the unified groups would include only those physiologic races which produce identical or similar infection types on the five more stable differential varieties. This approach would have merits from the plant breeding position because quite often if a wheat plant carries a factor for resistance for one race within a unified group, this same factor will govern resistance to other races in the group.

The problem of environmental variations might be solved by the use of the plant response instead of infection types. Simons (21) of Iowa State University now uses this method for the identification of physiologic races of crown rust of oats. In

this case "R" would stand for all of the resistant types of infection. This would include everything from only flecks to the 2-type of infection. "I" would be used for all intermediates, including the "X" and "Y" types of infections. Susceptible types of infection would be designated as "S" which would include infection types 3 and 4. This would prevent the effort made in trying to separate small variations within the infection types.

On the basis of results shown by data analyzed, it seems advisable that more work should be done with determinations of leaf rust physiologic races under changing environmental conditions. Adequate facilities should be available so that environmental conditions could be controlled accurately. The concept of physiologic specialization complicates the efforts made in obtaining sources of plant resistance. More information on the physiologic races of rusts should contribute to a better understanding of the host-parasite relationship and promote further development in breeding for resistance.

SUMMARY

Using the standard set of differential varieties, nine physiologic races of leaf rust were tested at different temperature levels. It was found that the rust reactions on the varieties Malakof, Democrat, Webster, Mediterranean, and Loros appeared to be fairly stable under temperature differences. The rust reactions on the varieties Carina, Brevit, and Hussar, appeared to be very unstable when temperature levels were changed.

In many instances the same culture of rust appeared to be a different physiologic race when the infection types were produced at a different temperature level. In view of these results, it seems probable that similar strains of rust have frequently been described as different physiologic races merely because the environmental conditions differed in the places at which identifications were made or in the seasons in which the work was performed.

ACKNOWLEDGMENT

The writer wishes to express his thanks to C. O. Johnston, U.S.D.A. plant pathologist, for his guidance, advice, and criticism on this work; and to Dr. J. C. Frazier of the Department of Botany and Plant Pathology for help with the measurements of light intensities. The writer also wishes to thank the Department of Botany and Plant Pathology for space and equipment provided in the greenhouse so that this work might be carried on, and the members of the staff for suggestions offered.

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EFFECT OF CERTAIN ENVIRONMENTAL CONDITIONS ON THE IDENTIFICATION OF PHYSIOLOGIC RACES OF *PUCCINIA RECONDITA TRITICI*

by

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B. S., Kansas State University, 1951

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Botany and Plant Pathology

KANSAS STATE UNIVERSITY

1960

The purpose of this research was to determine the cause of variations in leaf rust infection types that seem to appear on the differential wheat varieties under different environmental conditions. Also, a measure of the stability of leaf rust reactions on each of the eight differential varieties was to be obtained at different temperature levels.

Sets of the differential wheat seedlings were inoculated with known physiologic races of leaf rust, and kept at different temperature levels during uredial development. In the first test, five replications of nine physiologic races were used at three slightly fluctuating temperatures averaging 60°. Another test was made at low constant temperatures using three physiologic races. Studies were also made on the effect of light intensities and age of infection on physiologic race identification.

It was clear from the results obtained that temperature must be taken into consideration in the identification of physiologic races of leaf rust. Eight of the nine physiologic races used appeared to be a different race at the high temperature level. One race appeared to differ only at the low temperature, and two of the rust cultures were identified to be a different physiologic race at each of the three temperatures employed.

The reactions on all the varieties were not influenced by temperature in the same way. Carina, Brevit, and Hussar increased in susceptibility at higher temperatures. Malakof and Democrat increased in susceptibility only slightly at temperatures below 60° F. At higher temperatures Loros had a tendency

to increase in susceptibility; however, it would never increase from complete resistance to complete susceptibility. Reactions on Webster and Mediterranean were nearly constant for all three temperature levels.

Leaf rust reactions on Malakof, Democrat, Webster, and Mediterranean appeared to be nearly stable under changing environmental conditions. Except at high temperatures, Loros was just as stable in its reactions. Carina and Brevit were somewhat erratic in their reactions and, depending upon the physiologic race used, changed from a resistant type of reaction at lower temperatures to a totally susceptible reaction at higher temperatures. It appeared that the infection types on the variety Hussar were affected more by changing temperatures than any of the other differential varieties. Hussar increased in susceptibility at higher temperatures with eight of the nine physiologic races used. Also the "Y" type of reaction would often appear on Hussar when the temperature was maintained at an average of 60° F.

The following plans were offered as possible routes to correct the variations in physiologic race identification due to different environmental conditions: (1) the establishment of a standard set of environmental conditions for all leaf rust testing stations, (2) have all race identification made at one central laboratory, (3) select more stable varieties to replace the very unstable Carina, Brevit, and Hussar, (4) use the unified race group system to classify the races, and (5) use the plant response instead of the infection type to identify races.