COMPARISON OF METHODS OF ANALYSIS OF FIELD COLLECTIONS OF LEAF RUST OF WHEAT *Puccinia recondita* Rob. ex Desm. FOR THEIR PHYSIOLOGIC RACE CONTENT

by

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INTRODUCTION

There are two economically important rusts of wheat in this country. The least spectacular, but in Kansas, the more destructive of the two is Puccinia recondita Rob. ex Desm. (9), the causal organism of leaf rust of wheat and the subject of this paper. Leaf rust, as this organism will be referred to for convenience throughout this paper, seldom totally destroys the crop and its effect on yield is caused by a reduced number of kernals and a smaller kernal size (51).

The second of these rust fungi, Puccinia graminis Pers. which causes stem rust of wheat, is much more noticeable in its effect which results in severe shriveling of the caryopsis and lodging in the area of the pedicel.

Natural infection of the alternate host of leaf rust is known only from Russia and Portugal (6). In this country it must survive in the uredial stage alone. Regardless of this, leaf rust is able to produce new physiologic races and biotypes and these remain a successful parasite of wheat despite the efforts of breeding for resistant varieties. The knowledge of the races and biotypes of leaf rust present aids the plant breeder in selection of resistant parents to be used in the breeding program.

The present procedure used at the leaf rust laboratory of the United States Department of Agriculture at Manhattan, Kansas is to establish composite cultures from each field collection. Preferably the variety on which each collection was made is known but many collections are made from unknown varieties. When cultures have been established an attempt is made to obtain 4 single-pustule isolates from each culture. However there frequently are fewer than 4 uredia with spores available for transfer.

The relatively small number of isolates per culture has caused some

investigators to question the reliability of data on the abundance and distribution of physiologic races of P. recondita. There also has been some question of the value of race analysis of collections from individual fields as compared with those of composites of uredospores collected in several fields in an area.

The purpose of the present research was to ascertain whether 4 single-pustule isolates from each collection is sufficient to give a satisfactory picture of the race and biotype content of a given collection. Several different methods of sampling were tried to ascertain whether they would give better or similar results to the methods currently used. Another objective was to learn the value of the use of highly resistant varieties to locate new races or biotypes.

REVIEW OF LITERATURE

Physiological Specialization

Stakman and Piemeisel (72) described a new strain in Puccinia graminis triciti in 1917. This was the beginning of an era of research with rust fungi in a field that became known as physiologic specialization. This discovery led to a large amount of work not only with the rusts but with other diseases caused by fungi.

The original paper by Stakman and Piemeisel was followed by reports by Melchers and Parker (45) 1918, Levine and Stakman (35) 1918, Stakman (68) 1919, on specialized races in Puccinia graminis tritici, Hoerner (26) 1919, on forms in P. coronata, Stakman, et al. (71) and Murphy (48) on forms of oat stem rust (P. graminis avenae), and Mains and Jackson (41) (42) on forms in P. triticina. This is by no means all the work on physiologic specialization in the cereal rusts but it shows some examples of the early work in the field.

Greenhouse Culture Methods

Before physiologic specialization could be studied, culture methods of growing rust in the greenhouse had to be developed. Some of the early work along that line was done by Carleton (5) 1903, who used a scalpel to scrape a little material from some of the spots on the leaves and applied it to seedlings which had been atomized with water. The plants again were atomized with water and placed under a bell jar for 2 days. Carleton noted that in the summer inoculations should be done in the evenings and that if the sunlight was very bright the bell jars should be covered.

Melhus (46) in 1912, inoculated young seedlings by spraying them with a suspension of water and spores. For a moist chamber he used a humidity box with glass sides and top and with a moss bottom which was kept wet when in use. Melhus attempted to maintain 95% relative humidity in the box and a temperature of 16°C (60.8°F). The plants were left in the box for 24 hours.

Methods used by Fromme (15) were similar to those of Melhus except he used a bell jar for a moist chamber. Fromme found that plants which were atomized with water could be inoculated by shaking above them a pot containing plants with sporulating rust so that the spores fell and stuck to the wet leaves. The newly inoculated plants were covered with a bell jar.

Stakman (67) in 1914, used methods similar to those used by Carleton. He set the pot in a shallow pan of water to maintain wet soil and high humidity while the plants were under the bell jar.

Melchers (44) found in 1915 that he could obtain an abundance of large uredia by wetting the leaves of the plants to be inoculated by passing the leaf blade between the thumb and index finger which had been dampened by dipping them in a dish of distilled water, then applying uredospores with a scalpel. The scalpel used by Melchers was a dissecting needle which had been

bent and flattened on the end. He reported that the inoculum could be placed on either the upper or lower surface of the leaves with satisfactory results.

Melhus and Durrell (47) in 1919, sprinkled plants with water in a moist chamber and then blew spores on them as a satisfactory method of inoculation. They also developed a glass tube into which spores were placed and then blown onto the plants by attaching a rubber bulb to the tube and squeezing the bulb. Melhus and Durrell also reported tap water which they used had a toxic effect on the germination of uredospores of Puccinia coronata.

Hunt (28) in 1919, suggested an iceless refrigerator as an inoculation chamber. Plants were put in a container constructed of a wood frame covered with the water in such a manner that the water moved by capillary action to keep the cloth constantly moist. This resulted in a chamber in which the temperature was approximately 10°F cooler than on the outside. When kept outdoors in the shade the inside temperature was 15°F cooler than that in glass moist chambers in the greenhouse.

In 1923, Mains (39) summarized greenhouse culture methods used in his rust studies. He stated that dried leaves with uredial material could be mailed and these collections could be stored up to 6 months in an icebox at 48° to 54°F. He sprayed his moist chambers to keep them moist and cool and left the plants in moist chambers for 48 hours. Hungerford and Owens (27) 1923, used a battery jar for a moist chamber. They also found that a moist chamber made from a shallow tub covered with window sash with 2 inches of water in the bottom made an excellent moist chamber.

Johnston (30) in 1931, used tap water to moisten seedling leaves after which uredospores were dusted on the plants. Johnston and Mains (32) in 1932, used small galvanized-iron cylinders as moist chambers. The bottoms were covered with sphagnum moss and kept wet, the top was covered with glass or

with wet newspaper covered with glass. They found that a 24-hour period in the moist chamber was sufficient to get excellent infections of leaf rust of wheat.

Fischer (12) 1935, found that he obtained better infection if, instead of shaking sporulating plants above the plants to be inoculated, they were brushed with the sporulating plants. Pots containing sporulating seedlings were inverted and used to brush the leaves of seedlings to be inoculated.

Tervet (75) in 1950, used a cyclone separator to collect spores. In the greenhouse it was powered by an electrically driven portable blower and vacuum pump and in the field by a rubber bulb or lung pressure. Tervet and Cassell (76) (77) , used spores collected by a cyclone seperator and then reversed the apparatus to blow the spores onto the leaves of stem rust differential varieties using talc as a carrier. Tervet, et al. (78) in 1951, described the types of cyclone separators that they had worked with in collecting spores and other small particles.

Rowell and Hayden (57) in 1956, and Rowell (56) in 1957, found that a mixture of light petroleum oil plus Sovaspray 100, produced by Socony Vacuum Oil Co. Inc., an isoparaffinic low viscosity spray oil made an excellent carrier for field inoculation of stem rust. They discovered also that spores stored in oil remained viable longer than dry spores. Rowell and Olien (58) in 1957, outlined procedures for the use of oil inoculation in greenhouse studies.

Geis, et al. (19) in 1958, suggested that more than one race could be inoculated on a single leaf. They accomplished this by the use of small pieces of filter paper immersed in an aqueous spore suspension and then taped to the leaf. Infection in field was satisfactory if temperature and moisture conditions were right. A moist chamber was used with this method in the greenhouse.

Differential Varieties and Infection Types

After satisfactory techniques to secure infection had been established it was necessary to select good differential hosts and to establish and describe easily recognizable types of infection denoting resistance and susceptibility. Mains and Jackson (40) called attention to two races of leaf rust of wheat in 1921.

Mains and Jackson (41) in 1923, published a brief account of their work on leaf rust of wheat. They found that 31 of 200 varieties of wheat tested had differential reaction. Using the 7 differential varieties Malakof, Mediterranean, Democrat, CI 3756, CI 3778, CI 3779 and CI 3780 they found 12 strains of leaf rust.

In 1926 Mains and Jackson (42) published a detailed report of their work. They described 5 classes of host reaction as determined by the types of infection as follows:

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

4- very susceptible	Uredinia abundant, large. No necrosis or chlorosis immediately surrounding the uredinia. infected areas sometimes occuring as green islands surrounded in each case by a chlorotic ring.

These classes of reaction are still used. Two additional types of infection have been adopted. Stakman and Levine (70) described the X-type reaction for stem rust. The X-type reaction has also been used in leaf rust work. Stakman and Levine described X reaction as follows:

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

Heyne and Johnston (25) described the Y-type reaction in 1954 as follows:

> The tip of primary leaf fully susceptible (type 4) but the base showed more resistance type (0;).

In addition to the types of infection Stakman and Levine (35) adopted certain symbols which also frequently are used for leaf rust. They are as follows:

= Trace	Uredinia very few in number and covering a limited space surface; development of rust generally poor and decidedly subnormal.
- Slight	Rust development below normal but somewhat better than "trace".
± Moderate	Variation in rust development from "slight" to "considerable"; when infection is uniform but only medium in quanity the symbol is omitted.
+ Considerable	Infection better than normal; uredinia fairly numerous and scattered.
++ Abundant	Luxuriant development of rust; uredinia very many, covering large area of affected host.
; Hypersensitive flecks	
. Necrotic flecks	

Johnston (31) placed infection types 0, 0;, 0-1, 1, 1^+, 2^-, 2, 2^+, 2^{++}, 1-2, 0-2, in the resistant category. Types X, 1-3, 1-4, 2-3, 2-4, 1-X, 2-X, X-3, X-4 in the intermediate or variable class, and types 3, 3^+, 4^-, 4, 4^+, 3-4, $3-4^+$, 4-3 in the susceptible class.

In 1932, Johnston and Mains (32) revised the key for forms of leaf rust and adopted one based on the 8 differential varieties which still are used. They listed in the key 53 forms but said form 1 and 16 were probably the same. Johnston (31) lists 183 races in the 6th revision of the international register of physiologic races of Puccinia recondita.

The Effect of Temperature on Reaction Types

Temperature has been long known to have an effect upon incubation period of the fungus and the reaction of certain host varieties. Fromme (15) noted in 1913 that higher temperature speeded up the incubation period. Mains and Jackson (42) stated in 1926 that Hussar was highly resistant to a form of leaf rust during the fall and winter and only moderately or slightly resistant to the same form in the late spring. Waterhouse (79) working in Australia reported that two of the differential varieties, Carina and Hussar, were fully susceptible in the summer and resistant in the winter. Gassner and Straib (18) of Germany noted in 1932, that Malakof, Democrat and Mediterranean were highly resistant to form 14 at normal temperatures but was very susceptible at 46.4° to 53.6°F, while Webster remained resistant at all temperatures. Roberts of England (55) stated in 1936 that Hussar was affected by seasonal changes but she felt temperature was not the only factor involved.

Newton and Johnson (49) made in 1937 an extensive study showing that in general Malakof and Loros were fairly constant in reaction between 60° and 75°F while Carina, Brevit and Hussar were more resistant at lower temperatures.

They recommended a greenhouse temperature of 65°F as optimum for the development of proper infection types.

Hassebrauk (24) showed in 1939 that in his experiments in Germany the lower temperatures down to 42.8°F increased the susceptibility of Malakof, Mediterranean, Democrat and Hussar while it increased the resistance of Carina and Brevit. There also was a slight increase in the resistance of Webster. Loros was uneffected by the temperature change.

During the epiphytotic of 1938, Chester and Jamison (7) studied 98 cultures from Oklahoma and found race 13, 49 times; race 19, 10 times; race 9, 6 times; race 10, 5 times and race 31 once. Thus 71 of the 98 cultures would fall into the race 9 group. While in the 1938 epiphytotic in Kansas, C. O. Johnston found race 9 to be most common. With no natural barriers Chester and Jamison therefore felt that greenhouse conditions might have made the difference in the race analysis.

Chester and Temple (8) in 1941, cultured races 9, 13, 19, 20 and 31 side by side on different days. They found all races reacting in a similar manner when inoculated on the same date and concluded that the differences in these five races were primarly due to the variation in temperature during the inoculation and incubation periods.

In 1941, Newton and Johnson (30) in Canada reported on a detailed study on the effect of temperature on physiologic race identification. Loros was not effected by changes between 57° and 69°F. Malakof with one exception remained the same, once it showed an increase in susceptibility with an increase in temperature. Mediterranean was inconsistent in its variation depending on the race with which it was inoculated. Democrat became more susceptible with a decrease in temperature and Carina, Brevit and Hussar became more susceptible with a higher temperature. Webster was fairly stable with most races.

Williams (80) in 1960, found Malakof, Webster, Mediterranean and Democrat were nearly constant for all temperature ranges. Loros became more susceptible at the higher temperatures while Carina and Brevit were considerably more susceptible at high temperatures. Hussar gave a Y-type reaction above 60°F and more resistant reaction as the temperature was lowered.

Temperature effects have been noted in many other rusts, a few examples are: stem rust of wheat, Pltier (53) 1923, Waterhouse (79) 1929, Harrington (22) 1931, Johnson (29) 1931, Melander (43) 1935, Shukla (63) 1953, Forsyth (13) 1956, Patterson, et al. (52) 1957, Silverman (64) 1959 and Bromfield (3) (4) 1961; crown rust of oats, Peturson (54) 1930, Simons (65) 1954, Futrell and Rivers (16) 1955 and Zimmer and Schafer (81) 1961; stem rust of oats, Gordon (20) 1930 (21) 1943, and Waterhouse (79) 1929; bean rust, Schein (60) 1961; Puccinia simplex on barley, Waterhouse (79) 1929 and Puccinia sorghi on corn, Syamanada and Dickson (74) 1959.

The Effect of Light on Reaction Types

The effect of light on reaction types has not been studied as extensively as has temperature possible due to the difficulties involved in getting artifical light to simulate sunlight. The seasonal reaction changes may be due to not only temperature changes but to the changes in the day length. Fromme (15) noted in 1913 that light deficiency slowed the incubation periods of the rusts. Mains and Jackson (42) in 1926, Waterhouse (79) in 1929, Roberts (55) in 1936 also noted the effect or possible effect of light on reaction type. Roberts also found that with certain races of leaf rust the resistance of Webster and Carina veried with light intensity.

Hart and Forbes (23) reported in 1935 that darkness does not hinder infection by Puccinia triticina or P. antirrhini but it did hinder P. coronata on certain varieties of oats. Darkness reduced the infection, prevalence and

severity of P. graminis and Uromyces appendiculatus. A slight reduction in severity occured in darkness with P. sorghi and P. helianthi.

Hassebrauk (24) in 1939 found that the lack of light increased the resistant of most varieties except Carina and Brevit which were more susceptible at lower light intensities. According to Chester (6) in 1946, Hassebrauk found in 1940 that with stronger than normal light for the greenhouse they obtained a preponderance of race 13 instead of race 20, and race 19 acted as race 9. In general the tendency for susceptibility to increase as the days were longer and the light was more intense was reported by Newton and Johnson (50) in 1941.

The effects of light on the infection type of stem rust on wheat were reported by Johnson (29) in 1932, Forward (14) in 1932, Melander (43) in 1935, Shukla (63) in 1953 and Forsyth (15) in 1956. Bever (2) reported in 1934 that stripe rust of wheat was little affected by light intensity but varied from resistant at long day lengths of 15 hours, to susceptible at shorter day lengths of 6 to 12 hours. Syamanada and Dickson (74) in 1959 studied the influence of light on inbred corn lines with Puccinia sorghi. Supplemental light during the winter tended to result in more resistant reactions.

The Effect of Plant Nutrition on Reaction Types

Doak (11) in 1931, studied the effect of plant nutrition on the reaction type. He used sand culture and found that nitrogen increased the susceptibility of the plant while phosphorus and potassium decreased the susceptibility. Excessive nitrogen caused large pustules and decreased chlorosis. Excessive phosphorus increased chlorosis while excessive potassium increased chlorosis and decreased size of uredinia. Deficiency of nitrogen caused a decreased per cent of infected plants. Phosphorus deficiency caused decreased chlorosis.

Gassner and Hassebrauk (17) of Germany reported in 1934 that soil fertility modified reactions and they questioned if ordiniary field or garden soil gave sufficiently uniform results in rust studies. Hassebrauk (24) found in 1939 that nitrogen increased the susceptibility of Carina and Brevit wheat while nitrogen deficiency resulted in X-type reaction.

According to Chester (6) in 1946, Naumov recommended in 1939 the addition of 1.5% NH_4NO_3 or $NaNO_3$ to increase the strength of reactions.

Chester (6) states that a culture when grown in the usual greenhouse soil mixture gave typical reactions on the standard differentials. When this culture was checked against the differentials which were grown in a rich sandy loam from the Oklahoma Red River Valley the reaction of Mediterranean and Democrat changed from 0; to 2-3 and 2 respectively. In stem rust of wheat Johnson (29) in 1931, Darley and Hart (10) in 1944, and Shukla (63) in 1953, made studies on some of the effects of mineral nutrition on reaction types.

Effect of Humidity on Reaction Types

Chester (6) states that Gassner and Straib in 1931 found no difference in reaction type of leaf rust infection of plants grown under bell jars at high humidity, and those in the greenhouse at low humidity. Hassebrauk (24) found that Carina, Brevit, and Hussar grown in poor soil under bell jars enhanced the susceptibility of those varieties. Chester (6) tested race 13 under different relative humidities and found Brevit and Democrat increased in susceptibility with an increase in humidity the change in Brevit was sufficient to change the race identification.

Effect of Age of Plant on Reaction Types

Johnston and Melchers (33) in 1929 stated that resistance to leaf rust depended on the age of the plant in many varieties. Some varieties highly

susceptible in the seedling stage were resistant to the same race at flowering time. Mains and Jackson (42) also noted this type of resistance. Chester (6) stated that Hassebrauk (1939) compared infection types on primary leaves of different ages. He found the youngest plants of Carina and Brevit were most resistant to races 19 and 31 while the reverse was true when races 20 and 14 were used. Chester (6) in a similar experiment found the youngest plants of Brevit and Democrat were more susceptible and that Democrat was enough so that it changed the race identification.

Recent Developments in Leaf Rust Race and Biotype Identification

All of the instances of a change in type of reaction due to change in temperature, light, nutrition, humidity and age of seedling, caused most workers to recommend changes in the system of race identification. Basile (1) in 1957 proposed an unified numeration scheme. It was based on the reactions of five of the standard differentials which were thought to be the most stable. This combines many of the old standard races, which differed only in the reactions of the unstable varieties Carina, Brevit and Hussar, into unified groups under unified numeration (UN) numbers. Studies on the physiologic races of P. recondita soon clearly indicated that they could be subdivided by the use of additional differentials. As early as 1929 Waterhouse (79) further divided standard races of leaf rust by the use of the variety Thew. Scheibe (59) noted in 1930 that what appeared as a single biotype on the standard differentials could sometimes be further subdivided on another variety of wheat. Levine, et al. (34) reported in 1957 on an extensive study of the reaction of some races of P. recondita on certain wheat varieties which further divided the races. Stakman, et al. (69) in 1953 reported on biotypes in stem rust.

Loegering et al. (36) in 1959 proposed as modification of the system of identification of leaf rust which subdivided the standard physiologic races by the use of supplemental differentials to detect differences in virulence not detected by the standard differentials. The system of numbering was to retain the standard race number followed by NA (North American) and the last two digits of the year followed by a dash and a number indicating a specific line. In 1961 Loegering et al. (37) reported on the NA61 set of supplemental differentials consisting of the varieties Lee CI 3384, Webster CI 12100, Sinvaloche CI 12595 and Waban CI 12992. The universal resistant varieties were Agrus CI 13228, Aniversario CI 12578, Transfer CI 13483, Klein Lucero D.I.V. 8386, Exchange CI 12635 and Wanken CI 13659. The last two have been added since 1961. The 4 test varieties now used are Wardal 2 CI 13628, Triumph x Triticum-Agropyron elongatum CI 13523, Lerma Rojo CI 13651 and Dular CI 13373.

Storage of Uredospores

Mains (39) in 1923 stored leaf rust uredospores in the icebox and since that time workers have stored spores under refrigeration. The uredospores were either on dired leaves or stored as loose spores in vials or other suitable containers. This method was satisfactory for short periods but not for long term storage.

Sharp and Smith (61) in 1952 made the first break in the long term storage problem. They used the lyophilization process to store uredospores. After several trials they found that the usual process of freezing done with other biological materials was not necessary. They found storage was possible if the spores were dried under vacuum for 3 hours and there was no change in pathogenicity. Tubes were opened and placed in a moist chamber for 24 hours

before use. Stewart (73) in 1956 vacuum dried 5 mg. of fresh spores in 8mm. pyrex vials for 30 minutes under 3 inches of mercury. He added hemin to his spores before vacuum drying stating that it enhanced viability during storage. Sharp and Smith (62) in 1957 reported on collections they had vacuum dried 5 years before. They found approximately 20% loss in viability in 5 years of storage if they were kept in the refrigator after vacuum drying. They also found that vacuum dried tubes could be broken and then stored up to 2 weeks in a desiccator at 35.6° to 41°F without change in viability.

Another important discovery was made by Loegering et al. (38) in 1961 in which they reported rust spores could be stored for long periods in liquid nitrogen. A glass vial containing one mg. of spores was immersed into liquid nitrogen for 20 minutes and then removed to a liquid nitrogen refrigator at -160° to -196°C for storage. Upon removal for use the tubes were immediately defrosted in a 37°F water bath for 4 minutes.

MATERIALS AND METHODS

The studies reported herein were based on 63 field collections of leaf rust of wheat. (Table 1) Most of them were collected in Kansas during 1961, 1962 and 1963, with the greatest number of collections being made in the fall of 1962. There were 48 dried-leaf samples, 4 loose-spore samples from a single plot or field and 8 loose-spore composite samples. There was also 1 sample each from Fargo, N. D., Madison, Wis., and St. Paul, Minn.

The uredospores for the experiments were collected either on severed leaves or by use of a large cyclone collector to obtain loose spores. With the exception of collection numbers 1 thru 7 both methods of collections were used in each field. The collections of uredospores made by the cyclone separator are referred to as "composite" collections. With the exception of

collections 14, 16, 18 and 59 (see Table 1) the loose-spore collections were composites of spores collected in many fields in various areas of the state and hereafter will be called "composites".

Dried-leaf collections were made by picking 6 to 12 leaves infected with leaf rust in each field and placing them in a glassine envelope. The leaves were flattened in the envelopes and kept under pressure to prevent rolling. These samples were dried in the laboratory and then stored at 45°F in a refrigator until used.

The composite collections were made by the use of a cyclone separator which was powered by the vacuum from the manifold of a motor vehicle. The cyclone separator was connected to the vehicle by means of approximently 25 feet of plastic hose. The spores from one area of the state were all collected into one vial. The loose spores were dried for 24 hours at room temperature. After drying, the collections were stored in the refrigerator at 45°F until used. Collections 1 to 3, 6 and 7 (see Table 1) were collections sent to the United States Department of Agriculture Leaf Rust Laboratory at Manhattan, Kansas for race identification.

The original cultures were made using the methods of Melchers (44) with minor modifications. Bison CI 12518 or RedChief CI 12109 served as the susceptible hosts. Six to 12 plants were grown in 3 or 4-inch plastic pots. The plants were inoculated when 8 to 12 days old depending on environmental conditions. The cultures were kept under lantern globes to help maintain purity.

Several types of moist chambers were used. One type was a galvanized-iron cylinder 12 inches in diameter lined with cotton and covered with glass. The largest moist chamber was a large aluminum painted metal storage cabinet 36 x 18 x 72 inches, containing several shelves and lined with cotton. This

moist chamber was most frequently used. Another moist chamber was made from white denim with a redwood frame 3 x 5 x 1 foot placed inside a cooled room at temperatures of 65° to 70°F. This moist chamber was used June thru August 1963 when the moist chambers in the greenhouse was too hot to use, even at night.

The plants were left in the moist chambers 8 to 12 hours depending on time and space. Two time trials were made and it was found that 4 hours in the moist chamber was sufficient for satisfactory infection.

Single-pustule isolates were made from the original inoculations when the cultures were 10 to 12 days old. Well isolated pustules near the upper portion of the leaf were selected to avoid contamination. Spores were collected with a scalpel and transferred to susceptible seedlings. Bison or RedChief planted in 4-inch plastic pots were used as the susceptible host. Twenty isolates were made from each collection and numbered consecutively. The first 4 represented the 4 isolates normally used in leaf rust studies at Kansas State University. These 4 plus 6 more represented the 10 isolates for comparison and these plus 10 more made up the 20 isolates.

A statistical test of homogeneity was performed on each collection comparing the first 4 isolates against the next 6 and the last 10 isolates.

The standard leaf rust differentials were planted in bread pans approximately $5\frac{1}{2}$ x $9\frac{1}{2}$ x 3 inches in size. Six to eight seeds of each differential variety was planted in spaced clumps. The plants were 9 to 15 days old when inoculated. The single-pustule isolates served as a source of inoculum and were brushed over the moistened differentials in a 3 x 3 x 3-foot inoculation booth covered with plastic. The infections on the differentials were recorded when the pustules were fully developed, usually 8 to 12 days after inoculation depending on environmental conditions.

All race identifications were made using the Sixth Revision of the Register of Physiologic Races of Puccinia recondita (31) and a Diagnostic Key for the Identification of Physiological Races of Puccinia rubigo-vera tritici Grouped According to an Unified Numeration Scheme (1). The North American 1961 (NA61) Set of Supplemental Wheat Varieties for Leaf Rust Race Identificiation (37) also was used in the first 3 experiments. Due to the length of time required to complete an experiment and the changing environmental conditions all data were recorded on the basis of the differentials but when the data were compiled, the unified group scheme was used in all experiments except experiment 1 where the reactions were all typical.

The plants used for both the resistant-variety test and the test of loose-spore collections on differential varieties were grown in flats approximately 14 x 23 x 2½ inches in size made of galvanized metal. The differential varieties were planted in 13 rows 12 inches long, the 10 resistant varieties were planted at random in clumps 6 to 8 seeds per clump and 50 clumps per flat. The material grown in flats was inoculated by an oil spore-suspension. The oil used was a mixture of 1 part Californinia Spray Companys Spray Oil Stock B, to 1 part light weight mineral oil. This was sprayed on the plants at a pressure of approximately 35 pounds per square inch.

To find whether there was a change in pathogenicity due to the use of oil as a carrier for spores two original cultures were made from each of the composite collections 12, 14 and 18. One set of originals was inoculated by using oil as a carrier for the spores and the other set was inoculated using distilled water as a carrier.

A fertile clay loam and a very sandy loam soil low in fertility were used. The fertile soil was contaminated with a root rot pathogen so the soil was treated with methyl bromide or with steam at 3 to 7 pounds pressure for 3 hours as a control measure.

Greenhouse temperatures varied greatly during the course of the experiments ranging from 50°F in the winter to over 100°F in the summer. The winter temperatures averaged 70° to 75°F and the summer temperatures 80° to 95°F. Evaporative coolers were used in the greenhouses during June, July and August.

Table 1. Information on collections of the leaf rust of wheat used in these investigations.

Collection number	Type of collection	Variety collected on	Where collected	Date collected
1	dried-leaf	Lee	Fargo, N. D.	8/25/1961
2	dried-leaf	Trumbull	Madison, Wisc.	7/ 7/1961
3	dried-leaf	Rushmore	St. Paul, Minn	7/23/1961
4	dried-leaf	Kaw	Stockton, Kan.	11/28/1961
5	dried-leaf	Bison	Stockton, Kan.	11/28/1961
6	dried-leaf	Kharkof	Experiment, Ga.	?/ ?/1961
7	dried-leaf	American Banner	Knoxville, Tenn.	6/31/1961
8	dried-leaf	unknown	Westmoreland, Kan.	5/31/1962
9	dried-leaf	unknown	Frankfort, Kan.	5/31/1962
10	dried-leaf	unknown	Seneca, Kan.	5/31/1962
11	dried-leaf	unknown	Blaine, Kan.	5/31/1962
12	composite		same as 8-11	5/31/1962
13	dried-leaf	Bison	Manhattan, Kan.	10/23/1962
14	composite	Bison	Manhattan, Kan.	10/23/1962
15	dried-leaf	Triumph	Manhattan, Kan.	10/23/1962
16	composite	Triumph	Manhattan, Kan.	10/23/1962
17	dried-leaf	Columbia	Manhattan, Kan.	10/23/1962
18	composite	Columbia	Manhattan, Kan.	10/23/1962
19	dried-leaf	unknown	Marion Co., Kan.	10/25/1962
20	dried-leaf	unknown	Marion Co., Kan.	10/25/1962
21	dried-leaf	unknown	Geary Co., Kan.	10/25/1962
22	dried-leaf	unknown	Marion Co., Kan.	10/25/1962
23	dried-leaf	unknown	Rice Co., Kan.	10/25/1962
24	dried-leaf	unknown	McPherson Co., Kan.	10/25/1962
25	dried-leaf	volunteer	McPherson Co., Kan.	10/25/1962
26	composite		same as 19-25	10/25/1962
27	dried-leaf	unknown	McPherson Co., Kan.	11/ 2/1962
28	dried-leaf	unknown	McPherson Co., Kan.	11/ 2/1962
29	dried-leaf	unknown	McPherson Co., Kan.	11/ 2/1962
30	dried-leaf	unknown	Sedwick Co., Kan.	11/ 2/1962
31	dried-leaf	unknown	Chase Co., Kan.	11/ 2/1962
32	dried-leaf	unknown	Reno Co., Kan.	11/ 2/1962
33	dried-leaf	unknown	Reno Co., Kan.	11/ 2/1962
34	dried-leaf	unknown	Sedgwick Co., Kan.	11/ 2/1962
35	dried-leaf	unknown	Sumner Co., Kan.	11/ 2/1962
36	composite		same as 27-35	11/ 2/1962
37	dried-leaf	unknown	Rooks Co., Kan.	10/26/1962
38	dried-leaf	unknown	Rooks Co., Kan.	10/26/1962
39	dried-leaf	unknown	Graham Co., Kan.	10/26/1962

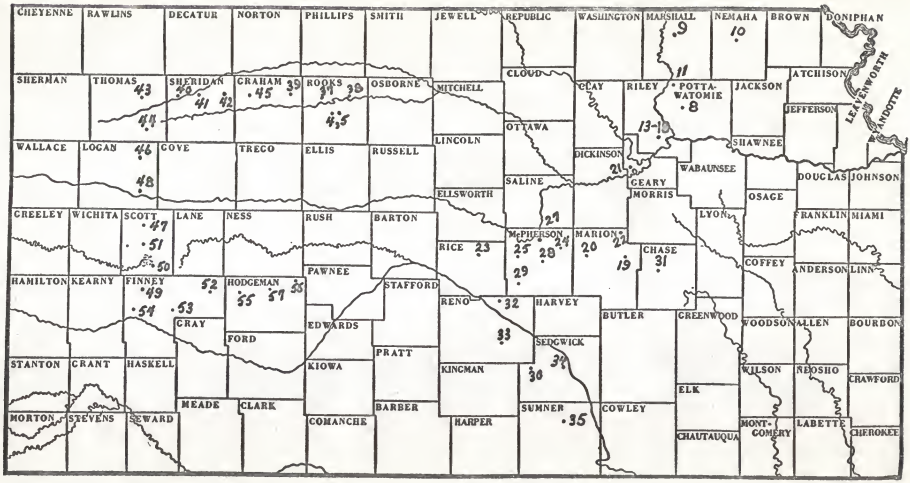
Table 1. (concl.)

Collection number	Type of collection	Variety collected on	Where collected	Date collected
40	dried-leaf	unknown	Sheridan Co., Kan.	10/26/1962
41	dried-leaf	unknown	Sheridan Co., Kan.	10/26/1962
42	dried-leaf	unknown	Sheridan Co., Kan.	10/26/1962
43	dried-leaf	unknown	Thomas Co., Kan.	10/26/1962
44	dried-leaf	unknown	Thomas Co., Kan.	10/26/1962
45	dried-leaf	unknown	Thomas Co., Kan.	10/26/1962
46	dried-leaf	unknown	Logan Co., Kan.	10/26/1962
47	dried-leaf	unknown	Scott Co., Kan.	10/26/1962
48	dried-leaf	unknown	Logan Co., Kan.	10/26/1962
49	dried-leaf	unknown	Finney Co., Kan.	10/26/1962
50	dried-leaf	unknown	Scott Co., Kan.	10/26/1962
51	dried-leaf	unknown	Scott Co., Kan.	10/26/1962
52	dried-leaf	unknown	Finney Co., Kan.	10/26/1962
53	dried-leaf	unknown	Finney Co., Kan.	10/26/1962
54	dried-leaf	unknown	Finney Co., Kan.	10/26/1962
55	dried-leaf	unknown	Hodgeman Co., Kan.	10/26/1962
56	dried-leaf	unknown	Hodgeman Co., Kan.	10/26/1962
57	dried-leaf	unknown	Hodgeman Co., Kan.	10/26/1962
58	composite		same as 37-57	10/26/1962
59	composite	Ottawa	Ashland Farm, Kan.	5/20/1963
60	composite	unknown	East Central, Kan.	5/21/1963
61	composite	unknown	West Central, Kan.	5/29/1963
62	composite	unknown	Southeast, Kan.	5/21/1963
63	composite	unknown	Northwest, Kan.	5/20/1963

EXPLATION OF PLATE I

A map of Kansas showing the approximate location where the dried-leaf collections were made. Numbers refer to the collection numbers given in Table 1.

PLATE 1


CHEYENNE
RAWLINS
DECATUR
NORTON
PHILLIPS
SMITH
JEWELL
REPUBLIC
WASHINGTON
MARSHALL
NEMAHA
BROWN
DONIPHAN
SHERMAN
THOMAS
SHERIDAN
GRAHAM
ROOKS
OSBORNE
MITCHELL
CLOUD
CLAY
RILEY
POTTAWATOMIE
JACKSON
ATCHISON
JEFFERSON
LEAVENWORTH
WYANDOTTE
WALLACE
LOGAN
GOVE
TREGO
ELLIS
RUSSELL
LINCOLN
OTTAWA
DICKINSON
SHAWNEE
WABAUNSEE
DOUGLAS
JOHNSON
GEARY
SALINE
MORRIS
OSAGE
GREELEY
WICHITA
SCOTT
LANE
NESS
RUSH
BARTON
ELLSWORTH
LYON
FRANKLIN
MIAMI
MCPHERSON
MARION
RICE
CHASE
COFFEY
ANDERSON
LINN
HAMILTON
KEARNY
FINNEY
HODGEMAN
PAWNEE
STAFFORD
RENO
HARVEY
BUTLER
GREENWOOD
WOODSON
ALLEN
BOURBON
GRAY
EDWARDS
FORD
SEDGWICK
STANTON
GRANT
HASKELL
KIOWA
PRATT
KINGMAN
ELK
WILSON
NEOSHO
CRAWFORD
MEADE
CLARK
BARBER
SUMNER
COWLEY
MORTON
STEVENS
SEWARD
COMANCHE
HARPER
CHAUTAUQUA
MONTGOMERY
LABETTE
CHEROKEE

EXPERIMENTAL RESULTS

Comparisons of 4, 10 and 20 Single-Pustule Isolates from Collections made in 1961

The first 3 experiments were based on 3 collections of leaf rust made at points outside of Kansas. The collections were dried leaves from which cultures were obtained. Single-pustule isolates were made and races determined during greenhouse season of 1962-1963.

Experiment 1. A dried-leaf collection made on the variety Lee, CI 12488, was received from Fargo, N. D. during the summer of 1961. An original culture was established in the normal manner but, instead of the usual 4 single-pustule isolates, 20 single-pustule isolates were made. They were numbered consecutively as they were made. The physiologic races then were determined for the first 4, the first 10 and all 20 isolates. The results of this experiment are shown in Table 2. Of the first 4 isolates 75% was race 5 and 25% race 15. In the first 10 isolates 70% was race 5 while 30% was race 15. The full 20 isolates consisted of 5% of race 1, 35% of race 15 and 60% of race 5.

When the first 4 isolates were used to inoculate the NA61 supplemental differentials it was found that 25% was sub-race NA61-12 and 75% was NA61-14. Among the first 10 isolates sub-race NA61-5 and NA61-12 each were found 10% of the time and NA61-14 was found 80% of the time. The 20 isolates consisted of 5% of sub-race NA51-5, 10% each of NA61-12 and NA61-7, 20% of NA61-13 and 55% of NA61-14. The following combinations of standard and NA61 sub-races were found: Standard race 5 and NA61-5, -12, -13 and -14; standard race 1 and NA61-7; and standard race 15 and NA61-7, -12, -13 and -14. The data indicated that as few as 4 single-pustule isolates in this case gave a clear indication of the most abundant races and sub-races in a collection. Four

isolates were nearly as good as 10. However 20 isolates gave a more complete picture of the race content of the collection.

Table 2. Standard and NA61 races found in a dried-leaf collection of leaf rust from Lee, CI 12488, in 4, 10 and 20 single-pustule isolates. (collection 1)

Number of isolates	Per cent of indicated race							
	Standard race			NA61				
	1	5	15	-5	-7	-12	-13	-14
4	0	75	25	0	0	25	0	75
10	0	70	30	10	0	10	0	80
20	5	60	35	5	10	10	20	55

Experiment 2. This experiment was similar to experiment 1. The collection was from the variety Trumbull, CI 5657, in the Uniform Rust Nursery at Madison, Wis. in 1961. The results of experiment 2 are shown in Table 3, in terms of unified races instead of standard races. Five of the unified race-groups were found and 5 of the NA61 supplemental races. Among the first 4 isolates there were 25% each of unified race-groups (UN) 2 and 9 and 50% of UN 13. The first 10 isolates revealed the presence of UN 2 and 9 each 10% of the time while UN 13 appeared 80% of the time. The 20 isolates consisted of 5% each of UN 3, 9 and 17, 10% of UN 2 and 75% of UN 13. The supplemental differentials revealed the presence of sub-races NA61-2, -4, -5 and -7 in the first 4 isolates. The first 10 isolates contained 70% of NA61-4 and 10% each of NA61-2, -5 and -7. The 20 isolates revealed NA61-4 75% of the time; 10% NA61-7 and 5% each of NA61-2 and -4. The following combinations of unified race groups and NA61 sub-races were found: UN 2, NA61-7 and -14; UN 3, NA61-7; UN 9, NA61-5; UN 13, NA61-2 and -4 and UN 17, NA61-4. In this experiment also 4 single-pustule isolates indicated the dominant race as well as 20 isolates but minor races which were not evident with 4 or 10 isolates appeared when 20 were used. The preponderance of supplemental race NA61-4 was clearly shown only by 10 and 20 single-pustule isolates.

Table 3. Unified race-groups and NA61 supplemental races found in a dried-leaf collection of leaf rust from Trumbull, CI 5657, among 4, 10 and 20 single-pustule isolates. (collection 2)

Number of isolates	Per cent of indicated race-group or race											
	Unified race-group						NA61 race					
	2	3	9	13	17	-2	-4	-5	-7	-14
4	25	0	25	50	0	25	25	25	25	0
10	10	0	10	80	0	10	70	10	10	0
20	10	5	5	75	5	5	75	5	10	5

Experiment 3. This experiment was similar to experiments 1 and 2. The dried-leaf collections was made on Rushmore, CI 12273, at At. Paul, Minn. Three unified race-groups were found. The data are shown in tabular form in Table 4. The first 4 isolates consisted of 25% of UN 5 and 75% of UN 13. The first 10 isolates disclosed 30% of UN 5, 20% of UN 6 and 50% of UN 13. Among 20 isolates there was 40% of UN 5 and 30% of each of UN 6 and 13. Six NA61 sub-races were found. NA61-4, -3, -5 and -7 were each found 10% of the time in the first 10 isolates, NA61-4 occured 20% of the time and NA61-14 appeared 50% of the time. When considering 20 isolates NA61-3, -5, -7 and -8 each occured 5% of the time while NA61-4 appeared 20% of the time and NA61-14 appeared 60% of the time. The following combinations of Unified Race groups and NA61 sub-races occured: Unified race 5 contained NA61-5, -8 and -14; UN 6 contained NA61-14; and UN 13 contained NA61-3, -4, -7 and -14. In this experiment the 4 single-pustule isolates gave markedly results different from those obtained using 10 or 20 isolates.

Table 4. Unified race-groups and NA61 races found in a culture from a dried-leaf collection from Rushmore, CI 12273, in 4, 10 and 20 single-pustule isolates. (collection 3)

Number of isolates	Per cent of indicated race-group or race									
	Unified race-group				NA61 race					
	5	6	13	-3	-4	-5	-7	-8	-14
4	25	0	75	25	25	25	25	0	0
10	30	20	50	10	20	10	10	0	50
20	40	30	30	5	20	5	5	5	60

Experiment 4. Table 5 shows the results of physiologic race analysis of 4 dried-leaf collections number 4 to 7. Collection 4 was made on Kaw, CI 12871, in the fall of 1962, was 100% UN 9 for all isolates. Collection 5 was made on Bison, CI 12518, within ¼ mile of collection 4 on the same day. The first 4 isolates comprised 25% of UN 2, 75% of UN 9. The first 10 isolates were comprised of 20% of UN 2 and 80% of UN 9. The 20 isolates indicated the culture consisted of 20% of UN 2, 5% of UN 5 and 75% of UN 9. Collection 6 made from Kharkof, CI 1442 showed 100% of the first 10 isolates to be UN 13. In the 20-isolate analysis 5% were UN 2 and 95% were UN 9. Collection 7 was made on American Banner, CI 6943. The first 4 isolates consisted of 50% each of UN 3 and 13. Based on 10 isolates, there was 10% of UN 2, 50% of UN 3 and 40% of UN 13. Twenty isolates consisted of 10% of UN 2, 35% of UN 3 and 55% of UN 13. All of the isolates from Kaw were UN 9 regardless of the number of isolates. UN 9 was the dominate group from Bison and UN 13 was the most prevalent on Kharkof regardless of the number of isolates. However, the minor race UN 5 from Bison, UN 2 from Kharkof and American Banner did not appear when only 4 isolates were used.

Table 5. Unified race-groups found in 4 dried-leaf collections using 4, 10 and 20 single-pustule isolates. (collections 4-7)

Collection number	Variety	Race group	Per cent of race-group indicated: Number of isolates 4	10	20
4	Kaw	9	100	100	100
5	Bison	2	25	20	20
		5	0	0	5
		9	75	80	75
6	Kharkof	2	0	0	5
		13	100	100	95
7	American Banner	2	0	10	10
		3	50	50	35
		13	50	40	55

Comparison of 4, 10 and 20 Single-Pustule Isolates
from Dried-Leaf and Composite Collections

Experiment 5. This experiment was made using dried-leaf collections, 8-11 and the composite (collection 12). Data are given in Table 6. Only 12 isolates from collection 9 and 5 from collection 10 were obtained. In the 4 dried-leaf collections 5 unified race-groups were found. The most prevalent were UN 2 and UN 9. The average of the 57 isolates made from the dried-leaf collections revealed 23% of UN 2, 3% of UN 3, 5 and 13 and 68% of UN 9. The twenty isolates from the composite collection showed 55% of UN 2, 10% of UN 5 and 35% of UN 9.

Among the 4 single-pustule isolates UN 2 was less abundant than among 10 or 20 single-pustule isolates, in all but collection 8. Cultures from dried-leaf collections contained UN 3 and UN 13 but these were not found in the composite. The standard method of analysis involved 4 single-pustule isolates from dried-leaf collections in Table 6, the average of the first 4 isolates from dried-leaves (total of 16 isolates) was 12% of UN 2, 6% of UN 3 and 75% UN 9. As a comparison the first 4 isolates of the composite revealed 25% UN 2, 25% UN 5 and 50% UN 9. The first 10 composite isolates showed 40% UN 2, 20% UN 5 and 40% UN 9. The 20 composite isolates showed 55% UN 2, 10% UN 5 and 35% UN 9.

Experiment 6. This experiment was designed not only to compare 4, 10 and 20 single-pustule isolates from composite and dried-leaf collections but also the effect of using oil as a carrier on race identification. Dried-leaf and composite collections were made from 3 susceptible varieties of wheat. The varieties, Bison (collection 13 and 14), Triumph (collections 15 and 16) and Columbia (collections 17 and 18) were planted in a block randomly arranged and in 4 repliciations. The results are shown in Table 7.

Table 6. Per cent of unified race-groups found in 4 dried-leaf collections made in Northwestern Kansas counties and in the composite collection from the same fields. (collections 8-12)

Unified race group	Collection number: 8 (No. of isolates: 4)	8 (No. of isolates: 10)	8 (No. of isolates: 20)	9 (No. of isolates: 4)	9 (No. of isolates: 10)	9 (No. of isolates: 12)	10 (No. of isolates: 4)	10 (No. of isolates: 5)	11 (No. of isolates: 4)	11 (No. of isolates: 10)	11 (No. of isolates: 20)	12 (composite) (No. of isolates: 4)	12 (composite) (No. of isolates: 10)	12 (composite) (No. of isolates: 20)	Average % based on 4 dried-leaf collections (57 isolates)
2	25	10	10	25	40	33	25	40	0	30	25	25	40	55	23
3	0	0	0	0	0	0	0	0	25	10	5	0	0	0	3
5	0	10	5	0	10	8	0	0	0	0	0	25	20	10	3
9	75	80	85	75	50	58	75	60	75	60	60	50	40	35	68
13	0	0	0	0	0	0	0	0	0	0	10	0	0	0	3

Table 7. Composite and dried-leaf collections made on 3 susceptible varieties with comparison between 4, 10 and 20 single-pustule isolates; and originals made by scalpel and water, water spray and oil spray. (collections 13-18)

Variety and CI no.	Unified race group	Per cent of race group: Dried-leaf collection: water carrier and scalpel inoculation: No. of isolates: 4	Dried-leaf collection: water carrier and scalpel inoculation: No. of isolates: 10	Dried-leaf collection: water carrier and scalpel inoculation: No. of isolates: 20	Composite collection: water carrier and spray inoculation: No. of isolates: 4	Composite collection: water carrier and spray inoculation: No. of isolates: 10	Composite collection: water carrier and spray inoculation: No. of isolates: 20	Composite collection: oil carrier and spray inoculation: No. of isolates: 4	Composite collection: oil carrier and spray inoculation: No. of isolates: 10	Composite collection: oil carrier and spray inoculation: No. of isolates: 20	Average % based on 60 isolates
Bison, CI 12518	2	100	80	70	75	60	65	75	70	75	70
	5	0	0	5	0	0	5	0	20	10	7
	9	0	20	25	25	40	30	25	10	15	23
Triumph, CI 12132	2	75	70	80	100	90	85	100	90	85	83
	5	0	20	10	0	0	5	0	0	0	5
	9	25	10	10	0	10	10	0	10	15	12
Columbia, CI 12629	2	75	80	90	50	80	85	75	60	70	82
	5	0	0	5	25	10	10	0	0	5	6
	9	25	20	5	25	10	5	25	40	25	12

Each collection was made from a single variety. All varieties were planted on the same date. The collections were made by collecting a few leaves from all 4 replications and a composite made by running the cyclone seperator in all 4 blocks. The composite collections were handled in two ways. In one method, spores were placed in water and sprayed onto the host to establish the original culture. The other method involved mixing the spores with oil and spraying them onto the leaves.

Based on the 20 single-pustule analysis for the dried-leaf collections from Bison, 70% were UN 2; 5% UN 5 and 25% UN 9. The composite collection when water was used as a carrier was 65% UN 2, 5% UN 5 and 30% UN 9. The composite collection with oil used as a carrier was 75% UN 2, 10% UN 5 and 15% UN 9. The dried-leaf collection from Triumph was 80% UN 2 and 10% each of UN 5 and 9. The composite collection using oil as a carrier was 85% UN 2 and 15% UN 9. The dried-leaf collection made on Columbia was 90% UN 2 and 5% each of UN 5 and UN 9. The composite collection with water as a carrier was 85% UN 2, 10% UN 5 and 5% UN 9. The composite collection with oil as a carrier was 70% UN 2, 5% UN 5 and 25% UN 9. Based on the total of 60 isolates from Bison they averaged 70% UN 2, 7% UN 5 and 23% UN 9. Collection from Triumph averaged 83% UN 2, 5% on UN 5 and 12% of UN 9, while those from Columbia averaged 82% UN 2, 6% UN 5 and 12% of UN 9.

The data in Table 7, shows considerable similarity between dried-leaf collections and the composite collections regardless of method of collection or inoculation. This experiment, contrary to experiment 3 demonstrated that, 4 isolates gave nearly as good results as 10 or 20 isolates for the major race. However minor race 5 appeared only among 20 pustule isolates in 5 out 9 cases.

Experiment 7. In this experiment 7 dried-leaf collections, numbers 19 to

25, from fields in Geary, Marion, McPherson and Rice counties were studied with composite collection 26. Data were taken on 140 isolates which are given in Table 8. Unified race-groups 2, 3, 5, 9 and 13 were found. The most prevalent were UN 2 and UN 9. Resistance was noted in the field in which collection 23 was made and no isolates of UN 9 were found in this collection. Neither was UN 9 isolated from collection 23. Only a very small amount from 21 and a relatively small amount from collection 19. These were from fields in the northeast corner of the sampled area. (see Plate I) The other collections, 20, 24 and 25, had between 25% and 40% of UN 9 based on 20 isolates each. Based on the total of 140 single-pustule isolates from the dried-leaf collections 78% were UN 2 while in the composite collection 80% were UN 2. On the other hand 17% of 140 isolates from dried-leaves were UN 9, as compared with 15% of 20 single-pustule isolates from the composite. Among the minor races the average from dried-leaf samples was 1% of UN 3 and 13, and 3% of UN 5 while 5% of UN 5 was found in the composite. UN 3 and UN 13 did not appear in the composite.

The 7 dried-leaf collections in Table 8, based on the average of the first 4 isolates from each collection (total of 28) show 75% UN 2, 4% UN 5, and 21% UN 9. The first 4 isolates from the composite revealed 75% UN 2 and 25% UN 9. Ten isolates from the composite showed 70% UN 2, 10% UN 5 and 20% UN 9. The 20 isolates from the composite were 80% UN 2, 5% UN 5 and 15% UN 9.

In this experiment 4, 10 and 20 single-pustule isolates, from the composite, revealed practically the same information as the average of the first 4 isolates from the 7 dried-leaf collections (28 isolates). Also, 4 isolates indicated the presence of the major races UN 2 and UN 9 as well as 10 or 20 isolates.

However at least 10 isolates were necessary to find UN 3 and UN 5 except

in the case of collection 21. It required 20 isolates to locate UN 13 in collection 24.

Table 8. Per cent of unified race-groups found in 7 dried-leaf collections and the composite collection from the same fields in 4 central Kansas counties. (collections 19-26)

Collection number	Number of isolates	Unified race-group 2	3	5	9	13
	4	75	0	0	25	0
19	10	80	0	0	20	0
	20	90	0	0	10	0
	4	50	0	0	50	0
20	10	60	0	10	30	0
	20	70	0	5	25	0
	4	75	0	25	0	0
21	10	90	0	5	5	0
	20	100	0	0	0	0
	4	100	0	0	0	0
22	10	100	0	0	0	0
	20	100	0	0	0	0
	4	100	0	0	0	0
23	10	80	10	10	0	0
	20	90	5	5	0	0
	4	50	0	0	50	0
24	10	80	0	0	20	0
	20	50	0	5	40	5
	4	75	0	0	25	0
25	10	80	0	0	20	0
	20	60	0	0	40	0
Total or average of above	140	78	1	3	17	1
26	4	75	0	0	25	0
(composite)	10	70	0	10	20	0
	20	80	0	5	15	0

Experiment 8. This experiment involved the comparison of dried-leaf collections 27 through 35 from McPherson, Sedgwick, Chase, Reno and Sumner counties and composite collection 36. The results of a study of 9 dried-leaf collections compared with a composite collection from the same fields are shown in Table 9. Race UN 2 was the most prevalent in 8 of the dried-leaf

collections while UN 9 was the most prevalent in 1 collection. Four collections had 5 to 15% of UN 5. Among the 180 isolates from dried-leaf collections 62% were UN 2, 3% UN 5 and 35% UN 9. The composite collection based on 20 isolates yielded 70% UN 2 and 30% of UN 9. The average of the first four isolates from dried-leaf collections (a total of 36 isolates) revealed 64% UN 2, 6% of UN 5 and 31% UN 9. The first 4 isolates from the composite revealed 25% of UN 2 and 75% of UN 9. The first 10 isolates from the composite showed 50% each of UN 2 and UN 9. The 20 isolates from the composite showed 70% of UN 2 and 30% of UN 9. In this experiment the 4 single-pustule isolates from the composite did not resemble the average of the first 4 isolates from the 9 dried-leaf collections (36 isolates). The 10 isolates from the composite were better than 4 isolates and the 20 isolates from the composite revealed about the same data as the average of the first 4 isolates from the 9 dried-leaf collections except UN 5 was not found in the composite.

In all 9 cases the first 4 isolates disclosed the major race. In the case of collection 28, 4 isolates showed 100% UN 2 while the 20 isolates revealed the collection had 40% of other unified race-groups present. This is one instance when 4 isolates was definitely not adequate. In the 9 collections the 10 single-pustule isolates showed the same unified race-groups present as did 20 isolates.

Table 9. Per cent of unified race-groups found in 9 dried-leaf collections and the composite collection from the same fields in 5 Southcentral, Kansas counties. (collections 27-36)

Collection number	Number of isolates	Unified race-group 2	5	9
	4	50	25	25
27	10	80	10	10
	20	75	15	10
	4	100	0	0
28	10	60	10	30
	20	60	5	35

Table 9. (concl)

Collection number	Number of isolates	Unified race-group 2	5	9
	4	75	0	25
29	10	70	0	30
	20	70	0	30
	4	25	0	75
30	10	50	0	50
	20	45	0	55
	4	75	0	25
31	10	50	0	50
	20	55	0	45
	4	50	25	25
32	10	50	0	50
	20	50	5	45
	4	50	0	50
33	10	50	10	40
	20	70	0	30
	4	75	0	25
34	10	70	0	30
	20	60	0	40
Total or average of above	180	62	3	35
	4	25	0	75
36 (composite)	10	50	0	50
	20	70	0	30

Experiment 9. Table 10 shows 21 dried-leaf collections compared with a composite collection. These represented collections made in fields in Rooks, Graham, Sheridan, Thomas, Logan, Scott, Finney and Hodgeman counties on Oct. 26, 1962 and assigned collection numbers 37 to 58. UN 2 and UN 9 again were the most numerous, and UN 5 occured once each in collections 55 and 57. Collections 55 and 57 were 2 of the collections made in Hodgeman county. Among dried-leaf collections UN 2 ranged between 0 and 95% of the total isolates. The average of 420 isolates made from dried-leaf collections was 36% UN 2 and 63% UN 9. The composite was found to be 65% UN 2 and 35% UN 9.

Comparing the first 4 isolates of the 21 leaf collections (a total of 84

isolates) the following averages for the unified race-groups were obtained: 43% of UN 2 and 57% of UN 9. The first 4 isolates from the composite collection showed 50% of each of UN 2 and UN 9. Ten isolates from the composite gave 60% of UN 2 and 40% of UN 9, while the 20 isolates showed 35% of UN 2 and 65% of UN 9. In this experiment 4, 10 and 20 single-pustule isolates from the composite revealed similar information to the average of the first 4 isolates from 21 dried-leaf collections (84 isolates).

The first 4 single-pustule isolates revealed the major unified race-group 17 out of 21 times. The minor unified race-groups were not found by the first 4 isolates 9 times. The 10 isolates failed 3 times to show the major race and 6 times not all of the minor unified race-groups were found.

Table 10. Per cent of unified race-groups found in 21 dried-leaf collections and the composite collection from the same fields in 8 Western, Kansas counties. (collections 37-58)

Collection number	Number of isolates	Unified race-group 2	5	9
	4	25	0	75
37	10	50	0	50
	20	40	0	60
	4	75	0	25
38	10	70	0	30
	20	60	0	40
	4	50	0	50
39	10	20	0	80
	20	40	0	60
	4	75	0	25
40	10	40	0	60
	20	30	0	70
	4	100	0	0
41	10	100	0	0
	20	95	0	5
	4	0	0	100
42	10	30	0	70
	20	25	0	75

Table 10. (cont.)

Collection number	Number of isolates	Unified race-group 2	5	9
	4	50	0	50
43	10	30	0	70
	20	25	0	75
	4	25	0	75
44	10	60	0	40
	20	40	0	60
	4	25	0	75
45	10	50	0	50
	20	40	0	60
	4	0	0	100
46	10	10	0	90
	20	10	0	90
	4	0	0	100
47	10	0	0	100
	20	0	0	100
	4	0	0	100
48	10	10	0	90
	20	20	0	80
	4	0	0	100
49	10	0	0	100
	20	5	0	95
	4	50	0	50
50	10	50	0	50
	20	50	0	50
	4	50	0	50
51	10	30	0	70
	20	35	0	65
	4	75	0	25
52	10	40	0	60
	20	40	0	60
	4	0	0	100
53	10	0	0	100
	20	20	0	80
	4	0	0	100
54	10	30	0	70
	20	35	0	65
	4	100	0	0
55	10	70	0	30
	20	50	5	45

Table 10. (concl.)

Collection number	Number of isolates	Unified race-group 2	5	9
	4	75	0	25
56	10	60	0	40
	20	45	0	55
	4	75	0	25
57	10	60	0	40
	20	45	5	50
Total or average of above	420	36		63
	4	50	0	50
58 (composite)	10	60	0	40
	20	65	0	35

Test of Composite Collections on Ten Selected Varieties of Wheat

In the past the search for new biotypes or strains was performed by inoculating selected resistant varieties with single-pustule isolates (progeny of a single uredospore). Another method involves inoculating with relatively few uredospores from the field which had been increased in the greenhouse to obtain sufficient spores for inoculation. The use of composite collections results in each infection to be caused by a different uredospore collected in the field.

Ten varieties of wheat which had shown seedling-resistance to a number of races of leaf rust in earlier tests were used in this experiment in an attempt to locate new biotypes or strains of the fungus. The varieties were replicated five times and sown in a complete block arrangement in metal pans. Six of the varieties were Agrus, Aniversario, Exchange, Klein Lucero, Transfer and Wanken. Transfer probably is the most resistant although Agrus also shows very high resistance. Klein, Lucero and Wanken are resistant but sometimes traces of sporulation occur. Aniversario usually is resistant but traces of

sporulation may occur. Exchange frequently has 2-type pustules surrounded by a large chlortic area although 4-type pustules occasionally are found.

The other 4 varieties were Triumph x T.-Ae. CI 13523; Ponca x TAP 45; Ottawa, CI 12894 and Justin, CI 13462. During the winter CI 13523 in previous tests had commonly showed a 0;-2 type reaction. However, in these tests run during the summer its reaction was 0; to most of the cultures tested. Ponca x TAP 45 was choosen on basis of limited tests by the writer. It was 0;-1 to all cultures tested. Ottawa was added to the group because it was resistant to all known strains of UN 9 but susceptible in the seedling stage to UN 2. In these tests Ottawa seems to be more resistant than would be expected. Justin was checked in limited tests and found to give a 0;-1 type reaction to the cultures to which it was tested. Justin in these tests gave a varied reaction but was more susceptible than expected.

The seedlings were inoculated by spraying the leaves with a mixture of spores suspended in an oil carrier. The reactions of the varieties and selections are recorded in Table 11. Susceptible-type infections were found only in Exchange, Justin and Ottawa. Some susceptible reactions were found in at least one replication of Justin inoculated with all collections. Some susceptible-type infections also were found on Exchange. Ottawa showed some susceptibility to collections 14, 16 and 26, complete susceptibility to collection 59, both low and high resistance to collections 2, 18, 36, 58, 60, 61 and 62 and high resistance to 63. It is not known whether or not the susceptible infection on Justin, Exchange and Ottawa are new races or biotypes of known races. Ottawa is known to be susceptible to some of the standard physiologic races. The remaining 7 varieties all exhibited some type of resistance. Transfer gave an 0; reaction to all cultures tested. The reaction of Agrus was 0; except in one replication inoculated with collection

Table 11. Reaction of 10 selected varieties of wheat to 12 composite collections of leaf rust from different varieties or different areas in Kansas in 1962 and 1963.

Variety	Average infection types observed for composite collection number											
	12	14	16	18	26	36	58	59	60	61	62	63
Agrus	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;
Aniversario	0;-2	0;-1^{+}	0;-1^{-}	0;-1	0;-1^{+}	0;-1	0;-1^{+}	0;-1^{-}	0;-1^{-}	0;-1	0;-1	0;-1$^{=}$
Exchange	0;-1^{-}	0;-3	0;-3	0;-3	1-4	0;-2	0;-1	0;-1	0;-2	0;-1	0;-2	0;
Justin	0;-2,4	2-4	2-4	0;-4^{-}	2-4	2-3	0;-3	2-3	0;-3	0;-2	3-4	2,4
Ottawa	0;-2	0;-3	0;-3^{+}	0;-2	0;,4	0;-2	0;-2	3-4	0;-2	0;-2	0;-2	0;
Lucero	0;	0;	0;	0;	0;-1	0;-1^{-}	0;-1$^{=}$	0;	0;-1	0;-1$^{=}$	0;-1$^{=}$	0;-1$^{=}$
Pnc x TAP 45	0;	0;	0;	0;-1$^{=}$	0;	0;	0;	0;	0;	0;	0;	0;
Transfer	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;
CI 13523	0;	0;	0;	0;	0;-1$^{=}$	0;-1$^{=}$	0;	0;	0;	0;	0;	0;
Wanken	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;

Table 12. Pustule counts made from composite collections inoculated directly to selected differential varieties. (collections 12, 14, 16 and 18)

Variety	Collection number											
	12			14			16			18		
	No. of pustules			No. of pustules			No. of pustules			No. of pustules		
	R[1]	S[2]	% R	R	S	% R	R	S	% R	R	S	% R
Malakof	126	1,086	10.1	1,294	237	74.8	1,728	290	85.6	1,544	453	76.9
Carina	1,522	2,421	38.6	1,529	471	76.4	1,705	295	85.2	1,535	465	76.8
Brevit	871	2,205	28.3	1,563	437	78.2	1,735	275	86.8	1,601	399	80.0
Webster	1,461	2,476	37.1	1,447	553	72.4	1,610	390	80.5	505	97	83.9
Loros	791	1,209	39.6	1,522	470	76.4	1,735	262	86.9	1,577	423	78.8
Democrat	1,322	678	66.1	376	1,624	18.8	301	1,799	15.0	131	1,247	9.5

[1]R Resistant
[2]S Susceptible

26. The reaction of Wanken was from 0-0; except in one replication inoculated with collection 36 where it was 0;-1^{m} and in one replication of collection 12 where it was 0;-1^{-}. CI 13523 was 0; except in 3 of the 5 replications of collection 26, 2 of the 5 replications of collection 36 and 1 replication of collection 58. Ponca x TAP 45 was 0; to all collections except collection 26 where one replication was 0;-1^{m} and another 0;-1^{-}. Lucero and Aniversario reacted as expected i. e. the reaction was always resistant but varied in level of resistance. Exchange was resistant to all collections but there were some susceptible-type pustules from some collections. It is more susceptible than Lucero and Aniversario.

Collection 26 was a little more virulent on CI 13523, Ponca x TAP 45 and Agrus. Collections 14, 16, 18 and 26 were more pathogenic to Exchange than the rest of the collections. Collection 59 was made at the Ashland Agronomy Farm in an Ottawa seed-increase block of several acres in which nearly 100% of the plants were heavily infected. This probably is a new biotype since Ottawa was thought to have good adult-plant resistance under field conditions. Of the ten varieties in this test only two showed any change in reaction to this collection. Ottawa was mostly susceptible and Justin did not show any highly resistant plants as it did to at least part of some of the other collections.

Test of Composite Collections on Selected Differential Varieties of Wheat

Counting the percentage of resistant and susceptible pustules on a differential variety would enable the worker to identify the major race in an area, providing some information on the races normally in the area was available. This would eliminate the time consuming task of making many

original and single-pustule isolates if a composite collection from many fields could be used directly for race identification.

This experiment was designed to determine whether the relative amount of the major standard races in a composite could be identified by infection counts. Malakof, Carina, Brevit, Webster, Loros and Democrat were the differentials used in this study. This study was run in duplicate and the information is given in Table 12. Hussar was not included as its reaction varies too much even with the same culture from tip to base of leaf (Y-type). Mediterranean was not included as the reactions are similar to those of Democrat. Carina and Brevit were included, even though unstable, as the resistant reactions, including the 0; are easy to see. Composite collections 12, 14, 16 and 18 were used in this test. The inoculations were made by spraying on spores using oil as a carrier.

All visible infections were counted on collection 12. This took too long to be practical so up to 1,000 infections were counted on each variety. Collection 12 (Table 6) was 55% avirulent on Malakof, UN 2; 65% avirulent on Carina, Brevit, Webster and Loros, UN 2 and 5; and 35% avirulent on Democrat, UN 9, as based on 20 isolates. Infection counts showed 10.1% avirulent on Malakof, UN 2; 38.6% on Carina, 28.3% on Brevit, 37.1% on Webster and 39.6% on Loros, UN 2 and 5 and 66.1% on Democrat UN 9. The low count of resistance in Malakof is due to the dull fleck caused by UN 2 which is difficult to see.

Collection 14 (Table 7) was found to be 70% avirulent on Malakof, UN 2; 77% avirulent to Carina, Brevit, Webster and Loros, UN 2 and 5; and 23% avirulent to Democrat, UN 9; based on 60 isolates. The infection count test showed 74.8% avirulent on Malakof, UN 2; 76.4% on Carina, 78.2% on Brevit, 72.4% on Webster and 76.4% on Loros, UN 2 and 5 and 18.8% on Democrat, UN 9. In these tests the secondary leaves of Malakof were read because the resistant flecks

show there more clearly. The resistant infections on Democrat were faintly developed and many probably were missed. Collection 16 (Table 7) was found to have 83% avirulent on Malakof, UN 2, 88% on Carina, Brevit, Webster and Loros, UN 2 and UN 5 and 12% on Democrat, UN 9. The infection count showed 85.6% avirulent on Malakof, UN 2, 85.2% on Carina, 86.8% on Brevit, 80.5% on Webster and 86.9% on Loros, UN 2 and UN 5 and 15% on Democrat, UN 9. Collection 18 (Table 7) was found to have 82% avirulent on Malakof, UN 2; 89% on Carina, Brevit, Webster and Loros, UN 2 and UN 5; and 12% on Democrat, UN 9. The infection count showed 76.9% avirulent on Malakof, UN 2; 76.8% on Carina, 80% on Brevit, 83.9% on Webster and 78.8% on Loros, UN 2 and UN 9 and 9.5% on Democrat, UN 9.

DISCUSSION

Four single-pustule isolates are normally made in the USDA leaf rust laboratory from each field collection in race identification. C. O. Johnston and some other investigators have often wondered if this was sufficient to reveal the most prevalent race or races. In this study a comparison was made between 4, 10 and 20 single-pustule isolates of 63 collections of leaf rust of wheat. Although the number of uredospores in any area being sampled approaches infinity it seems safe to assume that 20 single-pustule isolates would be a better measure of the physiologic race population than 4 isolates.

All race identifications were made by the use of the Sixth Revision of the Register of Physiologic Races of Puccinia recondita (31). The data were grouped into unified race-groups by the use of a diagonstic key (1). The use of an unified numeration scheme was required due to the fluctuations in reaction types caused by varing environmental conditions during the experiments (6) (49) (80).

There was a total of 59 comparisons between 4 and 20 isolates. In these the major race in the first 4 isolates was the major race in the full 20 isolates 44 times (74%). Eight times, two isolates of each of two unified race-groups appeared in the first four isolates, only once were these two not the two major race-groups. The first 4 isolates gave the correct major unified race-group or groups as measured by 20 isolates 86% of the time. In only 7 of 61 comparisons did the first 4 isolates fail to give the correct major unified race-group.

In the 60 comparisons between 10 and 20 single-pustule isolates the unified race-group indicated as most prevalent by the first 10 isolates was also the one which was most prevalent among the 20 isolates 47 times (78%). Seven times the number of isolates were equal for two unified race-groups and all 7 times these were the major two unified race-groups. This makes 90% of the time the most prevalent unified race-group or groups based on 10 isolates were the same as when based on 20 isolates.

A statistical test of homogencity, (Snedocor 66) was performed on each collection testing the first 4 isolates against the next 6 and the last 10 isolates. These were 61 tests using the standard races or the unified race-groups, 56 times the chi-squares were not significant at the 90% level. In the 3 tests involving the NA61 races (37) the chi-squares were found not to be significant at the 90% level 2 out of 3 times. The 60 isolates made using dried-leaf collections with scalpel inoculation, and composite collections using either water or oil as a carrier showed no significant differences was found between collections made on Bison, Columbia and Triumph varieties at the 90% level.

The first 4 isolates revealed all of the unified race-groups identified by the 20 isolates 27 out of 59 times. Twenty five times (42%) 20 isolates

revealed 1 more unified race-group and 7 times (10%) 2 additional unified race-groups were found. The first 10 isolates revealed the same unified race-groups as did 20 isolates 42 out of 60 times (70%). However 17 times (28%) 1 additional race was found and once (2%) 2 additional groups were found in 20 isolates.

When the first 4 single-pustule isolates from each field in which the composite was made, are averaged and compared with the composite collection, 7 out of 8 times the major unified-race group was revealed by the first 4 isolates from the composite. Once the first 4 isolates from the composite yielded two cultures and these were the two major groups. In only one case did the first four isolates from the composite fail to reveal the major unified race-group. When the first 10 isolates from the composite collections revealed the major race 6 times. Twice there were two cultures each of the major two groups. Once the major group was not the most prevalent in the first 10 isolates. When all twenty isolates from the composite collections are considered the major race was revealed 7 of 9 times.

Comparing the first 4 of the isolates from the composite collection with all the dried-leaf isolates from the fields which the composite was made, the 4 isolates from the composite revealed all unified race-groups 5 out of 9 times. Once 1 additional unified race-group was found in the leaf collections that was not found in the first 4 isolates from the composite. Comparing the first 10 isolates from the composite collection with the average of all the dried-leaf collections from the fields in which the composite was made twice the 10 isolates revealed all of the unified race-groups that the dried-leaf collections did. Six times all but 1 unified race-group and once all but 2 unified race-groups were found in the 10 isolates from the composite that were found in all the isolates from dried leaves. When the 20 isolates from

the composite collections were compared with all the dried-leaf collections 5 times all of the race groups were found in the composite, 3 times 1 additional group was found in the leaf collections and once 2 additional groups were found in the leaf collections that 20 isolates from the composite failed to show.

Making a few composite collections and a large number of isolates from each may be as satisfactory for race analysis as using a large number of collections and a small number of isolates. Regardless of the type of sample the more isolates the better the analysis. One advantage of the composite collection is that it is relatively easy to handle and results in the making of fewer original cultures. Better composite collections could be made. One way would be to run the cyclone separator over the same number of leaves in each field as this would allow a field heavily infected to represent a greater portion of the composite than one with less infection and this portion should be similar to number of spores collected. Another way would be to run the collector for a fixed period of time in each field. Tervet et al. (75) (76) (77) (78) have used cyclone separators to collect spores from a single pustule in the field and then used these spores directly to inoculate a set of stem rust differentials.

In recent years investigators have begun working on methods to detect biotypes of leaf rust not revealed by the standard leaf rust differentials (36) (37). The writer feels that composite collections used to inoculate selected resistant varieties is an excellent way to find new or unusual biotypes or strains quickly. If the plants were on hand for inoculation when the collection was made, 10 days would be sufficient to obtain results.

The use of a composite collection on differential varieties seem to be of limited value at the present since resistant flecks on Webster and Democrat

under certain enviromental conditions, and high infection with 2 or more races are difficult to see and on Malakof they are practically impossible to see on the primary leaves.

Mains and Jackson (42) noted that seasonal changes caused a fluctuation in the resistance of Hussar to a culture of leaf rust. Since then many others have reported that temperature has an effect on the resistance of Hussar (24) (49) (55) (79) (80). Doak (11) found that by adding nitrogen to soil increased the susceptibility of wheat to leaf rust. Gassner and Hassebrauk (17) had similar results. The writer noted during the course of these experiments that Hussar usually gave a type-4 reaction when inoculated with race 9 or 15 at temperatures between 60° and 80°F and especially when grown in fertile clay loam soil. However if very sandy loam soil, low in fertility, was used the reaction usually was a Y-type or 4-type at the higher end of the temperature range and a 2-type at the lower temperature.

SUMMARY

Studies on 63 field collections of leaf rust of wheat using 4, 10 and 20 single-pustule isolates from each collection indicated that the first 4 isolates from each culture revealed the major race as well as 20 isolates 86% of the time.

Ten isolates were only a little better, revealing the major races as well as 20 isolates 90% of the time. However, 10 isolates revealed the presence of a few minor races that 4 isolates did not reveal. Only in 6 instances did 20 isolates reveal the presence of a race or races that neither 4 or 10 isolates had revealed.

Ten and 20 single-pustule isolates sometimes gave more accurate results than 4 isolates but it is doubtful whether the added accuracy is commensurate

with the added time, space, and expense to test the larger numbers of isolates.

Results of 4, 10 and 20 single-pustule isolates from dried-leaf collections from individual fields and loose-spore composites collected in the same fields were approximately the same. Again 4 isolates usually clearly indicated the major races.

There was no evidence that use of sprays of either water or oil suspensions of spores had any effect on race analysis.

Inoculation of 10 selected resistant varieties with 12 composite loose-spore collections revealed that 7 of the varieties did not show any susceptible pustules but the varieties Justin, Exchange and Ottawa had many. These may indicate susceptibility to some races or to some biotypes of races already known. There also was good evidence that inoculating highly resistant varieties with field collections of leaf rust was a good way to locate new or unusual biotypes.

Inoculating differential varieties with composite collections and counting 1,000 infections to estimate the major race content was not very satisfactory. The resistant reactions on Malakof, Webster and Democrat, under certain enviromental conditions were very difficult to see and count.

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COMPARISON OF METHODS OF ANALYSIS OF FIELD COLLECTIONS OF LEAF RUST OF WHEAT *Puccinia recondita* Rob. ex Desm. FOR THEIR PHYSIOLOGIC RACE CONTENT

by

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The object of these investigations were to determine whether 4 single-pustule isolates from each field collection of leaf rust give a satisfactory picture of the race and biotype content of the collection. Some other methods also were tried to see if they would give better results or similar results quicker and/or cheaper than the current methods.

Two types of field collections were made. The normal dried-leaf collection was made by collecting leaves with sporulating leaf rust on them. The other type of collection was made by the use of a cyclone seperator operated by the vacuum off the manifold of a vehicle. Both types of collections were made in each field but loose spores from several fields in an area were composited. The collections were stored in the refrigerator until used.

Cultures were established on a susceptible variety from each dried-leaf and composite collection. When infections were sufficiently developed spores from isolated pustules were placed on a susceptible variety. Twenty isolates were made from each collection and numbered consecutively. The first 4 represented the 4 isolates normally used in leaf rust studies at Kansas State University. These 4 plus 6 more represent the 10 isolates for comparison and these plus 10 more made up the 20 isolates. The 4 isolates revealed the major unified race-group or groups as well as 10 or 20 isolates 86% of the time. The 10 isolates revealed the major unified race group or groups 90% of the time.

When the 20 isolates from the composite collections were compared with the average of all the isolates from the dried-leaf collections from the same fields it was found that 8 out of 9 times the isolates from the composite revealed the major unified race-group as well as all the single-pustule isolates from dried-leaves.

No significant differences were found between using oil or water as a

carrier for spraying on uredospores.

Ten varieties of wheat, with seedling resistance to many races, were inoculated with 12 of the composite collections. This appeared to be a rapid and satisfactory method to determine whether new races or biotypes were present.

Composite collections were used to inoculate 6 of the differential varieties and 1,000 infections were counted on each differential. Some counts were similar to the data obtained from 20 single-pustule isolates. However, the tendency of Democrat, Malakof and Webster to have resistant flecks which are very dull under certain environmental conditions and having both susceptible and resistant pustules made counting very difficult.

A fertile loam soil caused Hussar to give a susceptible reaction with races 9 and 15, at temperatures between 60° and 80°F, but when grown in a low fertility sandy loam soil it showed a Y or 4-type reaction at 80°F and a 2-type at 60°F.