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*Cereal Diseases - Rust*

STUDIES IN CEREAL DISEASES

VIII

Specialization and Hybridization of Wheat  
Stem Rust, *Puccinia graminis tritici*,  
in Canada

By

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DOMINION OF CANADA

DEPARTMENT OF AGRICULTURE

BULLETIN No. 160—NEW SERIES

630.4  
C212  
B 160  
n. s.  
1932  
c. 3

Published by direction of the Hon. Robert Weir, Minister of Agriculture,  
Ottawa, 1932



Agriculture  
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# Specialization and Hybridization of Wheat Stem Rust, *Puccinia graminis tritici*, in Canada<sup>1</sup>

BY

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## INTRODUCTION

Annual rust surveys have been made in Canada for the past twelve years to determine the prevalence of physiologic forms of *Puccinia graminis tritici*, but the information gathered in this period has never been brought together in published form. Much of the data collected have appeared from time to time in the annual reports of the Dominion Botanist or in papers dealing with certain phases of the work by the present authors and Mr. A. M. Brown, but some of the results have not yet been published. One of the objects which the writers had in view when this bulletin was undertaken was to give a comprehensive account of the results of the stem-rust surveys carried out during the period 1919 to 1930.

It was also thought worth while to discuss in some detail the methods used and the technique involved in the determination of physiologic forms. In previous papers by the present authors and others, it has often been assumed that readers were familiar with the technique employed in rust investigations. It was not until inquiries had been received from various sources that the authors realized how many phytopathologists were unacquainted with the technique of determining physiologic forms.

As the determination of physiologic forms is not an end in itself, but is rather a means for the attainment of other objectives, it seemed desirable to include in this paper also a discussion of the practical importance of physiologic forms in relation to plant breeding and agronomic problems. In this connection may be mentioned the role played by these forms in determining the rust resistance of wheat varieties, their use in studies on the genetics of rust resistance, and the value of physiologic-form surveys in explaining certain facts relating to the development of rust epiphytotics.

The discovery of heterothallism in the rust fungi has given rise to investigations which have thrown much new light on the nature of physiologic forms and their possible origin. In the investigation reported in this bulletin particular attention has been given to the inheritance of the pathogenic characters of these strains as revealed in hybridization studies; and, although these studies have, thus far, not yielded information as enlightening as that obtained from genetic studies of higher plants, yet enough knowledge has been acquired to warrant a discussion of physiologic forms in the light of recent investigations.

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<sup>1</sup> The writers wish to acknowledge their indebtedness to Mr. A. M. Brown for help with the photographs.

<sup>2</sup> Senior Plant Pathologist and Plant Pathologist, respectively.

## THE DETERMINATION OF PHYSIOLOGIC FORMS

### Historical Summary

The phenomenon of physiologic specialization in rusts has for many years attracted the attention of workers in pure and applied biology because of the light which it throws on the parasitic behaviour of fungi, and lately because of the practical application which these studies may have on the establishment of a basis for rust resistance.

Eriksson (6) was the first to show definitely that physiologic specialization occurs in cereal rusts. He worked with *Puccinia graminis* and in 1894 showed that what was usually considered as one species attacking all the common cereals, in reality consisted of several pathological strains or biologic races.

This discovery stimulated much research and various biologists in Europe and the United States began work in earnest upon this problem. The whole field of physiologic specialization has been carefully reviewed by Reed (44) and physiologic specialization in the cereal rusts has been summarized by Stakman (45). A detailed review, therefore, is not given here. Only those papers are referred to which are directly relevant to the context of this bulletin.

Until 1916 the existence of physiologic forms of *P. graminis* on wheat was not suspected. During that year a form of stem rust was collected by Stakman and Piemeisel (46) in the Palouse district of Washington and Idaho to which certain varieties of wheat were almost immune although these same varieties were readily susceptible to a form of rust collected at St. Paul, Minnesota. This variability in reaction of the same variety of wheat in different localities was most readily explained by assuming the existence of more than one physiologic form of the rust fungus, each form capable of affecting only certain wheats.

Since 1916, extensive work on physiologic specialization in *P. graminis tritici* has been done by Stakman, Levine and their co-workers at the University of Minnesota, in co-operation with the United States Department of Agriculture (24, 45, 46, 47, 48, 49, 50, 51, 52, 53). As early as 1922 these investigators (51) had published on the existence of thirty-seven physiologic forms of stem rust of wheat which differed from one another chiefly in their types of infection on wheat varieties. The standard technique developed by them has been adopted by workers in many other countries, e.g., by Waterhouse in Australia (56, 57) and McDonald in Africa (25), and it thus has been possible to compare and contrast the physiologic forms in all wheat-growing countries.

Until the present investigation was undertaken, no specific work had been done on physiologic forms of *P. graminis tritici* in Canada, although observations made by Dr. W. P. Thompson of the University of Saskatchewan, in his breeding experiments, had suggested very strongly that such strains did exist. In 1918, however, at the suggestion of Prof. W. P. Fraser, a study was begun by the senior author (30) at Macdonald College (McGill University) in the province of Quebec. This investigation was continued for two years by her at the Dominion Plant Pathological Laboratory, Saskatoon, in co-operation with Professor Fraser (8, 30). From that time until 1925 it was carried on at Saskatoon by him (8). With the establishment of the Dominion Rust Research Laboratory at Winnipeg, the work was continued there by the present authors, as facilities were afforded for stem rust research on a more adequate scale than had hitherto been provided in Canada.

## Methods of Identifying Physiologic Forms

### COLLECTING INOCULUM

(a) *From the Field.*—Extensive collections of rust were made annually in the field on wheat, barley and wild grasses, from the time the first pustules appeared in early summer until late in September.<sup>1</sup> When the inoculum was collected locally the spores were usually transferred at once to seedling plants of Little Club, but when it was obtained from distant localities, the rusted straws were dried overnight, placed in paper packets and forwarded through the mail. Contrary to some opinions, it was found advantageous to use plain paper packets rather than oiled paper as in the latter there was always a tendency for the spores to germinate in transit due to excessive moisture. The collections were often stored during the hot summer months in a refrigerator (fig. 1), at a temperature of 5° to 10° C., and a relative humidity of 50 per cent, and transferred later in the season when lower and more suitable temperatures for the identification of physiologic forms could be secured in the greenhouse. Good germination was obtained even after the spores had been stored in a refrigerator for several months (*Cf.* Peltier) (40).

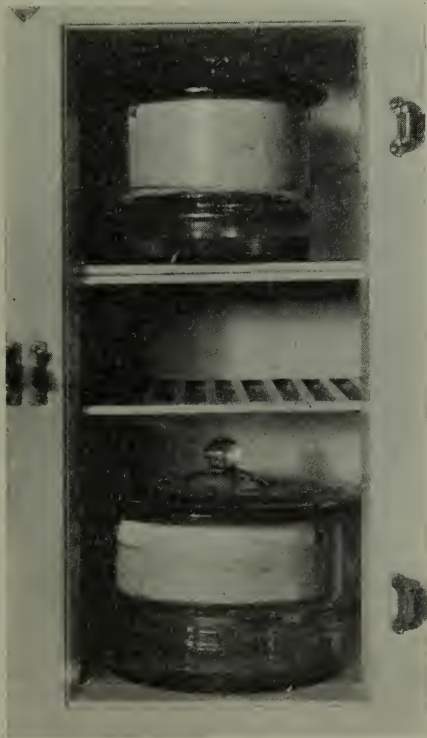


FIG. 1.—Interior of a Frigidaire refrigerator showing sealed containers in which cultures of stem rust are stored at a temperature of 5° to 10°C., and a relative humidity of 50 per cent.

<sup>1</sup> In the field surveys, the Dominion Laboratories of Plant Pathology, the agricultural faculties of several universities, and the various Dominion Experimental Farms and Stations co-operated.

(b) *From Uniform Rust Nurseries.*—In connection with the annual rust survey, mention should be made of the excellent collecting ground provided by the uniform rust nurseries situated at the Experimental Farms, Stations, and universities during 1920 to 1929. These nurseries were first established by Professor W. P. Fraser. In these plots a number of promising wheat and oat varieties from both Canada and the United States were planted late in the spring in rod rows and there tested for their relative resistance to stem rust.

The susceptible plants in these different rows were not always uniformly rusted throughout their length. For example, quite often the north side of the plant was heavily rusted while the south side was comparatively free from rust (fig. 2). This phenomenon has been observed by Aamodt<sup>1</sup> and others, but as yet it is not fully understood. It may be that owing to the heat from the direct rays of the sun upon the south side of the plant the dew disappears before the majority of the germinating spores have had time to enter the plant; or possibly, as has been pointed out by Dillon Weston (5) in his study of the germination of urediniospores, the direct rays of the sun may have an inhibiting effect upon germination. There may be, also, some local modification in the physiology of the plants due to the sun's rays. The problems arising from these field studies are now being further investigated.

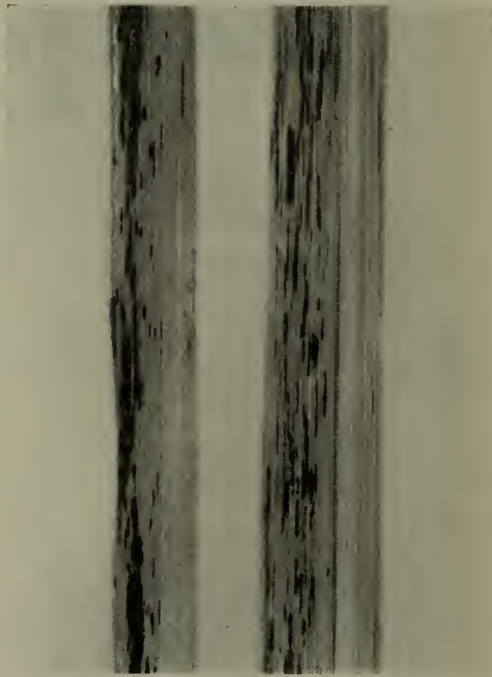


FIG. 2.—Sections from the culms of two wheat plants which have been split open and which show the heavy infection on the north side of the culms (left) and the light infection on the south side of the culms (right).

Although these plots were designed primarily for testing the relative resistance of varieties, they also acted as ideal places for collecting new and unusual physiologic forms, owing to the great variety of wheats planted in them. On

<sup>1</sup> Unpublished data by Dr. O. S. Aamodt of Alberta University, Edmonton, Alberta.



several occasions certain physiologic forms which had not been collected elsewhere in the vicinity were isolated from the rows.

As these nurseries were always planted later in the season than were the regular field crops, rust infections quite often developed in them when the nearby fields were practically free from rust. In this way many additional collections of rust were secured from the outlying parts of the general wheat area of Canada, and thus a better knowledge of the geographical distribution of physiologic forms was obtained. A rust nursery was placed at Beaver Lodge (lat. N. 55·2, long. W. 119·24) in the Peace River district in 1927. In the autumn an examination of the varieties in this nursery revealed stem rust on five wheat varieties, although no trace of rust could be discovered in any of the nearby fields. Had this nursery of late-sown varieties not been placed there, it might not yet be known that viable rust spores could be carried as far north as the Peace River district and there cause infection.

#### INOCULATION

The identification of a physiologic form is based upon the host reactions of certain wheat varieties in the seedling stage and is essentially the comparison of the host reactions of a group of wheat varieties to different strains of stem rust. To secure as reliable a comparison as possible, a standardized method of inoculation has been adopted.



FIG. 3.—The inoculation of wheat seedlings with stem rust.

The seedlings, which are usually grown in four or five-inch flower pots, are inoculated when three or four inches high. At that stage of growth only the first seedling leaf has emerged and consequently this leaf only is inoculated. Before the inoculum is applied, each leaf is gently rubbed between the moistened fingers to remove from the epidermis the waxy bloom which prevents the moisture from adhering to the leaf. The inoculum is applied to the lower surface of the leaf—the surface possessing the greater number of stomata—by means of an inoculating needle with a spatulate metal tip (fig. 3). The flattened shape of the needle renders it suitable for the gathering of inoculum from either uredinial pustules on other seedlings or pustules on stems or leaves of specimens collected

in the field. After the plants have been inoculated, they are subjected to a fine spray of water from an atomizer and are then placed immediately in the incubation chamber (fig. 5).

Certain precautions, of course, are necessary to prevent the contamination of one rust strain by another. The inoculation needle must be sterilized in an alcohol flame and the hands must be carefully washed with soap and water before each inoculation to prevent the contamination with spores of another strain of rust.

When wheat varieties are to be tested for rust resistance, it frequently becomes necessary to infect plants in later stages of growth. For this purpose the inoculating needle may be dispensed with, unless the supply of inoculum is very limited. The leaf and stem surface area of an adult plant is so great that inoculation by means of a needle becomes very tedious. The task can be performed much more quickly and with less danger of injury to the tissues by applying the rust spores with the fingers. Providing the hands are washed carefully with soap and water between the operations, the danger of contamination may be largely eliminated. If the supply of inoculum is limited, it may be necessary to use the inoculating needle, but, to prevent injury to the plant tissues, it is advisable to wrap the metal tip of the needle in a thin layer of cotton-wool. Fig. 4 shows the method of inoculating adult plants.



FIG. 4.—The inoculation of an adult wheat plant with stem rust.

#### INCUBATION

The plants remain in the incubation chamber for a period of about forty-eight hours. During this time the high humidity of the air in the chamber maintains a film of moisture on the surface of the leaves.

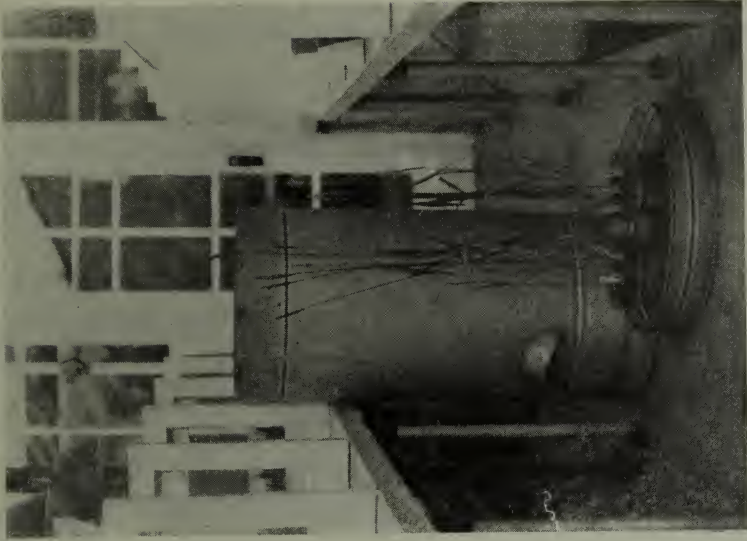


FIG. 6.—Incubation chamber for adult plants.

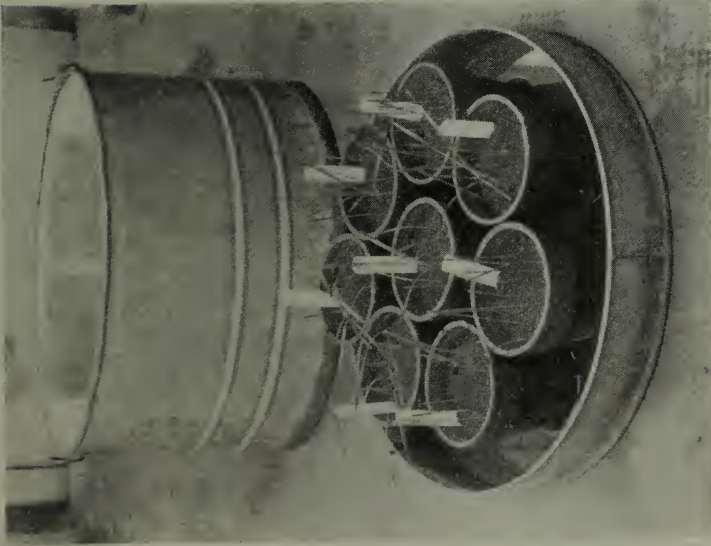


FIG. 5.—Incubation chamber containing inoculated seedlings ready for incubation.

The incubation chamber generally used consists of two parts: the bottom, a shallow tray-like receptacle in which the pots of inoculated seedlings are placed, and the cover, a cylinder closed at the upper end with window glass. The cover fits inside the bottom like an inverted petri dish. An inch or two of water in the bottom acts as a seal and furnishes enough water-surface to preserve a high atmospheric humidity in the chamber. Such a chamber is shown in fig. 5. It is eighteen inches in diameter and twelve inches in height and can accommodate about eight, five-inch flower pots.



FIG. 7.—Interior of greenhouse showing two rows of compartments.

During incubation, it is important to place the chamber so that the sun does not shine directly into it, as otherwise the temperature within the chamber may rise rapidly to a point at which spore germination is inhibited. Many failures to obtain infections are undoubtedly due to this cause.

A type of chamber used for adult plants is shown in fig. 6. It has been found much more difficult to maintain a film of moisture on adult plants than on seedlings. The volume of the chambers used for adult plants is so much greater than that of the chambers used for seedlings that the water surface in the bottom section does not suffice to maintain a saturated or nearly saturated atmosphere. In order to secure this degree of saturation, it has been found necessary to line the inside walls of the chambers with wet paper. If the greenhouse temperature is high, it may even be necessary to spray the plants at intervals of a few hours to maintain the humidity sufficiently high.

#### IDENTIFICATION

The types of rust infection (fig. 8) on which physiologic form identification is based have been described by Stakman and Levine (51) in the following terms:—

##### *Types of Infection*

###### (O) Immune:

No uredinia developed; hypersensitive flecks usually present, but sometimes there is apparent absolutely no trace of mycelial invasion in the host tissues.

###### (1) Very resistant:

Uredinia minute and isolated; surrounded by sharp, continuous, hypersensitive, necrotic areas.

###### (2) Moderately resistant:

Uredinia isolated and small to medium in size; hypersensitive areas present in the form of necrotic halos or circles; pustules usually in green, but slightly chlorotic, islands.

###### (3) Moderately susceptible:

Uredinia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present, especially under unfavourable cultural conditions.

###### (4) Very susceptible:

Uredinia large, and generally confluent, true hypersensitiveness, entirely absent, but chlorosis may be present when cultural conditions are unfavourable.

###### (X) Heterogeneous:

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

##### *Degrees of Infection*

###### (=) Trace:

Uredinia very few in number and covering a limited surface, development of rust generally poor and decidedly subnormal.

###### (-) Slight:

Rust development below normal, but somewhat better than trace.

*Degrees of Infection*—Con.

(±) Moderate:

Variation in rust development from “slight” to “considerable”; when infection is uniform but only medium in quantity 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.

*Miscellaneous Symbols*

(; ) Hypersensitive flecks.

(. ) Necrotic lesions.

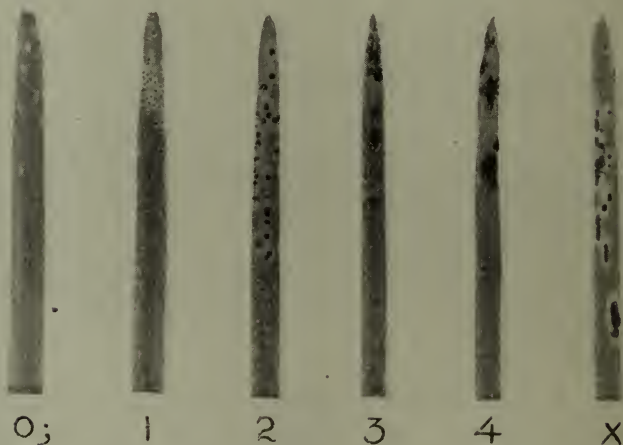


FIG. 8.—Six types of infection caused by physiologic forms of *Puccinia graminis tritici* on different wheat varieties.

*The Separation of Individual Forms from Cultures Containing Several Physiologic Forms*

It is a common experience, when cultures originating from field material are being studied, to find that two or more physiologic forms are present in the same culture. The problem of separating the forms then immediately arises. A mixture of physiologic forms can usually be detected through a casual inspection of the reaction of the differential hosts. One or another of the twelve wheat varieties is almost certain to show evidences of more than one type of infection. Thus, for example, a mixture of forms 21 and 36 would result in two types of infection in the varieties Arnautka, Mindum, Speltz Marz, and Einkorn. One each of these varieties there would be pustules of the “1” type, intermixed with pustules of the “4” type. A separation can be made by selecting isolated

pustules of the "4" type on Arnautka, Mindun, or Speltz Marz and transferring the spores by means of the inoculation needle to seedlings of any one of these varieties. The infections resulting from this inoculation should all be of the "4" type; in other words, a pure culture of form 21 will have been established (fig. 9). In the same manner the other form, namely, form 36, may be obtained in pure culture by inoculating Einkorn seedlings with spores from an isolated "4" type of pustule on Einkorn; or a susceptible variety like Little Club may be inoculated by the spores from a "1" type of infection on Arnautka, Mindun, or Speltz Marz to initiate a pure culture of form 36 (fig. 9). This method which may be designated as the "Mechanical Method" of separation is continually resorted to in the isolation of physiologic forms.

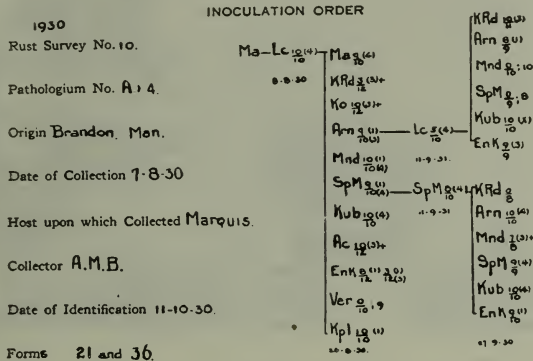


FIG. 9.—Inoculation order showing method of separating two physiologic forms.

Another method of separation may be illustrated by reference to the above-mentioned mixture of forms 21 and 36. This method is based on the fact that certain wheat varieties are immune to certain physiologic forms and susceptible to others. The variety Kanred, for example, is immune to form 21 but susceptible to form 36. Hence an isolation of form 36 may be effected by inoculating a number of differential hosts with spores from the pustules on Kanred. This variety has, through its physiological reaction, accomplished a separation of the two rust strains.

It sometimes happens that the experimenter is confronted with mixtures in which neither of the above-mentioned methods of separation is effective. A mixture of forms 11 and 17 may serve as an example. These two forms produce identical reactions on all the differential hosts except Kanred, which is susceptible to form 11 but immune to form 17. Consequently form 11 may be isolated by infecting the differential hosts with the rust developed on Kanred. Form 17, however, cannot be isolated in this manner or by a process of mechanical separation, as there is no visible difference in the reactions of the two forms on the other differential hosts. To obtain a pure culture of form 17 the investigator may either establish a number of cultures from each of several isolated sori on the chance that some of these sori represent form 17 in a pure state, or, as is preferable, may resort to the method of monosporous isolation.

The technique of single-spore isolation is relatively simple. The spores are dusted sparsely on to a glass slide which is then placed under the low-power objective of a microscope. A fine sewing needle, sharpened to a very fine point and fitted into a holder, is satisfactory for this purpose. Before each transfer the needle is sterilized by passing it through an alcohol flame. The point of the needle is then dipped into vaseline and passed through one or two layers of thin paper. The needle is brought cautiously under the low-power objective until the shadow of the point becomes visible. Its point is then moved downwards

until it touches the slide in the vicinity of a spore. A little further manipulation will bring the point into contact with the spore which, adhering to the film of vaseline, may then be removed and deposited in a small droplet of water on a seedling leaf. Fig. 10 shows the apparatus required for this operation.



FIG. 10.—Apparatus employed in the isolation of single urediniospores.

An important precaution should be mentioned in connection with single spore isolations. The seedlings which are to be inoculated must be grown under absolutely spore-proof conditions. Otherwise air-borne spores may fall on the seedlings prior to inoculation and cause infections which would be erroneously attributed to the individual spores. The seedlings may be protected from such extraneous spores by covering them before the time of emergence with lamp chimneys surmounted by petri dish covers.

*The Differential Hosts and Their Use in the Determination of Physiologic Forms of P. graminis tritici*

The twelve differential hosts selected by Stakman and Levine have been universally adopted for the determination of physiologic forms. Representing, as they do, several distinct *Triticum* species, these differential hosts exhibit a variety of infection types and are thus valuable in differentiating rust strains. Table 1 lists the varieties and selections at present in use at the Dominion Rust Research Laboratory.

TABLE 1.—Differential hosts used for the determination of physiologic forms of *Puccinia graminis tritici*

Species of Triticum	Variety	Source
<i>T. compactum</i>	Little Club	C. I. 4066.
<i>T. vulgare</i>	Marquis	Ottawa No. 15
<i>T. vulgare</i>	Marquis x Kanred	R. L. 226
<i>T. vulgare</i>	Kota	C. I. 5878
<i>T. durum</i>	Arnautka	C. I. 4064
<i>T. durum</i>	Mindum	C. I. 5296
<i>T. durum</i>	Speltz Marz	C. I. 6236
<i>T. durum</i>	Kubanka	C. I. 5639
<i>T. durum</i>	Aeme	C. I. 5284
<i>T. monococcum</i>	Einkorn	C. I. 2433
<i>T. dicoccum</i>	Vernal emmer	C. I. 3686
<i>T. dicoccum</i>	Khapli	C. I. 4013



Stakman and Levine (51) have prepared an analytical key for the determination of physiologic forms of *P. graminis tritici*. Knowing the reactions of each of the twelve differential hosts, it is an easy matter to determine the forms by means of the key. The key is organized like the usual dichotomous botanical key; the characters considered are the resistance, susceptibility, or indeterminacy of the differential varieties. The antithetic statements: infection homogeneous on all differential hosts; infection heterogeneous on some differential hosts—divide the forms into two classes. Within each class the reactions of each host are considered separately, as: Little Club resistant, and Little Club susceptible, and so forth. By this process the forms are grouped into smaller and smaller divisions until they are ultimately designated by number. After a culture has been identified as a certain physiologic form by means of the key, its infections must be compared with the known infections of the form indicated by the key. For this purpose the infections of all known physiologic forms have been tabulated. Thus, the infections of 37 physiologic forms have been tabulated by Stakman and Levine (51), while the infections of forms which have been described more recently are included in table 13. If the infections of the culture under consideration agree with those given in the table for the form indicated by the key, the identification is complete. If the infections of the culture do not agree with those of any known physiologic form, the investigator is faced with two alternatives. Either the culture is a mixture of forms or it is a form hitherto undescribed. A further study of the culture is then necessary in order to ascertain its purity. If further work should prove that the culture is pure it may be classified and described as a new physiologic form.

## THE DISTRIBUTION OF PHYSIOLOGIC FORMS IN CANADA FROM 1919 TO 1930

In tables 2 to 12 and figs. 11 to 16 are given summaries of the information relating to the physiologic forms isolated from cereals and grasses in Canada from 1919 to 1930. Table 2 includes a statement of the number of times each form was isolated annually and the total number of isolations of all forms of stem rust made each year. Tables 3 to 9 contain summaries of the distribution by provinces of the physiologic forms of stem rust from 1926 to 1930 only, as previous collections were scarcely large enough in number or sufficiently well distributed to give an accurate indication of the prevalence and distribution of physiologic forms in Canada. The area in which these collections were made is shown in fig. 11; while the frequency of occurrence of the different forms is diagrammatically presented in figs. 12 to 16.

### Discussion of Results

From 1919 to 1925, collections of wheat stem rust were made chiefly in the grain-growing areas of the three western provinces, Manitoba, Saskatchewan and Alberta; but, in 1926, a systematic search for physiologic forms was begun in all the provinces of Canada and in all sections of each province. As a consequence of this endeavour, in the eastern provinces, rust was collected on the barberry as well as on cereals and grasses. The number of collections made annually was also greatly increased and many new forms were discovered. Most of the new forms originated in Eastern Canada from aeciospores or from urediniospores collected in areas where barberries were common. In 1927 Craigie (2, 3) demonstrated that *P. graminis tritici* is heterothallic and furnished a technique by which the possibility of hybridization between species and physiologic forms might be experimentally investigated. By means of that technique the present authors and others have shown that physiologic forms do hybridize freely on the barberry with the production of new forms. As a result

of these researches it has been necessary to take into account the source of origin of the different physiologic forms and to study separately (a) the forms arising from barberries and (b) those arising from cereals and grasses. Tables 2 to 12 and figs. 11 to 16 deal only with the latter study.



FIG. 11

From them it will be seen that forty-one physiologic forms have been isolated from cereals and grasses in Canada during the twelve-year period, 1919 to 1930. From every three collections of rust made in the field approximately four physiologic forms were isolated, or, in other words, 1.3 forms were isolated from each field collection. This figure would have been considerably higher had the collections been made only in the late season, as at that time each collection consisted of many pustules and was composed usually of two or more forms. The collections made in the very early season, on the other hand, consisted usually of but one pustule and seldom contained more than one form.

All the forms of rust collected in Canada up to 1926 were identified as one or other of the forms found in the United States by Stakman and his colleagues, but since that year a number of forms different from those described by them have been isolated in Canada (34).

Another striking feature made evident by tables 2 to 8 is the fact that different physiologic forms predominate in different years (figs. 12, 13, 14, 15). From 1919 to 1921, form 17 was the predominating one and form 21 occurred only rarely. From 1922 to 1929 the reverse was true: form 21 became one of the most prevalent forms, while form 17 was rarely present. In the same way a form, for example, form 24, may appear for a season or two and then disappear for a period of years; or, again, as with form 11, it may appear so consistently year after year as to be looked upon as a permanent form, and then, without any apparent cause, it may disappear not to reappear for a number of years. In 1925, form 36 suddenly became the predominating form, comprising over sixty-four per cent of all collections made that year. It has continued to be the most common form, although it was found less and less frequently from year to year until 1930, when once again it seemed to be on the increase (fig. 12).

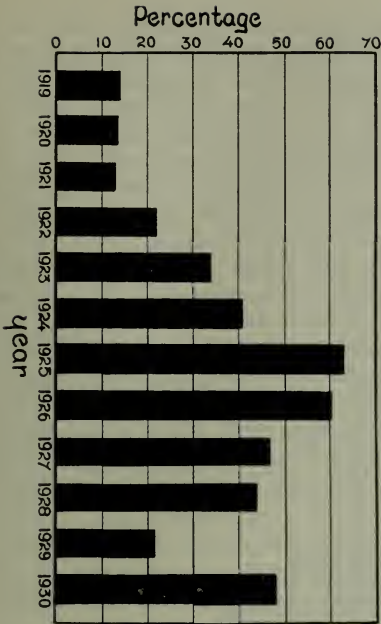


FIG. 12.—Frequency of occurrence of forms 3, 18 and 36 in Canada from 1919 to 1930, expressed as a percentage of the total isolations made each year. Form 36 becomes form 3 at a low temperature and form 18 at a high temperature.

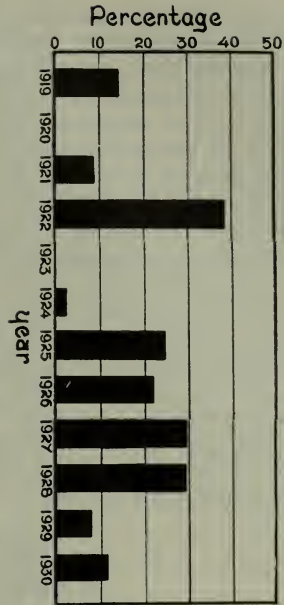


FIG. 14.—Frequency of occurrence of form 21 in Canada from 1919 to 1930, expressed as a percentage of the total isolations made each year.

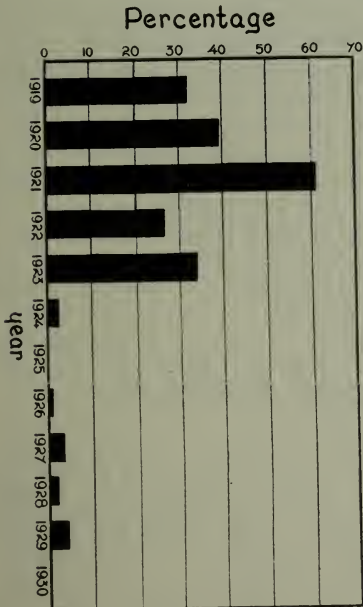


FIG. 13.—Frequency of occurrence of form 17 in Canada from 1919 to 1930, expressed as a percentage of the total isolations made each year.

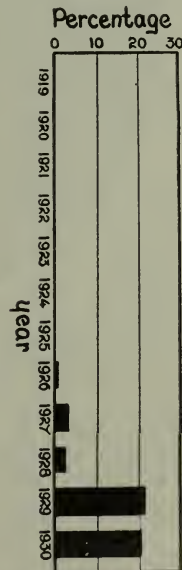


FIG. 15.—Frequency of occurrence of form 38 in Canada from 1919 to 1930, expressed as a percentage of the total isolations made each year.

Whether or not form 36 actually appeared for the first time in 1925 is now questionable. It is known that forms 36, 3 and 18 can be differentiated from one another only by their infection types on Kubanka wheat. Form 36 produces an "X" type of infection on Kubanka, form 3, a "1" type of infection, and form 18, a "4" type of infection. The recent work of Johnson (23) has shown that a variation in temperature produces striking changes in the reactions of some wheat varieties to certain physiologic forms. At a relatively low temperature, about 60°F., when the light intensity is from low to medium, Kubanka wheat is highly resistant to form 36. At a higher temperature, about 75°F., with the same light intensity, it is completely susceptible to this form. As form 36 may be identical with form 3 at a relatively low temperature and with form 18 at a relatively high one, and as all these early identifications were carried on in greenhouses that did not have temperature control apparatus, the probabilities are that from 1919 to 1924, form 36 was incorrectly identified as form 3 or form 18, and that it did not suddenly appear in 1925. In order to eliminate as much as possible the errors in identification that may have arisen through these temperature fluctuations, the frequencies of occurrence of forms 3, 18 and 36 in Canada from 1919 to 1930 have all been added and expressed in Fig. 12 as if the three forms were but one.

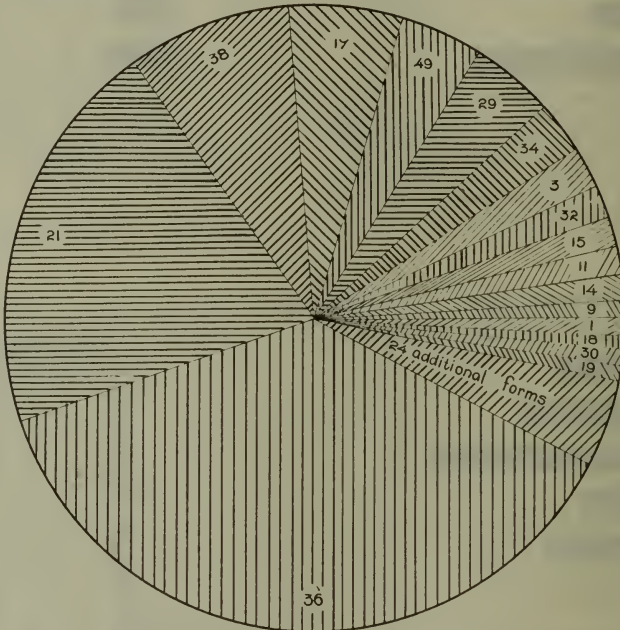


FIG. 16.—Frequency of occurrence of 41 physiologic forms of *P. graminis tritici* isolated from cereals and grasses in Canada from 1919 to 1930, expressed as a percentage of the 2,171 isolations made in that period.

Naturally the question arises: If no record was kept of the temperatures at which these early determinations were made, is it not possible that a number of them were incorrect? It is highly probable that some wrong determinations were made, but they may not be as numerous as might at first be supposed. Johnson (23) has shown that temperature affects the infections of less than 50 per cent of the physiologic forms, viz., those that produce an "X" reaction on certain hosts, and, therefore, 50 per cent of the determinations would stand as they are. Of the 50 per cent that give the "X" infection and, therefore, are affected by temperature, the vast majority differ so widely in their infection types that the forms could not possibly be confused. The only forms that might

have to be re-identified are those whose infections differ only slightly. As a very small number of forms are embraced in such a group, we can safely assume that the vast majority of the determinations reported in tables 2 to 8 are perfectly reliable. A full discussion of the factors affecting the determination of physiologic forms is included in a later part of the present paper.

Although Canada often suffers very severe losses due to the ravages of wheat stem rust, a small number of physiologic forms is apparently responsible for most of the injury. From 1919 to 1930, 2,171 isolations of wheat stem rust were made in distantly separated regions in Canada (fig. 11). Upon identification, forms 36 and 21 comprised over 50 per cent of the isolations, and these two forms together with three others, forms 38, 17, and 49, made up 75 per cent of them (fig. 16).

As forms 36 and 21 have been the prevailing forms in Canada since 1925, it might be supposed that they have been the first to arrive each season, and in that way become well established in the wheat-growing areas. There is no evidence to support this supposition. For five years careful records as to the date of appearance of each form have been kept, and these records show that no specific form arrives first each year: sometimes it is one form, sometimes another. The observations made in Manitoba and Saskatchewan during those years (table 12) indicate that approximately the same number and kinds of physiologic forms are collected early in the season, June 30 to July 31, as are collected late in the season, August 1 to October 31.

## THE FACTORS LIMITING THE DISTRIBUTION AND PREVALENCE OF PHYSIOLOGIC FORMS

There is every reason to believe that a number of more or less complicated factors must govern the distribution and prevalence of physiologic forms of wheat stem rust in Canada, such as, climate, the proximity of the wheat areas to barberry bushes, the length of the uredinial periods of the different forms, the suitability of the wheat varieties grown for spreading the forms, and the general contour of the land in different parts of the country.

In Canada there are three possible sources of stem rust from which inoculum may be distributed in the spring: (1) urediniospores and hibernating mycelium which survive the winter on volunteer grain and on wild grasses; (2) aeciospores from barberry bushes; (3) wind-borne urediniospores.

In Eastern Canada and in British Columbia, it seems quite possible that all three sources of stem rust infection may exist, but, owing to great natural barriers, the spores may be prevented from passing from these provinces into the great wheat-growing belt of the West. In British Columbia, a relatively small number of collections have been made during the past twelve years, but of the isolations made, one-half have proved to be forms quite unknown in any other part of Canada (tables 4, 5, 7, 8, 9). It seems probable, therefore, that although there is a prevailing wind from the Pacific, the spores never reach the wheat areas of Alberta, but are precipitated by the heavy rains on the steep slopes of the Rocky Mountains. This supposition seems to be supported by the results given in table 9. During the past five years approximately half as many new forms have been found in Alberta as have been found in any other province of the Dominion. That spores from the numerous barberries in British Columbia reach Alberta seems improbable, since so few new forms have been discovered there. Whether physiologic forms are also as effectively prevented from spreading into Western Canada from the east, as they appear to be from the west, is not quite clear. A barrier of some twelve hundred miles of dense woodland country separates the nearest wheat fields of Ontario from those of the West,

and the results presented in tables 9 and 10 seem to show that there can not be a very free interchange of spores across this region, for the forms isolated in Eastern Canada are often distinctly different from those isolated in Western Canada. For instance, in 1926, form 38 was one of the three prevailing forms in Eastern Canada and comprised almost sixteen per cent of all isolations made there; while in Western Canada it formed less than a half per cent of the isolations, having been isolated only once. Again, in 1929, forms 49 and 36 were the most commonly occurring forms in Western Canada, whereas those in Eastern Canada were 38 and 21, and forms 49 and 36 were comparatively rare. It is unlikely that there was much interchange of inocula between the West and the East, as otherwise, some similarity should have been found between the relative amounts of the different forms in both sections of the country.

In the great central wheat plain of the West, the uredinal stage of stem rust does not persist from one year to the next, except, possibly, under very exceptional circumstances. Fraser and his colleagues (8) found that the percentage of viable urediniospores dropped quickly from around ninety per cent in the late summer to less than ten per cent in the early winter, and that the alternate freezing and thawing in the early spring quickly reduced the percentage of living spores to a mere trace.

It is improbable also that the barberry has been an important factor in the spread of rust in Western Canada. It is not a native shrub here and has proven so poorly adapted to Western Canadian conditions that it has never been very widely introduced. It was outlawed in 1916 and since then fairly extensive barberry surveys have been carried out to destroy the bushes that were introduced prior to that year. A small number were discovered in towns and cities, but apart from these, comparatively few plantings appear to have been made. For practical purposes, these two sources of rust, *ie.*, aeciospores and overwintering urediniospores, can be disregarded in Western Canada.

There remains, however, the possibility of infection from wind-borne aeciospores and urediniospores from the south. A systematic attempt has been made by members of the staff of the Rust Research Laboratory to obtain evidence of such movements.<sup>1</sup> Three methods were employed: (1) the rust spore content of the air was studied by means of spore traps exposed near the ground, on high buildings, and from aeroplanes; (2) direct observations were made in the field on the progressive development of the rust; (3) and physiologic form surveys of stem rust were made each year to see whether or not the forms were moving in a northerly direction with the prevailing wind.

Briefly stated, the results of these epidemiological studies show that each year some spores appeared in southern Manitoba and southern Saskatchewan before any rust could be found in the fields, and also, that usually the greatest number of spores were caught when a southerly wind was blowing, or but a short time afterwards. The evidence indicated, therefore, that the initial infections in southern Manitoba and southern Saskatchewan were due to wind-borne spores from farther south. Once these infections were established, the rust spread westward and northward across Western Canada, and the date of occurrence of a physiologic form in a given locality varied more or less with the remoteness from the point of origin of the infection, the migration being gradual in certain seasons and more sudden in others. Again, the spread could be traced in advance of the infections in the field by spores caught on slides exposed in different parts of Saskatchewan and Alberta. In the more northern and western districts of Alberta, and the more northern districts of Saskatchewan, rust infections seldom occur early enough in the season to cause material damage.

<sup>1</sup> These experiments were carried out in co-operation with the Dominion Laboratory of Plant Pathology at Saskatoon, and the wing of the Royal Canadian Air Force stationed at Winnipeg.

The forms in the extreme northern and western sections of the great grain-growing areas of Canada were of frequent occurrence in the south, a circumstance which points strongly to the conclusion that the rust moves in a north-westerly direction. Moreover this conclusion was further strengthened by the fact that in addition to the forms common to both the north and the south, there occurred in southern Saskatchewan and Manitoba a greater number of new physiologic forms than were found in Alberta and in the north. For example, from 1926 to 1930, 16.2 per cent of all forms isolated in Saskatchewan and 14.8 per cent of all forms isolated in Manitoba proved to be new forms, whereas only 7.7 per cent of all isolations in Alberta were new (table 9). To draw any final conclusion as to how these new forms originated would be unwarranted. They may have been present in previous years and merely escaped detection, or they may have arisen through mutation or hybridization, but in view of the recent work on hybridization on the barberry, and of the known frequency with which new forms can arise on it, it seems much more probable that these new forms originated in the barberry area south of the Canadian border, and were blown northward.

Just why these new forms are less common in the more remote areas of the Canadian West is not perfectly clear. It is well known, however, that a number of them cannot attack hard spring wheats, and that, therefore, they may have been prevented from spreading westward and northward by the scarcity of suitable host plants. Considerable evidence have been found to suggest a possible relationship between the introduction of new wheat varieties into an agricultural area and the introduction there of new physiologic forms. For instance, form 38, a form to which Marquis is resistant, was discovered in 1926. It was collected only once in Western Canada that year, but from that time onward it so increased in amount (fig. 15) that in 1930 it had become the second most prevalent form in the three Prairie Provinces (table 7). In 1927, owing to the unusually late spring and the consequent fear of rust, durum wheats and varieties other than Marquis, such as Garnet, were planted in many localities, and as greenhouse experiments had already shown that some of these were susceptible to certain forms to which Marquis was resistant (32, 35) the question naturally arose: Would an extensive introduction of these wheats over a period of years bring about an appreciable change in the prevalence of the forms in the West? The results reported in tables 4, 5, 6, 7, 8 and 10 seem to indicate a definite change in the proportion of forms to which Marquis is resistant. They are apparently on the increase in Western Canada, for prior to 1926 a form to which Marquis was resistant was found only once in Western Canada. In 1926, out of the two hundred and sixty-nine isolations of physiologic forms made in the Prairie Provinces, Marquis was resistant to only three. Four years later, in 1930, out of one hundred and sixty-one isolations from the Prairie Provinces, Marquis was resistant to thirty-two. That is, the proportion of forms to which Marquis was resistant had increased from less than one per cent in 1926 to twenty per cent in 1930. Their relative number, however, has been considerably greater in the East than in the West, but these forms have not increased with the same rapidity as they have in the West. The reason for the presence of greater numbers of such forms in the East than in the West is not entirely clear, but is known that, whereas, prior to 1926, Marquis was grown almost exclusively in the West, a greater variety of wheats have always been grown in the East, and these forms may have been prevented from increasing in the West by the lack of congenial hosts.

Another factor which may have influenced the spread of these new forms, and which must influence the spread of all physiologic forms, is the fact that different physiologic forms differ greatly in the length of their respective uredinium-producing periods. During the course of the routine work of identify-

ing physiologic forms, it has frequently been observed that one form will begin to form telia about two weeks after the uredinia have appeared, while another form will show no sign of telia for a month or more. It seemed probable that forms having long uredinial periods in which to propagate themselves must have a much better chance of survival than have those with short uredinial periods. Johnson (23) studied the periodicity of telium formation in a large number of forms and from his results (table 11) it is clear that the five physiologic forms which have occurred most frequently in Canada during the past twelve years, forms 36, 21, 38, 17, and 49 (fig. 16), all have relatively long uredinial periods, and that the only four very rarely occurring forms tested by Johnson, forms 83, 53, 48 and 33, all have much shorter uredinial periods.

Notwithstanding these results, considerable more evidence must be accumulated before the distribution and prevalence of physiologic forms in Canada can be explained fully.

TABLE 2.—Physiologic forms of *P. gr. tritici* collected on cereals and grasses in Canada from 1919 to 1930, with a record of the number of times each form was collected annually.

Form	Number of times form was collected											
	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930
1	2	1								2	11	2
2					1							
3		4	3	10	10	16						
9	4	6	2	3				1	4			
11	2	5	2	3	5	9				5	2	
12		2				5						
14								2	16	2	5	4
15	1							2	8	21	1	
16									2			
17	9	31	27	16	10	1		1	15	6	13	
18	4	7	3	2								
19	1							1	1	6	4	1
21	4		4	24		1	44	84	133	103	26	22
23										4		
24	1		1									
27												1
29		17	1				13	28	4	11	15	4
30		1					1	7				
32		4					3	12	4		10	1
33			1								1	
34				1	3	7	1	4	10	15	4	14
35								1				
36				2			113	211	204	154	69	94
38								18	26	32	65	39
39											1	
40										1		
48								2			2	
49								3	8	14	68	8
50									4		6	
52								1	3	6		
53									1			
63										1		
64										1		
74										1		
75										1		
77										1		
78										4		
83											1	
84											1	
97											1	
113												2
Total number of forms..	9	10	9	8	5	6	6	16	17	21	20	12
Total number of isolations made in year....	28	78	44	61	29	39	175	378	450	391	306	192



TABLE 3.—Distribution by provinces of the physiologic forms of *P. graminis tritici* collected on cereals and grasses in Canada in 1926

Provinces	Physiologic forms																	Total number of isolations made	Total number of forms collected
	9	14	15	17	19	21	29	30	32	34	35	36	38	48	49	52			
P.E. Island.....					1	4			1		1	7			1		15	6	
Nova Scotia.....						1	1					5					7	3	
New Brunswick.....						8	1		1			18	6				34	5	
Quebec.....						2	3		2			8	4				19	5	
Ontario.....		1				8	4		2	1		10	7	1			34	8	
Manitoba.....			1	1		42	11	7	4	1		82	1	1	1	1	153	12	
Saskatchewan.....	1	1	1			18	8		2	2		75			1		109	9	
Alberta.....						1						6					7	2	
Total number of isolations made in year.....	1	2	2	1	1	84	28	7	12	4	1	211	18	2	3	1	378	.....	

TABLE 4.—Distribution by provinces of the physiologic forms of *P. gr. tritici* collected from cereals and grasses in Canada in 1927

Province	Physiologic forms																	Total number of isolations made	Total number of forms collected
	9	14	15	16	17	19	21	29	30	32	34	36	38	49	50	52	53		
P. E. Island.....												1						1	1
Nova Scotia.....							3		1			4	7					15	4
New Brunswick.....						1					1	2						4	3
Quebec.....					1	4					6	1	1					13	5
Ontario.....		1			1	7		1	2	1	7	3		1		1		25	10
Manitoba.....	1	10	4		4	44	1		1	4	57	5	2	1	1			135	13
Saskatchewan.....	3	1	3	1	7	50	3	5		5	81	6	3	1	2			171	14
Alberta.....		4	1	1	2	24				1	47	2	2	1				85	10
British Columbia.....						1												1	1
Total number of isolations made in year.....	4	16	8	2	15	133	4	7	4	10	204	26	8	4	3	1	450	.....	

TABLE 5.—Distribution by provinces of the physiologic forms of *P. gr. tritici* collected on cereals and grasses in Canada in 1928

Province	Physiologic forms																	Total Number of isolations made	Total Number of forms collected				
	1	11	14	15	17	19	21	23	29	34	36	38	40	49	52	63	64			74	75	77	78
Prince Edward Island.....		1						2		1	1	1					1	1	1	1		10	9
Nova Scotia.....			1	2		1	2				1	10										17	6
New Brunswick.....							2			1	3	2										8	4
Quebec.....						2				2	4	2		1								11	5
Ontario.....		1				3		3		2	5	8		2	1							23	8
Manitoba.....					1	11	5	42	5	9	59	7	8	3								150	10
Saskatchewan.....		2	1	5	5	38	4	2	61	2	1	3	2									126	12
Alberta.....	1	1			1	13	2		19					1								38	7
British Columbia.....						1	2		1													4	4
Total number of isolations made in year.....	2	5	2	21	6	6	103	4	11	15	154	32	1	14	6	1	1	1	1	1	4	391	.....

TABLE 6.—Distribution by provinces of the physiologic forms of *P. gr. tritici* collected on cereals and grasses in Canada in 1929

Province	Physiologic forms																	Total number of isolations made	Total number of forms collected			
	1	11	14	15	17	19	21	29	32	33	34	36	38	39	48	49	50			83	84	97
P. E. Island					1		1		2			1	3								8	5
Nova Scotia						2	2	1				1	4			2					12	6
New Brunswick					1		1					1	3								6	4
Quebec					1		1					11				1					14	4
Ontario	1		1	1	1	1	3		3	1	1	1	8		1	1		1	1		26	15
Manitoba	1		3		4		8	6	2		2	22	14	1	1	22	1			1	88	14
Saskatchewan	6	2	1		4	1	10	6	2		1	32	21			38	4				128	13
Alberta	3				1		1	1	1			11	1			5					24	8
Total number of isolations made in year	11	2	5	1	13	4	26	15	10	1	4	69	65	1	2	68	6	1	1	1	306	.....

TABLE 7.—Distribution by provinces of the physiologic forms of *P. graminis tritici* collected on cereals and grasses in Canada in 1930

Provinces	Physiologic forms											Total number of isolations made	Total number of forms collected		
	1	14	19	21	27	29	32	34	36	38	49			113	
Nova Scotia		1								1	2	1		5	4
New Brunswick				1						3	2			6	3
Quebec	1									3	5			9	3
Ontario				1						3	3	1		8	4
Manitoba		2		8	1	2		7	31	15	2			68	8
Saskatchewan	1	1		8		2	1	7	43	11	4			78	9
Alberta			1	3					10	1				15	4
British Columbia				1								2		3	2
Total number of isolations made in year	2	4	1	22	1	4	1	14	94	39	8	2		192	.....

TABLE 8.—Distribution by provinces of new physiologic forms of *P. graminis tritici* collected on cereals and grasses in Canada from 1926 to 1930

Provinces	Physiologic forms															Total number of isolations made	Total number of forms collected				
	38	39	40	48	49	50	52	53	63	64	74	75	77	78	83			84	97	113	
Prince Edward Island	4				1					1	1	1	1						9	6	
Nova Scotia	23				3														26	2	
New Brunswick	15																		15	1	
Quebec	23				2	1													26	3	
Ontario	29			2	4	1		1	1						1	1			40	8	
Manitoba	42	1		2	35	2	5										1		88	7	
Saskatchewan	40		1		49	5	4												99	5	
Alberta	4				7	1	1												13	4	
British Columbia														4			2		6	2	
Total number of isolations made in year	180	1	1	4	101	10	10	1	1	1	1	1	1	1	4	1	1	1	2	322	.....

TABLE 9.—Relative prevalence by provinces of new physiologic forms of *P. graminis tritici* collected on cereals and grasses in Canada from 1926 to 1930.

Provinces	Total number of forms isolated	Number of isolations of new forms	Number of isolations of all forms	Isolations of new forms in per cent of all isolations
Prince Edward Island.....	14	9	34	26.4
Nova Scotia.....	10	26	56	46.4
New Brunswick.....	9	15	58	25.8
Quebec.....	11	26	66	39.4
Ontario.....	21	40	116	34.5
Manitoba.....	19	88	594	14.8
Saskatchewan.....	19	99	612	16.2
Alberta.....	16	13	169	7.7
British Columbia.....	4	6	12	50.0

TABLE 10.—Relative prevalence of forms to which Marquis is resistant in Western Canada and Eastern Canada from 1926 to 1930

Year	Total number of isolations of all forms in Western Canada	Number of isolations of Marquis resistant forms in Western Canada	Isolations of Marquis resistant forms in per cent of all isolations	Total number of isolations in Eastern Canada	Number of isolations of Marquis resistant forms in Eastern Canada	Isolations of Marquis resistant forms in per cent of all isolations
1926.....	269	2	0.74	109	20	18.3
1927.....	392	33	8.4	58	16	27.6
1928.....	314	17	5.4	77	25	32.5
1929.....	240	48	20.0	66	35	53.0
1930.....	161	32	20.0	31	13	41.9

TABLE 11.—Relative length of the uredinal period of nine physiologic forms of *P. graminis tritici*, five of which are of frequent occurrence, and four, of rare occurrence.

Frequently occurring physiologic forms	Average number of days between inoculation and formation of first telia	Rarely occurring physiologic forms	Average number of days between inoculation and formation of first telia
36.....	29.5	83	25.0
21.....	31.0	53	14.0
38.....	28.0	48	14.0
17.....	47.0	33	12.0
49.....	27.5		

TABLE 12.—Frequency of occurrence of physiologic forms of *P. graminis tritici* in Manitoba and Saskatchewan in the very early and late seasons from 1926 to 1930

Physiologic Form	Percentage of collections from June 30- July 31						Percentage of collections from August 1- October 31					
	Year						Year					
	1926	1927	1928	1929	1930	Average for 5 years	1926	1927	1928	1929	1930	Average for 5 years
	%	%	%	%	%	%	%	%	%	%	%	%
1.....			1.7	8.3	1.6	2.3			0.6	0.7		0.3
9.....	0.9	1.5				0.5		0.7				0.1
11.....			0.9	2.8		0.7			0.6			0.1
14.....		3.4		2.8		1.2	0.7	3.5	0.6	1.4	2.0	1.6
15.....		0.6	11.5	2.8		3.0	1.4	4.3	1.3			1.4
16.....								0.7				0.1
17.....	0.9	2.4	1.8			1.0		5.0	1.9	4.2		2.2
19.....			0.9			0.2			2.6	0.7		0.7
21.....	20.5	25.5	21.2	6.9		14.8	25.0	37.1	35.2	9.0	16.2	24.5
27.....											1.0	0.2
29.....	12.3	0.6	1.8	4.1		3.8	2.8	2.1	3.8	6.3	4.0	3.8
30.....	1.6	1.2				0.6	3.6	2.1				1.1
32.....	2.5	0.6			1.6	0.9	2.1			2.8		1.0
34.....	1.6	3.4	1.8		10.9	3.5	0.7	2.1	5.8	2.0	7.1	3.5
36.....	59.0	52.5	46.9	19.4	62.5	48.1	60.7	37.1	41.0	27.7	52.5	43.8
38.....		3.4	4.4	15.3	21.9	9.0	0.7	3.5	2.6	16.6	12.1	7.1
39.....				1.4		0.3						
40.....									0.6			0.1
48.....							0.7			0.7		0.3
49.....		3.0	5.3	36.1	1.6	9.2	1.4		2.6	23.6	5.0	6.5
50.....		1.2				0.2				2.8		0.6
52.....	0.9	0.6	1.8			0.7		1.5	1.3			0.6
84.....										0.7		0.1
97.....										0.7		0.1

TABLE 13.—Mean reactions of wheat stem rust differential hosts to physiologic forms collected in nature in Canada since 1925 or produced through hybridization at the Dominion Rust Research Laboratory, Winnipeg, Man.<sup>1</sup>

Physiologic form	Differential hosts											
	L.C.	Ma.	Krd.	Ko.	Arn.	Mnd.	SpM.	Kub.	Ae.	Enk.	Ver.	Kpl.
38.	4	2 =	4-	3-	X+	X±	X+	X+	X++	4-	1 =	1+
39.	4-	2 =	4 =	3+	4+	3++	4-	4 =	3++	4 =	1 =	1-
40.	4+	4+	4	4+	4+	4+	4	4 =	4	0	4 =	1 =
48.	4+	1	0;	1+	X+	X±	X±	X+	4-	4 =	1 =	1+
49.	4	4-	0	4 =	1 =	0;	0;	X =	3+	1-	0;	1 =
50.	4+	2+	0;	2+	1-	0;	0;	X =	X±	0;	0;	0;
52.	4	4	4-	4	1 =	1 =	1 =	3++	4	4-	4+	1-
53.	4	2±	0	1	4	4	4	4	4	3±	3±	1
56.	4	3+	4-	3+	0;	0;	0;	3+	3+	1-	1-	1
57.	4	4-	0	4-	1	1	1	3+	4-	3+	3+	1
63.	4	X	3+	3+	4	4	4	4	3+	1	1	1
64.	4	4-	3-	1	1-	0;	0;	3±	3	1	0;	1
69*	4	2+	0	0;	1±	1±	1±	3+	3±	3	3+	1
74.	4	4-	3 =	2+	0;	0;	0;	X-	3+	3±	0;	1
75.	4	3+	2+	0;	3+	3+	3+	4-	3+	1	0;	1
76*	4	4-	0	3+	X	X	X	X	3+	3+	X-	1
77.	4	4	3 =	3-	3-c	3-c	3-c	4-	3+	1±	X	1
78.	4	X	0	3 =	3-c	3-c	3-c	3+	3+	3+	1	1
79*	4	4-	0	1-	1-	1-	1-	4-	3+	0;	3+	1
80*	4	3-c	0	3-	0;	0;	0;	X-	3+	1-	3+	1
81*	4	X	0	1+	4	4	4	4	4-	1-	1-	1-
82*	4	3+	3+	3+	0;	0;	0;	X	3+	3+	X	1
83.	4	1+	3-	1-	3+	3+	3+	3+	3+	3+	3+	1
84.	4	X	3-c	3-	0;	0;	0;	X	4	3+	X	1
85*	4	4-	0	3±	4-	4	4	4	4	3+	X	1
87*	4	4	4	3±	4	X	X	X	3+	3+	4	1
88*	4	X	0	1±	4	4	4	4	4	3+	1	1
89*	4	2	3+	0;	4	X	X	X	3±	3+	3+	1
90*	4	4	0	3±	4	X	X	X	3+	1	1	1
91*	4	X	0	X	4	4	4	4-	3+	3+	X	1
92*	4	X	0	X	1	1	1	4	4	1	1	1
93*	4	3+	0	3 =	0;	0;	0;	X	3±	3+	3+	1
94*	4	X	0	X	4-	4-	4-	4-	3+	1	1	1
95*	4	2	3±	1	4	4	4	4	3+	1	1	1
96*	4	X	4	X	4	4	4	4	3+	3+	1	1
97.	4	3+	4	3+	0;	0;	0;	X	3	0;	3+	1
104*	4	X-	0	0;	0;	0;	0;	1	0;	3c	X	0;
106*	4	X	3	3-	4	X	X	X	3	3+	4-	1-
107*	4	3±c	3-	0;	4	4	4	4	3	1-	0;	1-
108*	4	4	4-	0;	1±	0;	0;	X+	4-	3	3+	1-
109*	4	2±	4	3-	1±	1±	1±	4	3-	3+	4	1
110*	4	4-	3	3-	3+	3+	3+	3+	3	3	X-	1
111*	3±c	1-	0	0;	0;	0;	0;	0;	0;	1-	0;	1-
112*	4	X	0	0;	0;	0;	0;	X-	0;	3	0;	1-
113.	4	X	3 =	3±	3±	X-	X-	X-	4	3+	0;	1
114*	4	3+	X	3+	4	4	4	4	4-	3+	1-	1
115*	4	2-	3 =	3 =	4-	4-	4-	4-	4-	3+	3±	1-
116*	4	4-	0	3	4	4	4	4	4-	1	4-	1-
117*	4	4-	0	0;	4	4-	4-	4-	4	3+	3+	1-
118*	4	X	0	1-	1-	1-	1-	4	4	3+	1-	1-
119*	4	X	0	0;	4	4	4	4	4-	3+	3+	1-
120*	4	X	0	3 =	4	4	4	4	4-	3+	3	1-
121*	4	4-	0	0;	1-	1-	1-	4	3+	3	3+	1
122*	4-	3+	4-	0;	4	4	4	4	4-	3+	1-	1
123*	4	2-	0	3-	3+	4-	4	4	4-	3+	3+	1
124*	4	3-c	0	3=c	0;	0;	1 =	3+	X	3	X-	1

\*Physiologic forms not discovered in nature but produced through hybridization experiments.

<sup>1</sup>The writers are indebted to Dr. E. C. Stakman and Dr. M. N. Levine for assigning numbers to all new physiologic forms discovered in Canada.

## FACTORS AFFECTING THE DETERMINATION OF PHYSIOLOGIC FORMS

Rust investigators engaged in identifying physiologic forms have realized for many years that environmental conditions affect the development of rust. It is a common experience that the rust reactions of plants growing in greenhouses vary somewhat at the different seasons of the year. Under these circumstances, infections are usually at their optimum in spring and autumn when average conditions of light and temperature prevail. Consequently, greenhouses where rust investigations are conducted are usually maintained at a moderate temperature and a medium light intensity in order to minimize as much as possible fluctuations in rust development.

### Temperature

In general, it has been considered that these fluctuations were confined to variations in the length of the incubation period and in the degrees of infection, but that the types of infection were not altered. Peltier (39), for example, states that "the period of incubation of stem rust can be extended over a long period by subjecting the inoculated plants to a temperature of 5°C.", but with respect to the type of infection he concludes that "in no instance was the type of infection altered by any environmental factor or factors employed." Melander (26), who studied the length of the incubation period over a wide temperature range, reports that at 10°C. uredinia appear a week later than at 20°C. At 0° and 1°C., he finds that form 36 of *P. graminis tritici* produces uredinia after 59 days while form 15 fails to produce uredinia within 80 days. Regarding the type of infection, he states that, at 0° and 1°C., this type sometimes differed from the normal in that minute uredinia only were formed, but these when exposed to ordinary greenhouse temperature (20°C.), attained an almost normal size.

Waterhouse (57) appears to have been the first to call attention to the fact that fluctuations in rust development induced by environmental changes may involve not only the degree of infection but also the type of infection. He studied particularly the effect of seasonal variation upon the reactions of wheat seedlings to physiologic forms of *Puccinia graminis tritici*. Certain wheat varieties, when infected by some physiologic forms, exhibited different types of rust reaction at different seasons of the year. Form 34, for instance, which in the summer produced a "4" type of reaction on the durum differentials, Arnautka, Mindum, and Speltz Marz, was found during the winter months to give rise to an "0;" reaction on the same varieties.

Similar fluctuations in the types of infection were noted by Johnson (23) when certain physiologic forms of *P. graminis tritici* were cultured simultaneously at different temperatures, and a similar behaviour is recorded by Gordon (14) for *P. graminis avenae*. These investigators have demonstrated that differences in temperature alone are sufficient to bring about striking differences in type of rust infection, for, in their experiments, temperature only was varied while other factors in the environment such as light, humidity, and carbon-dioxide concentration, remained practically unchanged.

It is evident from the above-mentioned results that environmental changes in general, and temperature fluctuations in particular, are factors which must be considered in the identification of physiologic forms. It is especially important to keep temperature under control in greenhouses where physiologic forms, which normally produce "X" reactions on some of the differential hosts, are being determined. Such forms, which for the sake of brevity will be referred to as "X-forms," are peculiarly susceptible to environmental changes. An

experiment reported by Johnson (23) may serve to illustrate the sensitiveness of such forms to temperature as well as to emphasize the importance of controlling temperature when X-forms are being identified. A form, which at ordinary greenhouse temperatures (about 65°F.) had been identified as form 29, was cultured simultaneously in two greenhouse-sections, one of which was maintained at a mean temperature of 58.0°F., the other at a mean temperature of 73.1°F. The same differential varieties were infected in each greenhouse. The final reactions obtained at the two temperatures did not differ very appreciably except on the durum varieties Mindum, Speltz Marz, and Kubanka, on which markedly different reactions were observed. At the lower temperature the reactions were of the type "0"; and "1"; at the higher temperature infection types were recorded as "3+" and "4" (fig. 17). None of these infection types are, however, characteristic of form 29, which, under ordinary greenhouse conditions, produces an "X" type of reaction on these varieties. The bearing of this experiment on the determination of physiologic forms is obvious. If the form were cultured and identified at the lower temperature, it would be classified as a new form; if it were identified at the higher temperature, it would be classified as form 17. At a moderate temperature (about 65°F.), neither determination would be correct.

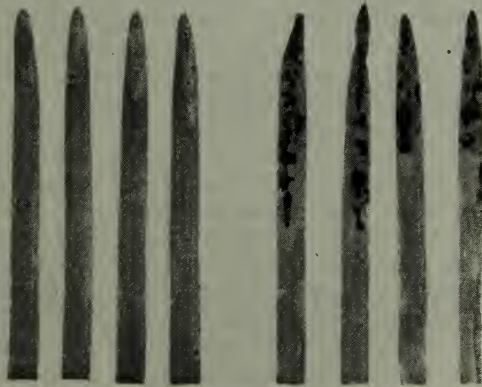


FIG. 17.—Reactions of Speltz Marz seedlings to physiologic form 29. Left: leaves of plants kept at a mean temperature of 58.0°F. Right: leaves of plants kept at a mean temperature of 75.1°F.

It should be stated that all of the wheat stem rust differentials do not exhibit this host-reaction response to temperature to an equal degree. The greatest range of response is to be found in the reactions of the durum varieties Mindum, Speltz Marz and Kubanka. The common wheats, Marquis and Kota, although influenced somewhat in their host reactions by temperature, exhibit a much narrower range of fluctuation. The authors have, thus far, observed no variety of common wheat which, in response to temperature, produced host reactions varying to the same extent as those of the above-mentioned durum varieties. Waterhouse (57), however, states that the *vulgare* wheat variety, Federation, when infected by form 46 produces a "4++" reaction during the summer season and a "1++" reaction during the winter.

It should furthermore be emphasized that the varieties which are influenced in their host reactions by temperature changes do so only when infected by certain physiologic forms. The same varieties when infected by other physiologic forms show much the same type of rust reaction at high and low temperatures. The forms which, in response to different temperatures, vary in their rust reaction are for the most part X-forms under normal conditions. Indeed any form which produces an "X" reaction on durum wheats at ordinary greenhouse temperatures is likely to produce on the same varieties a "4" type of reaction at high and a "1" or "0" type at low temperatures.

The physiology of this peculiar response to temperature variations is at present little understood. We are evidently dealing with a host-parasite relationship which is so delicately balanced that slight environmental fluctuations suffice to shift the balance one way or the other. Although this response to the environment appears for the most part to be characteristic of the reactions of certain host varieties, it does not seem possible to explain it as simply a response of the host, because the same host, when infected by other physiologic forms, *i.e.*, forms which do not produce the "X" reaction under ordinary greenhouse conditions, does not exhibit any appreciable response to temperature variations. A similar objection may be advanced against any attempt to explain the variations in rust infections as a direct response to temperature on the part of the fungus; for the reason, that a physiologic form which produces different infections at different temperatures on one host, will produce, on another host, the same type of infection at all temperatures. There seems to be only one other alternative, namely, that of considering that different temperatures affect the host-parasite relationship differently and thus bring about various types of infection.

The effect of temperature on rust development probably deserves greater recognition than it has been given hitherto. Indeed, *Puccinia graminis* is in no way exceptional in its behaviour in relation to temperature. Waterhouse (57) observed striking differences, perhaps mainly attributable to temperature, between the infections by *Puccinia triticina* and *Puccinia anomala* (*P. simplex*) in summer and winter when these rusts were cultured in the greenhouse. Both rusts showed greater virulence in winter than in summer. Peturson (42) demonstrated a similar behaviour for *P. coronata avenae*. Gassner and Straib (12) and Newton, Johnson, and Brown<sup>1</sup> have shown, in studies of *Puccinia glumarum*, that it is very susceptible to temperature fluctuations. *Puccinia glumarum*, however, is the exact antithesis of the other rusts mentioned in its relation to temperature, for hosts which are susceptible to it at low temperatures become resistant or immune at moderately high temperatures. *Puccinia glumarum* is apparently more sensitive to temperature than any other cereal rust and consequently in any greenhouse studies of this rust the control of temperature becomes of great importance.

### Light

The evidence concerning the influence of light on rust development is less conclusive than that relating to temperature. Peltier (41) studied the effect of different light intensities on the development of *P. graminis tritici* form 21, on the variety Little Club. Artificial illumination was used in his experiments, the source of light being Mazda bulbs of 50, 100, 200, 300, 500, 750, and 1,000 watts. Temperature fluctuations were eliminated in these experiments by the use of suitable control equipment. In his series of experiments, increasing intensity of light was accompanied by an increase in both number and size of pustules. At intensities above 500 watts, pustule development was normal but, below that, the development was increasingly subnormal. At the lowest

<sup>1</sup>Report of the Dominion Botanist for 1930 p. 56.



intensity (50 watts) the types of infection were recorded as "flecks and small pustules," the pustules having the appearance of those on resistant varieties.

It is difficult to state to what extent variations in light intensity are responsible for the striking differences reported by Waterhouse (57) between infections in the greenhouse in winter and summer. Undoubtedly, in his experiments, temperature, light intensity, and daily light duration varied and must all be considered as contributing to the modification in the infection type.

Johnson (23) attempted to determine the effect of gradually increased light intensity and duration on stem rust development by observing the host reaction of wheat seedlings kept at uniform temperatures from December until the end of March. In December and January, light intensity at Winnipeg, where the experiment was carried out, is very low and rust development was correspondingly subnormal. At the end of March, conditions for rust development in the greenhouse are usually near the optimum. The temperature in the greenhouses was controlled. Special attention was given to the infections of X-forms. Varieties other than the durum showed little change in reaction during this period; but the durum, Mindum, Speltz Marz, and Kubanka, showed a marked change in their host reactions. In March, these varieties showed a weak "X" reaction to the majority of the X-forms at 59° F., a temperature at which, during the middle of the winter, the reactions were almost without exception "0;" and "1". At a moderate temperature (about 65° F.), the infections of those forms which, during December and January, produced an "X" type of infection became gradually more pronounced until, at the end of March, they were frequently "X+" and "4". Fig 18 illustrated graphically the effect which an increase in light intensity and temperature produces on the development of X-forms on the above-mentioned durum varieties.

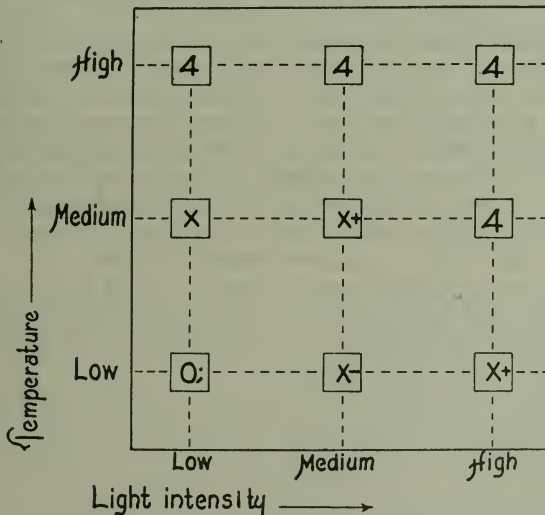


FIG. 18.—A diagrammatic representation of the effect of temperature and light intensity on the development of the "X" reaction.

Forward (7), however, in a careful investigation on the effect of prolonged darkness on the development of *P. graminis tritici* secured results, not dissimilar to those which Johnson (23) and others obtained through the influence of moderately low temperatures. By subjecting wheat seedlings infected with form 21 to periods of darkness varying from two to five days, she was able to modify the infection type on normally susceptible varieties. The plants were subjected to

darkness when the pustules began to take form under the epidermis, and, after remaining in the dark for a given period, were again placed in the light until the final readings were taken. The longer the period of darkness, the greater was the modification of the infection type. Varieties normally exhibiting a "4" type of infection showed, after a sojourn of two or more days in the dark, an ultimate reaction varying from "X" to "2" or "1", or even hypersensitive flecks. Moreover, this modification of the type of reaction was not confined to certain varieties as were the temperature effects reported by Johnson, but could be observed on any of the susceptible varieties under investigation. It is interesting to note that the reactions of seedlings in continuous darkness, except for daily interruptions during which the plants were exposed to light for a few hours, were not modified in the manner described above. The infection type remained the same, only the degree of infection being somewhat diminished.

This author concludes that her results are incompatible with the assumption that the interruption of photosynthesis is the sole effect of importance to rust development when infected plants are placed in the dark. She postulates that the disturbance of compatible relations between the host and parasite results from the establishment in the host cells of some definite metabolic state which is a consequence of subjecting the seedlings to prolonged darkness.

### Carbon-dioxide Concentration

Fluctuations in the carbon-dioxide concentration of the air in greenhouses are probably not sufficiently great to cause appreciable differences in infection type. Nevertheless, it has been shown that variations in the atmospheric concentration of carbon dioxide can produce corresponding variations in infection type. Gassner (10) and Gassner and Straib (11) have studied the effects of different carbon-dioxide concentrations of the air on the development of several cereal rusts, including *Puccinia graminis tritici*. They found that an increase in the carbon-dioxide concentration was accompanied by an increased rust development up to a certain point. An increase beyond this optimum led to a decrease in rust development, and, eventually, at very high concentrations, caused a cessation of pustule formation. The optimum concentration of carbon dioxide for *P. graminis tritici* was found to be between 0.3 and 0.75 per cent, and the upper limit of toleration was stated to be about 6 per cent. The stimulation due to increased concentration of carbon dioxide was manifested mainly in a shortening of the incubation period and an increase in pustule development.

Johnson (23) studied the response of certain X-forms of *P. graminis tritici* to carbon-dioxide concentrations over a range of 0.03 to 4.5 per cent. None of the concentrations used stimulated these physiologic forms to increased pustule development, but there was noted, on the contrary, a marked retardation at concentrations of 3.0 per cent and above.

Although increases in the carbon-dioxide concentration of the air can apparently bring about increases of rust development in some physiologic forms, it does not follow that this factor is of any special importance in greenhouse work. The concentrations of carbon dioxide which affect host reactions are so far in excess of those ordinarily prevailing in greenhouses that there is little danger that any fluctuations in atmospheric carbon-dioxide concentration which may occur in greenhouses will be great enough to affect appreciably rust infections.

### The Importance of Identifying Physiologic Forms under Controlled Conditions

Enough has been said about the influence of environmental factors on host reaction to indicate the importance of controlling the more important of these factors. Temperature and light at any rate should be kept under reasonable

control. It appears that for the identification of most physiologic forms of *P. graminis tritici*, greenhouses should be maintained at a temperature of about 65° F. if rust infections are to be comparable with those given in keys for the determination of such forms.

This precaution is particularly necessary when forms which give the "X" type of infection are being studied. At a higher light intensity, a lower temperature may probably be employed without obscuring the "X" reaction; and, conversely, at a low intensity, it is probable that a higher temperature will not eliminate this type of reaction. A progressive decrease in either temperature or light intensity will, of course, eventually bring about a condition at which the factor in the minimum becomes the *limiting factor*, and no amount of intensifying of the other factors will result in normal host reactions. The different types of infection vary considerably in their stability with regard to temperature. The minimal temperature which permits the development of the "X" type of infection is undoubtedly higher than the minimal temperatures for other infection types. The "X" type of infection commonly fails to appear at temperatures below 60° F. The other types of infection, such as "1", "2", "3", and "4" do not seem to be affected at this or even a lower temperature. As no careful study of these infection types has yet been made throughout the temperature range between 60° and 32° F., their behaviour in this region is more or less conjectural. Melander (26), however, has shown that a form which produces a "4" type of pustule at normal greenhouse temperatures gives rise to a "1" type at 0° to 1° C. When exposed to a temperature of 20° C. the mycellium resumes growth and sporulation and the pustule type becomes "3". Evidently, in this case, the low temperature was a limiting factor which prevented normal rust development.

## THE STABILITY OF PHYSIOLOGIC FORMS

The physiologic-form concept has undergone gradual changes in the last few years. Evidence has gradually been accumulating to show that physiologic forms are more likely to undergo genetic changes than was formerly thought. Theoretically, new physiologic forms may originate in three different ways, (1) by gradual adaptation of an existing form, (2) by mutation of an existing form, and (3) by hybridization of two existing forms on the barberry. These three possibilities will be discussed in the order given.

### Adaptation

The earlier rust investigators such as Ward (54, 55), Freeman (9), and others, devoted much attention to changes in the parasitism of rusts, which they considered to be the result of adaptation. They believed that a rust capable of attacking host A, but not host C, might acquire the power of parasitizing the latter by being grown for a number of uredinial generations on a taxonomically intermediate host B. Later investigators have failed to substantiate their findings and have, in the light of a wider knowledge of physiologic specialization, re-interpreted their results. Stakman, Piemeisel and Levine (49) have shown that the so-called adaptations can be explained by assuming that in each case the original rust culture was a mixture of two or more parasitic strains. At the present time, there seems to be no indisputable case of adaptation on record. If adaptation of rust strains is taking place, or has taken place, it should probably be measured in terms of geologic epochs rather than months or years.

### Mutation

The not infrequent occurrence of mutations in fungi lends support to the idea that mutation may be an important agency in the origin of physiologic

forms. However, owing to the fact that rusts cannot, as yet, be grown on artificial media, it is far more difficult to detect mutations in rusts than in most other fungi.

Moreover, mutations in the fungi are most commonly observed in haploid cultures on artificial media. In the haploid stage of a fungus, theoretically, any mutation may be detected because the phenomena of dominance and recessiveness do not come into play in haploid individuals. But in fungi, such as the rusts, which are studied for the most part in the diploid or dikaryotic phase, a recessive mutation might occur in one dikaryon without any evidence of it being furnished by pathogenic or other characters of the culture. Before such a mutation would be noticeable it would have to occur in both dikaryons unless, of course, the rust were already heterozygous for the character in question. Dominant mutations, which are rare in the higher plants and probably also rare in the fungi, are much more likely to be detectable in the uredinial stage of a rust than recessive mutations. The chances of discovery of any heritable change in a diploid rust are consequently very much smaller than in the case of a haploid fungus growing on artificial media.

The rust investigator is occasionally confronted with a mixture of two or more physiologic forms in a culture of a supposedly pure form. But, as he knows that the opportunities for contamination by other physiologic forms are many, he is hesitant about ascribing any such impurities to mutation. Even when circumstantial evidence for mutation seems strong, it is difficult to prove conclusively that such a change has taken place, as greenhouse compartments where rusts are grown are rarely secure from the entrance of extraneous rust spores.

Nevertheless, two mutations in *P. graminis tritici* have been recorded. Newton and Johnson (33), reported a colour mutation in physiologic form 9, which in all probability occurred while the rust was being cultured in the greenhouse. For six uredinial generations the rust had appeared to be a pure culture of form 9, with the characteristic red uredinia of stem rust. In the seventh generation, or approximately four months after the culture had been started in the greenhouse, an orange pustule was observed among the red. This was isolated, grown in pure culture, and identified as form 9 (orange). In colour the mutant is strikingly different from the parent form, but in pathogenicity the two are practically identical. Both the red parent form and the orange mutant have been cultured for a period of over four years without showing any further change.

This mutation may be considered retrogressive in the sense that it involved a loss of one of the two pigments responsible for the red colour of stem-rust uredinia. The brownish pigment in the epispore was lost while the yellow or orange carotinoid pigment in the centre of the spore remained unaltered.

A somewhat similar mutant has been recorded by Waterhouse (57). In a culture of *P. graminis tritici* form 34, of normal (red) uredinial colour, he observed a single yellowish pustule. A culture was obtained from this pustule. Determinations on the differential hosts showed that the atypically coloured rust was pathogenically identical with the culture in which it originated, namely, form 34. By a comparison with Ridgway's Colour Standards the uredinial colour was found to be "Mars yellow," while the colour of the original culture was classified as "Sanford's brown."

Further evidence of mutation for colour in *Puccinia graminis* is to be found in the occasional appearance of urediniospores of greyish-brown colour. These have been observed by the authors in both *P. graminis tritici* and *P. graminis secalis*, in the uredinial progenies of aecia on barberries infected with telial material gathered in the field. It is not known whether the atypically coloured

strains were present as such in the field material or whether the urediniospores of the preceding generation were of normal colour. The latter alternative is the more likely as urediniospores of abnormal colour have rarely been observed in nature, and then only in close proximity to barberries. In any case, the occurrence of spores of greyish-brown colour indicates that at some time or other a mutation for colour has taken place.

Another mutation is described by Stakman, Levine and Cotter (53). These authors state that "in a uredinial culture of *P. graminis tritici* form 1, constant pathogenically for more than 13 years, there suddenly appeared a new form (subsequently identified as *P. graminis tritici* form 60) different parasitically from anything the writers had hitherto known."

It appears then that *P. graminis tritici* is capable of mutating for both colour and pathogenicity. As neither of these mutations was, as far as is known, induced by any external agency, it is highly possible that *Puccinia graminis* has a certain capacity for mutating. Indeed, it is not improbable that that this phenomenon may account for a few of the parasitic strains of stem rust.

In rusts other than *Puccinia graminis*, the only known evidence of mutation is that presented by Johnston (21) who describes an aberrant physiologic form of *P. triticina* which may have arisen through mutation. This form, which was collected in nature, differed from other known forms in length of incubation period, spore colour, and size of uredinia on susceptible varieties. The incubation period was considerably longer than that of other forms of this rust; the spores were lighter orange in colour than those of other forms; and the pustules formed on susceptible varieties were unusually small.

Attention should, perhaps, be called to the fact that the mutants cited above have one thing in common, namely, that each one exhibits a loss of some characteristic which might be useful to the fungus in its struggle for existence. A pigment is lost in the mutation reported by Newton and Johnson and presumably also in the mutation recorded by Waterhouse. In the mutation recorded by Stakman, Levine, and Cotter, there is a loss of virulence, for the mutant has lost the capacity of infecting heavily the two differential hosts Kubanka and Einkorn. The supposed mutant reported by Johnston exhibits a loss in the intensity of spore colour and a loss of vigour, as shown by the increase in the length of the incubation period and the small size of uredinia on susceptible varieties.

## HYBRIDIZATION IN *PUCCINIA GRAMINIS*

The foundation for the work on hybridization in the rusts was laid by Craigie (2, 3, 4) through his discovery that *Puccinia graminis* and *Puccinia helianthi* are heterothallic. The technique of crossing two strains of rust is also essentially that developed and employed in his investigations.

The discovery of the function of the pycnia supplied rust investigators with a means of studying hybridization in the rusts, much as the discovery of the function of the male and female elements in higher plants supplied the earlier plant investigators with a means of studying hybridization. It is probable, however, that many difficulties have yet to be surmounted before the heredity of rusts can be studied with anything approaching the accuracy of such studies in higher plants.

### Objects of the Study

The objects of the work on hybridization can perhaps be stated most satisfactorily by listing some of the questions that arose when a means of crossing physiologic forms was discovered. The following questions are some of the more important.

1. What happens when physiologic forms are self-fertilized? Will each form break up into several physiologic forms, or will each form act like a pure line, and produce a progeny consisting of one form only? In other words, are physiologic forms heterozygous or homozygous?

2. Will physiologic forms hybridize, and if so, what is the nature of such hybrids?

3. Can crosses be made between physiologic races<sup>1</sup> of *Puccinia graminis*? If such crosses are made, what is the nature of the hybrids and how do they segregate?

4. Do Mendelian factors determine the nature of physiologic forms?

Although these questions have not yet been fully answered, enough information has been gathered to warrant a discussion of them. As such a discussion can be more satisfactorily undertaken if the technique of hybridization studies is first explained, it is desirable to give a resumé of the methods employed.

## Methods and Technique of Hybridization

### PRODUCTION OF THE TELIOSPORES

A resumé of the methods used in hybridization studies begins naturally with the production of the teliospores of the physiologic forms which it is desired to investigate. Unless the teliospores of each form be produced in *pure culture*, the subsequent crossing studies will be worthless. Consequently, it is essential to commence with a pure uredinal culture of the physiologic form in question and to carry this culture through into the telial stage uncontaminated by other rust strains. As most of the physiologic forms of *P. graminis tritici* do not develop teliospores on wheat seedlings, it becomes necessary to obtain teliospores from plants approaching maturity. While the rust on these mature plants is still in the uredinal stage, the purity of the form is checked, at least once, by inoculating the differential hosts of wheat stem rust. If any impurity is detected, the teliospores are immediately discarded.

### GERMINATION OF THE TELIOSPORES

Considerable difficulty was experienced at first in inducing teliospores which developed in the greenhouse to germinate. Such spores did not always germinate even after they had been over-wintered out of doors. Johnson (23) however, has succeeded in obtaining fairly consistent germination by a method which will be described briefly. The teliospores are formed on wheat plants approaching maturity. During the formation of the spores, the plants are kept in a greenhouse maintained at a low temperature (about 60° F.). Teliospores formed at higher temperatures germinate less readily. Immediately after the spores are formed, the culms bearing them are cut up into short pieces and frozen for a period of about two weeks in blocks of ice in a refrigerator maintained at about -5° C. The blocks of ice are thawed. The teliospore-bearing straws are then fixed firmly to a wooden frame and subjected to a spray of cold tap water, or placed under running tap water, for about one week. Thereafter, they are alternately dried and wetted (dry 2 days, wet 2 days) until germination commences. This procedure, which is merely a rough simulation of the conditions which bring the germination of teliospores in nature, usually results in germination after the first or second period of drying.

<sup>1</sup> The term "physiologic race" is here used—as adopted by J. C. Arthur in his "Plant Rusts"—to designate the original biologic forms of *Puccinia graminis*, as established by Eriksson, namely, *P. graminis tritici*, *P. graminis secalis*, *P. graminis avenae*, etc. The term "race" has been selected in preference to the term "variety" as the latter term has been frequently employed in this paper to denote varieties of cereals.

## INFECTION OF THE BARBERRY

Different methods of inoculating the barberry are undoubtedly employed by different rust investigators. The method here described, which has been in common use at the Dominion Rust Research Laboratory, generally produces satisfactory infections. A small barberry plant, growing in a four-inch flower pot, is covered by a lantern chimney (fig. 19) on top of which is placed a petri-dish cover lined with wet blotting paper to which are fixed pieces of straw bearing germinating teliospores of one physiologic form. The plant enclosed by the chimney and petri-dish cover, is placed in a damp incubation chamber. The petri-dish cover is revolved from time to time so as to secure an even distribution of sporidia on the leaves of the plant, and at intervals of a few hours the plant is sprayed with a fine spray of water in order to maintain a film of moisture on the leaves.

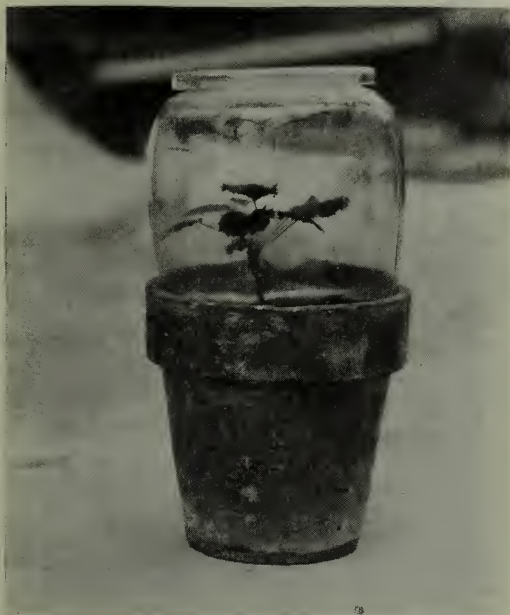


FIG. 19.—Barberry seedling ready for inoculation. Short pieces of straw bearing the germinating teliospores are suspended from the petri-dish covers.

The time during which the teliospores remain above the barberry plant depends on the amount of infection desired. If the physiologic form is to be "selfed", the petri-dish cover is not removed for forty-eight hours, so that the plant may become heavily infected. If the physiologic form is to be crossed with another form, a much lighter infection is desired, in which case the petri-dish cover is removed at the end of from four to six hours, but the plant remains in the incubation chamber for forty-eight hours.

## THE SELFING OF A PHYSIOLOGIC FORM

The selfing of a physiologic form involves the infection of a barberry with the sporidia of one physiologic form and the intermixing of the pycnial nectar of the resulting haploid pustules. About ten days after inoculation, or when pycnial nectar becomes abundant on the infections, the operation of selfing is

performed. This consists in carefully intermixing the nectar of the various pustules so that the haploid elements may enter into as many combinations as possible. The intermixing of the nectar may be done conveniently by means of a sterile platinum loop.

The study of the progeny derived from the selfing of a physiologic form is, of course, carried out through inoculation with the aeciospores which usually make their appearance within a week after selfing. As each aecial cup (aecium) commonly produces but one physiologic form it has been found convenient to work with mono-aecial cultures, *i.e.*, cultures each of which originates from a single aecium. The aecia, when used to inoculate susceptible wheat seedlings, give rise to uredinial cultures which may be studied for pathogenicity and resolved into their component physiologic forms.

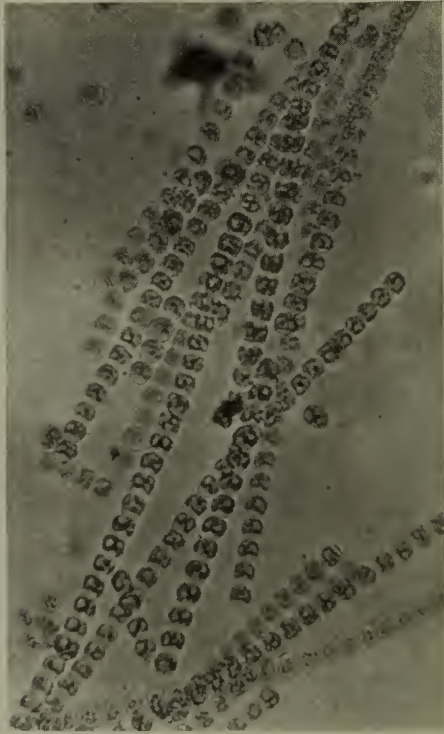


FIG. 20.—Chains of aeciospores separated through a rupture of an aecium which had been placed on a drop of water.

The method employed in establishing such cultures is briefly as follows: The aecia are removed from the barberry leaf as soon as they have grown to a length of one or two mm. but before they have opened to discharge their spores. They are detached from the leaf by a pair of fine forceps, each aecium being placed in a small drop of water on a sterile microscope slide and subsequently crushed by means of a small sterile metal spatulum. The crushing of the aecium is frequently unnecessary as it rapidly absorbs water and bursts open with a force which is often great enough to discharge chains of aeciospores far beyond the confines of the drop in which it is placed. (*Vide* fig. 20.) A single wheat seedling is then inoculated with the spores of each crushed aecium by drawing the microscope slide obliquely across the leaf so as to deposit the aeciospores on its surface. As soon as infections become apparent on the wheat



seedlings, the leaves bearing pustules are labelled and numbered. The pustules on each leaf now represent a uredinial culture of mono-aeial origin. When fully developed the pustules on each leaf are used for the inoculation of a number of plants of a susceptible variety in order to increase the inoculum in preparation for a study of the differential-host reactions and other characteristics of the mono-aeial cultures.

In this manner cultures of mono-aeial origin may be established without much difficulty. Considerable precautions must be taken however, to avoid contamination by extraneous spores. All wheat seedlings which are to be inoculated by mono-aeial spores are grown under spore-proof conditions, that is, the pots in which they are grown are covered by lamp-chimneys over which petri-dish covers have been placed. It is also important to remove the aecia before they open to liberate their spores, as aecia which have already opened may be contaminated by spores discharged by adjacent aecial cups.

One of the most important considerations in a study of the progeny of a selfed physiologic form is that of securing a good random sample of the aecia. Obviously all the aecia on a heavily infected barberry plant cannot be used to establish mono-aeial cultures owing to the immense amount of work which would be involved. There remains the alternative of selecting at random aecia which are representative of the whole aecial population. Consequently the barberry plant is heavily infected so that as many aecial pustules as possible may develop. A few aecia\* are then selected from each pustule. Another possible alternative which has, however, been rejected, is that of making mass inoculations with aeciospores derived from large numbers of aecia. This process may produce satisfactory results when the form studied is homozygous, as the progeny will then consist of but one physiologic form. If the form is heterozygous, as most physiologic forms are, the problem of separating the numerous strains composing the mass culture becomes extremely difficult. For this reason the writers have, in all later work, used mono-aeial cultures originating from single aecia selected at random from the aecial pustules of heavily infected barberries.

#### THE CROSSING OF TWO PHYSIOLOGIC FORMS

The first prerequisite to success in crossing two physiologic forms is to obtain haploid pustules of both forms on individual barberry plants. The fewer the infections on a barberry leaf the greater are the chances that the pustules will be haploid. A light infection of the barberry is, therefore, desirable. This is secured, as previously pointed out, by suspending the germinating teliospores above the barberry plant from four to six hours instead of for forty-eight hours, as is customary when heavy infections are desired.

After their removal from the inoculation chamber, the barberry plants are placed in a fine-meshed wire cage to protect them from flies and other insects which are attracted by the pycnial nectar. Here the plants are allowed to remain for at least three weeks after infection. By this time aecia will have developed on most or all of the bisexual pustules, and it may be concluded that the pustules which show no sign of aecial development are unisexual and may be used in crossing two physiologic forms. There remains, however, the possibility that a few of these seemingly mono-sporidial pustules may have arisen from infections by two sporidia of the same sex.

The method followed in crossing two physiologic forms is illustrated diagrammatically in fig. 21. The barberry on the left of the figure (A) is inoculated by form 17, the one on the right (B), by form 49. Some of the pycnial nectar from one of the unisexual pustules of (A) is successively transferred to the six unisexual pustules of (B) by means of a platinum loop which is sterilized by passing it through a flame after each transfer. To complete the cross, nectar from

pustule (6) of (B) is transferred back to the unisexual pustule of (A) from which the nectar had been first taken. Small tags showing the date on which the cross was made and diagrams of the distribution of the pustules on the leaves are attached to the leaves bearing the pustules used in the operation.

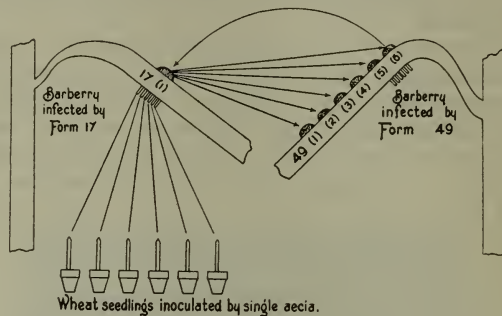


FIG. 21.—Diagram illustrating the method followed in crossing two physiologic forms.

As the amount of nectar produced by a single pustule is often limited, a modification of the above method is sometimes necessary. This consists in removing the nectar of the unisexual pustule from which transfers are to be made, and mixing it with a drop of distilled water on a watch-glass. The nectar thus diluted, is then successively transferred to other unisexual pustules in the manner previously described. It appears, from crosses in which this method has been used, that the diluted nectar is quite as efficacious in inducing the formation of aecia as the undiluted nectar. This method has, furthermore, the advantage that the nectar from one pustule may be transferred to a large number of pustules. Thus, in some crosses, the nectar of one pustule of one physiologic form has been applied to as many as twenty pustules of another physiologic form.

When a cross has been made between two physiologic forms and aecia have developed, ten or fifteen cups are usually selected from each pustule for transfer to wheat seedlings in order to increase the inoculum so that a study may be made of the nature of the hybrid forms.

### The Results of Hybridization Studies and Their Significance

#### THE RESULTS OF SELFING PHYSIOLOGIC FORMS OF *Puccinia graminis tritici*

Selfing experiments, conducted by Newton, Johnson and Brown (36, 37), have made it clear that most physiologic forms, when studied with respect to pathogenicity, do not behave like pure lines. Segregation of pathogenicity factors takes place during teliospore germination and these factors recombine in various ways to produce a number of physiologic forms in the next diploid generation.

Fig. 22 shows the result of selfing eight physiologic forms. Only one of the eight forms, bred true to type for pathogenic characters. One of the forms, namely, form 36, did not breed true for either pathogenicity or urediniospore colour. Evidently, then, if it is safe to generalize from these results, the majority of the physiologic forms of *P. graminis tritici* are in a heterozygous condition with reference to their pathogenicity. The number of possible factorial combinations is apparently large, as is indicated by the fact that the 219 uredinial cultures originating from the selfing of these eight forms were resolved into one or another of thirty different physiologic forms. Of these thirty forms, eighteen had been previously described but twelve were hitherto unrecorded.

The information gained from the selfing of these forms is inadequate for the purpose of establishing a factorial basis for the inheritance of pathogenicity. The progeny of each physiologic form would have to be studied much more extensively than was done. If the results are to be explained on a Mendelian basis, there is no doubt that, in some of the forms at least, several factors are involved. For example, eighteen different physiologic forms were identified from the progeny of form 53, although only 44 uredinal cultures arising from this form were studied. The number of factors necessary to explain the appearance of eighteen forms will vary according to whether dominance is complete or incomplete. If dominance were incomplete and the factors acted independently, three factors would suffice to account for this number of forms. But if dominance were complete it would be necessary to assume that at least five factors were operative. If, however, the progeny had consisted of 500 or 1,000 cultures, the probability is that many more forms would have been identified and an even greater number of factors would have to be assumed.

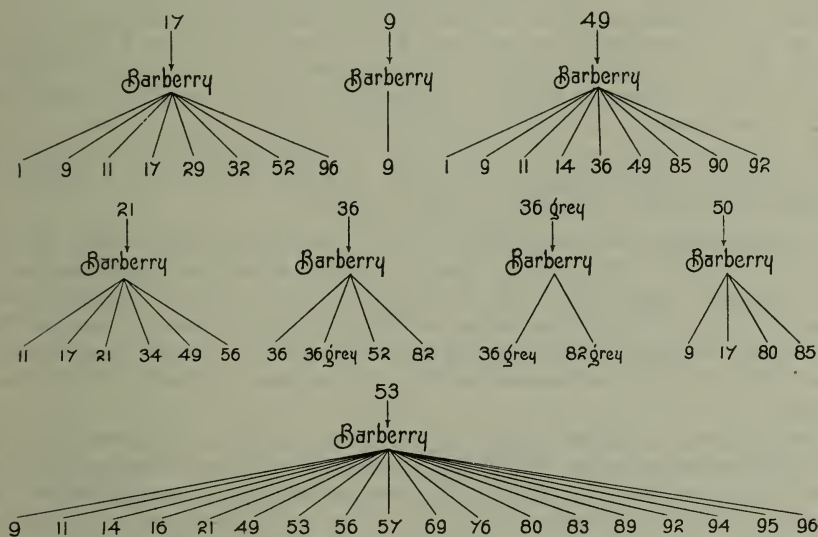


FIG. 22.—Diagram showing forms that arose from the selfing of eight physiologic forms.

#### CROSSES BETWEEN PHYSIOLOGIC FORMS OF *Puccinia graminis tritici*

**THE INHERITANCE OF PATHOGENICITY.**—The present knowledge of the inheritance of pathogenicity in crosses between physiologic forms of *Puccinia graminis tritici* is based chiefly on hybridization studies reported by Newton, Johnson and Brown (36, 37), and by Waterhouse (56). These studies have not yet been carried on long enough or on a scale sufficiently large to permit the laying down of a satisfactory genetic basis for the inheritance of pathogenic characters. Nevertheless, certain generalizations have become possible as a result of studies of first, second, and third-generation hybrids<sup>1</sup> of certain crosses.

<sup>1</sup>For the sake of convenience the term "hybrid" is used to denote any association of genotypes accomplished by means of crosses between physiologic forms or physiologic races of stem rust. While realizing that the validity of the term, when used in this sense, is open to argument, the authors have preferred to use it rather than to coin a new term for the designation of the above-mentioned genotypic associations.

*First-generation hybrids of crosses between physiologic forms.*—The  $F_1$  hybrid of a cross between two physiologic forms may be either pathogenically identical with one or the other of the parent forms, or, as is more frequent, it may differ from both. If the physiologic forms which are crossed are heterozygous for pathogenicity, it frequently occurs that different crosses between the same two forms give rise to pathogenically different hybrid strains. Thus, for example, three crosses between form 17 and form 49, both of which are heterozygous, gave rise to four different physiologic forms in the first generation, namely forms 30, 52, 87, and 93. Crosses between a homozygous and a heterozygous form have also been found to yield pathogenically different  $F_1$  hybrids. No cross has yet been made between two homozygous forms, but when such a cross is made it will probably be found that the different  $F_1$  hybrids are identical in pathogenicity.

A number of crosses have been made which produced  $F_1$  hybrids pathogenically identical with one or the other parent form. Thus, in a cross between form 9 and form 15, the  $F_1$  hybrids derived from the cross and the reciprocal cross were identified as form 9. Evidently form 9 was dominant over form 15. Similarly, in crosses between form 9 and form 52, all the  $F_1$  hybrids were identical with the form 9 parent in their reactions on the twelve differential hosts. In both instances, however, subsequent selfings of the hybrids proved that the hybrid strains of form 9 were geneotypically different from the parent form 9. Apparently, then, the Mendelian principle of dominance is exhibited in crosses between physiologic forms of *P. graminis tritici* in the same manner as in crosses between varieties of higher plants.

A certain degree of intermediacy is not uncommonly met with in hybrids which differ from both parent forms. Examples of such intermediacy are found in crosses between form 49 which produces a "1" type of infection on the durum and form 17 which produces a "4" type of infection. Some of the  $F_1$  hybrid forms exhibited an "X" type of infection on the same varieties. This infection type may be considered to be intermediate between the "1" and the "4" types.

Intermediacy in pathogenicity is not, however, by any means the rule in  $F_1$  hybrids between forms which are virulent and forms which are non-virulent on the same hosts. A number of crosses have been made between forms which give a "1" and forms which give a "4" type of infection on the durum varieties, Arnautka, Mindum and Speltz Marz. Ten different hybrid forms have been obtained from these crosses. Two of these showed an intermediate infection, namely "X," two produced a "1" infection, and six produced a "4" infection on the above-mentioned hosts.

Occasionally a hybrid form may show, on a certain host variety, a greater virulence than either of the parent forms. This phenomenon however, appears to be rare and has been noted only in hybrids derived from crosses between form 17 and form 49. The variety Kanred is immune from both forms and the variety Vernal is highly resistant to both. Of the four  $F_1$  hybrid forms derived from the above crosses, two possessed the capacity to attack Kanred heavily, and all four were capable of vigorous infection on Vernal.

*Second and third-generation hybrids.*—A study of the pathogenic characters of the second and subsequent hybrid generations constitutes one of the most interesting phases of the work on hybridization. Certain  $F_1$  hybrid forms have been selected for further study with reference to the pathogenicity of their progeny in the second and third generations. With this object in view, certain of the  $F_1$  hybrids were selfed, by means of the method already described in the discussion on selfing, and the resulting  $F_2$  hybrids identified as one physiologic form or another according to their reactions on the twelve standard differ-

ential hosts. The second and third-generation progeny of one of these hybrids will be discussed here in order to show what appear to be typical results of the selfing of hybrid forms.

The  $F_1$  hybrid selected for study was derived from a cross between form 9a<sup>1</sup> (orange) and form 36 (greyish-brown). The  $F_1$  progeny was all identified as form 17a of normal (red) uredinial colour. In the second generation, 126 uredinial cultures, arising from individual aecia, were studied for uredinial colour and pathogenicity. At present, only the pathogenic behaviour of these cultures will be considered. The  $F_2$  hybrids were resolved into one or another of seven different physiologic forms (table 14). Only one of the grand-parental forms,

TABLE 14.—A comparison between observed frequencies of physiologic forms in the second hybrid generation of the cross between form 9a and form 36, and frequencies calculated on the basis of a 27:9:9:9:3:3:3:1 ratio.

Physiologic forms	17	1	36	11	15	85	57	
Observed frequencies.....	69	22	14	15	1	4	1	0
Calculated frequencies.....	54	18	18	18	6	6	6	2

form 36 (greyish-brown), reappeared in the second generation. The  $F_2$  population studied was too small to permit of the establishment of a factorial basis for the inheritance of pathogenicity; but there appears to be little doubt that the inheritance obeys Mendelian laws. The distribution even suggests that three independently inherited Mendelian factors may be involved.

If pathogenicity is governed by Mendelian laws of inheritance, it would be expected that a number of homozygous strains would appear in the third and subsequent generations. At the present time, the study has not been carried farther than the third generation, and in this generation, only two  $F_2$  hybrids have, so far, been investigated. One of these, form 36a (greyish-brown), is evidently homozygous, as the twenty-six  $F_3$  cultures studied were all identified as form 36a (greyish-brown). The other  $F_2$  hybrid investigated, namely, form 1a (red) is heterozygous for pathogenicity, for its  $F_3$  descendants corresponded to two different physiologic forms. Of the thirty-eight  $F_3$  cultures studied, twenty-eight were identified as form 1a while ten were classified as a new physiologic form which differed but slightly in pathogenicity from form 1a. Manifestly some of the hybrids are already in a homozygous condition in the second and third generation while others still remain heterozygous.

*Cytoplasmic inheritance.*—This type of inheritance has already been noted in the smut fungi by Goldschmidt (13) who found evidence of it in a hybrid between two physiologic forms of *Ustilago violaceae* Pers. The pathogenic characters of the  $F_1$  hybrids of certain physiologic forms have led the writers to believe that cytoplasmic inheritance functions also in the rusts. The reasons for this belief will be set forth briefly.

When a cross and its reciprocal cross are made between two physiologic forms by transferring the pycnial nectar of a monosporidial pustule of form A to a monosporidial pustule of opposite sex of form B, the aecia formed on each of the pustules should give rise to one and the same physiologic form, providing pathogenicity is governed by Mendelian factors. Out of eight crosses made in both directions (each consisting of a cross and its reciprocal cross), three

<sup>1</sup> Forms to which the letter a is suffixed differ from forms designated by the same numeral in that they produce a lower infection type on the varieties Marquis and Kota; but these differences are not considered to be sufficiently great to justify the creation of a new form.

have conformed to this theory. In these three crosses the hybrids from both sides of each cross were identical in pathogenic characters. In each of the remaining five crosses, however, the hybrid arising from one side of the cross differed somewhat in pathogenicity from the hybrid arising from the opposite side. These eight crosses are represented diagrammatically in fig. 23.

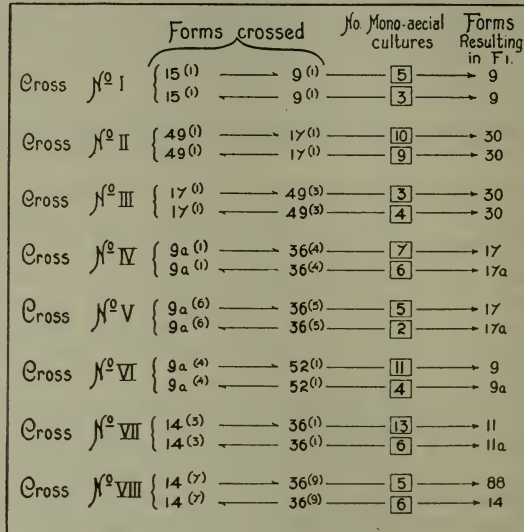


FIG. 23.—A diagrammatic representation of eight crosses between physiologic forms and their reciprocal crosses. In the first three crosses, there is no evidence of cytoplasmic inheritance; the forms arising from each cross and its reciprocal cross are the same. In the last five crosses, cytoplasmic inheritance is manifested; the forms arising from each cross and its reciprocal cross differ somewhat pathogenically.

The last of these crosses, No. VIII, fig. 23, affords a representative example of this type of inheritance, and may serve to illustrate the difference in pathogenicity between the hybrids arising from the two sides of the cross. The two parent forms used in this cross, form 14 and form 36, differ widely in the infection type, produced on the variety Marquis. Form 14 produces a "1" infection, form 36 a "4". The F<sub>1</sub> hybrids arising from the two sides of the cross produced identical infections on each of the differential varieties except Marquis. On this host the hybrid arising from the form 14 side of the cross produced a "1" infection, and was identified as form 14, while the hybrid from the opposite side of the cross produced an "X+" infection and was classified as form 88. Now, if pathogenicity is governed by Mendelian factors only, the infections of the two hybrid forms on Marquis should be identical, as were the infections of the hybrid forms on each of the other differential hosts. Apparently, the results can not be explained on a Mendelian basis alone. If it is assumed, however, that the cytoplasm of the maternal pustule—the one to which the pycniospores of the other parental pustule were applied—also contributes to the inheritance of pathogenicity, a plausible explanation can be reached. This explanation is the more plausible as there is good reason to suppose that very little, if any, cytoplasm reaches the maternal pustule by means of the pycniospores transferred to it, the nuclei of these spores being surrounded by only a very limited amount of cytoplasm.

The pycniospores of pustule 36 (9)<sup>1</sup> were transferred to pustule 14(7), the maternal pustule. It would then be expected that, as the nuclei of the pycniospores of pustule 36(9) and the nuclei of the haploid hyphae of pustule 14(7) contribute equally to the inheritance of the hybrid, the additional influence of the cytoplasm of the mycelium of pustule 14(7) would cause the hybrid originating in it to resemble form 14 in pathogenicity rather than form 36. This theory is confirmed by the results, for the hybrid is pathogenically identical with the form 14 parent. For the same reason, when the pycniospores of pustule 14(7) are transferred to pustule 36(9), it would be expected that the hybrid formed in the latter pustule would resemble form 36 in pathogenicity rather than form 14. Actually the resemblance of the hybrid to form 36 is confined to the reactions on the variety Marquis. The most plausible explanation of the resemblance of the infections of these two hybrid forms on Marquis to the infections of their respective maternal parents on this host is to be found in what might be called "maternal cytoplasmic inheritance."

Compared with the influence of the nucleus, the influence of the cytoplasm on rust reactions must be very slight. In all the crosses made, so far, the influence of the cytoplasm has only been detected in the reactions of the varieties Marquis and Kota. If there is any effect on the reactions of the other ten differentials, it is so slight as not to be easily discernible.



FIG. 24.—The reaction of Marquis to physiologic forms in the F<sub>2</sub> generation of the cross, form 14 x form 36, in which the F<sub>1</sub> hybrid originated on the form 14 side of the cross.

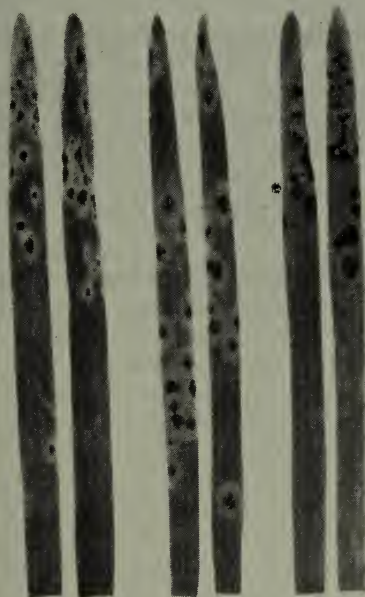


FIG. 25.—The reactions of Marquis to physiologic forms in the F<sub>2</sub> generation of the cross, form 14 x form 36, in which the F<sub>1</sub> hybrid originated on the form 36 side of the cross.

<sup>1</sup> The figures in parentheses refer to the haploid pustules used in the cross; each haploid pustule from which or to which nectar was transferred is designated numerically.

The behaviour of this type of inheritance in later hybrid generations is a matter of some interest. The evidence available from a study of  $F_2$  and  $F_3$  hybrids indicates that the cytoplasmic influence is perpetuated in later generations. A study of the  $F_2$  progeny of the above-mentioned hybrids of form 14 and form 36 (*vide* fig. 23, Cross VIII) may serve as an example of the influence of the cytoplasm in the second generation. The  $F_1$  hybrid-form 14 was selfed. In the  $F_2$  generation there arose eleven different physiologic forms. On Marquis nine of these forms produced "1" and "2" infections, which are characteristic of the parental form 14 (fig. 24). Two of the forms gave a slightly more vigorous infection, "3=c" (3= with sharp chlorosis). The  $F_2$  progeny of the other hybrid form, 88, was resolved into eight physiologic forms. Six of these forms produced on Marquis, an "X" reaction identical with the reaction of this host to form 88, while the remaining two forms produced a "4—" infection similar to that of the grand-parental form 36. Characteristic reactions of  $F_2$  hybrid-forms are shown in fig. 25.

#### THE INHERITANCE OF UREDINIOSPORE COLOUR

A certain amount of information concerning the inheritance of urediniospore colour has become available through crosses which have been made between atypically coloured strains of *P. graminis tritici*. One of these strains, physiologic form 9a (orange) was discovered as a mutant of form 9 of normal (red) uredinial colour. Two other strains of atypical uredinial colour, form 36 (greyish-brown) (and form 52 (greyish-brown) were discovered in cultures derived from barberries infected artificially with sporidia of teliospores collected in the field.

Two distinct pigments are present in normal urediniospores of *P. graminis tritici*, one, an orange pigment in the cytoplasm, the other, a brownish pigment in the spore-wall. The urediniospores of the orange strain lack the brownish pigment in the spore-wall, and the urediniospores of the greyish-brown strains lack the orange pigment in the cytoplasm.

#### *Crosses Between Orange and Greyish-brown Physiologic Forms.*

A number of crosses have been made between form 9a (orange) and form 36 (greyish-brown), and between form 9a (orange) and form 52 (greyish-brown). The majority of the  $F_1$  hybrids obtained were normal (red) in uredinial colour. A few exceptions, however, have occurred in which the urediniospores of the hybrids were orange in colour.

One of the normally coloured  $F_1$  hybrids between form 9a (orange) and form 36 (greyish-brown) was chosen for a study of its  $F_2$  progeny. In the second generation, observations for spore colour were made on 135 uredinial cultures. These cultures fell into four distinct colour groups: red, orange, greyish-brown, and white (table 15). There was some variation in uredinial colour among cultures in the red group. The spores of the majority of these cultures were normal (red) in colour and corresponded closely to the "amber brown," on Plate III of Ridgway's Colour Standard. Two cultures, however, were classified as "antique brown." The uredinia of the orange cultures corresponded to "orange" and were identical with those of the grand-parental form 9a (orange); those of the greyish-brown cultures corresponded in colour to the uredinia of the grand-parental form 36 (greyish-brown), and to "Prout's brown" on Plate XV. The spores of the white cultures were creamy-white in appearance when the uredinia first appeared, but assumed a light buff colour after they had been exposed to sunlight for several days. This colour corresponded to Ridgway's "ochraceous buff," on Plate XV.



TABLE 15.—A comparison between frequencies of second-generation hybrids from the cross between form 9a (orange) and form 36 (greyish-brown) in the four colour classes and frequencies calculated on the basis of a 9:3:3:1 ratio.

Urediniospore colour	Red	Orange	Greyish-brown	White
Observed frequencies.....	59	47	22	7
Calculated frequencies.....	75	25	25	8.3

The observed frequencies in the distribution of the 135 cultures among the four colour groups are recorded in table 15. For comparison, there are included in this table, frequencies calculated on the assumption that two independently inherited factors are necessary for normal urediniospore colour. The actual frequencies agree rather closely with the theoretical ones in the greyish-brown and white groups, but there are wide divergencies between the observed and the theoretical frequencies in the red and orange groups. It should be borne in mind that a relatively small number of cultures was studied and this possibly may account for the disagreement between the two frequencies. On the basis of these experiments it has not been possible to assign a two-factor basis to the inheritance of spore colour in this cross.

As stated above, some of the  $F_1$  hybrids of the orange and greyish-brown strains were exceptional in that the colour of their urediniospores was orange instead of red. One of these orange  $F_1$  hybrids was selfed and a study made of the  $F_2$  progeny. The hybrid under discussion was derived from a cross between form 9a (orange) and form 52 (greyish-brown). As the hybrid originated from the form 52 side of the cross—that is, the aecia were developed in a pustule of form 52 (greyish-brown) as the result of a transfer to it of pycnial nectar from form 9a (orange)—it is not possible to ascribe this deviation in colour to a selfing of one of the parent forms as might have been done if the hybrid had originated from the other side of the cross. Hence it was thought that this result was due to either mutation or heterozygosity for colour in the form 52 parent; and an effort was made to discover the cause by a study of the  $F_2$  progeny of the orange hybrid.

Observations were made on the uredinial colour of twenty-seven cultures. These fell into one or another of two colour groups: orange and white. Twenty cultures were orange and seven white in uredinial colour. In view of these results, it appears probable that form 52 (greyish-brown) is heterozygous for spore colour and not homozygous as had been supposed on the basis of a limited study of the progeny of this form when selfed. Form 52 (greyish-brown) would then contain factors for both greyish-brown and white spore colour. The combination of a factor for orange (Y), from form 9a (orange) with a factor for greyish-brown (G), from form 52 greyish-brown, would result in a hybrid of the normal red uredinial colour; but a combination of a factor for orange (Y), with a factor for white (g), would produce a hybrid of orange spore colour. This orange hybrid would, on selfing, produce orange and white  $F_2$  hybrids in the ratio of three to one. This expectation accords with the actual results obtained, as the ratio of yellow to white was 20 to 7.

CROSSES BETWEEN *P. graminis tritici* AND OTHER PHYSIOLOGIC RACES OF  
*P. graminis*

It is theoretically possible that new physiologic strains of rust may originate by means of the hybridization of different physiologic races of *Puccinia graminis*. This possibility has been tested experimentally by Stakman, Levine and Cotter (53), Levine and Cotter (24), and Newton, Johnson and Brown (38). A brief discussion of the results of these experiments will be undertaken.

*Crosses between Puccinia graminis tritici and Puccinia graminis secalis*

On the basis of theoretical considerations, crosses between *P. graminis tritici* and *P. graminis secalis* are, perhaps more likely to be successful than crosses between other physiologic races. With a few exceptions, their host ranges are similar; both have the capacity to parasitize barley and certain grass hosts. This similarity in parasitism is probably indicative of the close relationship of the rusts to each other.

A number of crosses between *P. graminis tritici* form 36, and *P. graminis secalis* form 11, are reported by Stakman, Levine and Cotter (53). In the uredinial progeny of these crosses, there occurred eight physiologic forms of *P. graminis tritici*, namely, forms 15, 21, 32, 36, 57, 67, 70, and 71, and two physiologic forms of *P. graminis secalis*, forms 9 and 11. As far as can be judged from the pathogenicity of these forms, only two of them, forms 70 and 71, show what might be expected to represent definite indications of hybrid origin. The remainder of the forms are either typical *tritici* or typical *secalis* forms. These forms may be hybrids in which *tritici* or *secalis* characters dominate, or they may be the result of accidental selfing of one or the other of the parent forms. Forms 70 and 71, however, are to a certain extent pathogenically intermediate between the parent forms. Although classified as *tritici* forms, they are exceptionally low in virulence on wheat varieties; neither do they possess any marked ability to attack rye. In fact, their pathogenic characters approach those which might be expected in a hybrid parasitically intermediate between the *tritici* and *secalis* races.

Hybrid forms of a somewhat similar character have been reported by Newton, Johnson and Brown (38), as a result of crosses between *P. graminis tritici*, forms 30 and 95, and a field culture of *P. graminis secalis*. Crosses between the *tritici* form, 30, and the *secalis* race yielded three physiologic forms obtained from the *tritici* side of the crosses. One of these, form 17, is a typical wheat stem rust form, and may have been the result of an accidental selfing of the *tritici* form. The other two forms are strikingly different in pathogenicity from the majority of the known physiologic forms of wheat stem rust. One of these forms is pathogenically identical with one of the above-mentioned hybrids obtained by Stakman, Levine and Cotter, namely, form 70; the other is parasitically different from any known *tritici* form and has been designated as form 112. Both give light reactions on wheat varieties as compared with the average *tritici* forms. Barley varieties were found susceptible but rye varieties resistant to both forms. Similar results were obtained from the cross between *P. graminis tritici* form 95 and *P. graminis secalis*. The uredinial hybrid progeny consisted of two physiologic forms, neither of which had previously been described. These forms, 111 and 104, were very low in virulence on the majority of the wheat varieties tested. Barley varieties were moderately susceptible, but rye varieties rather resistant. Fig. 26 shows the reactions of the twelve wheat differential hosts to physiologic form 111.

It is natural to raise the question of the significance of these non-virulent forms. Are they hybrids, or are they the result of accidental selfing of the *tritici* parent forms? The latter does not seem possible, as the *tritici* parent forms 30 and 95, did not yield any such forms in the selfing experiments carried out as a control. The forms obtained through the selfing of forms 30 and 95 were all characteristic *tritici* forms. The possible selfing of the *secalis* parent is, of course, not a point at issue here, since the hybrids arose on the *tritici* side of the cross; but it may be mentioned, incidentally, that the selfing of the *secalis* parent produced only *P. graminis secalis*. The evidence for the hybrid nature of these forms appears, therefore, to be reasonably strong. A study of their  $F_2$  progeny should establish this point beyond doubt, as typical *tritici* and *secalis* strains would be expected to occur in that generation along with other intermediate strains.

If, as the evidence indicates, these non-virulent forms are *tritici-secalis* hybrids, are they then, characteristic of all such hybrids? This does not, however, seem to be the case, as Levine and Cotter (24), have recently reported a *tritici-secalis* hybrid which attacks certain varieties of rye and wheat rather severely. Barley is also reported to be quite susceptible to this hybrid form.

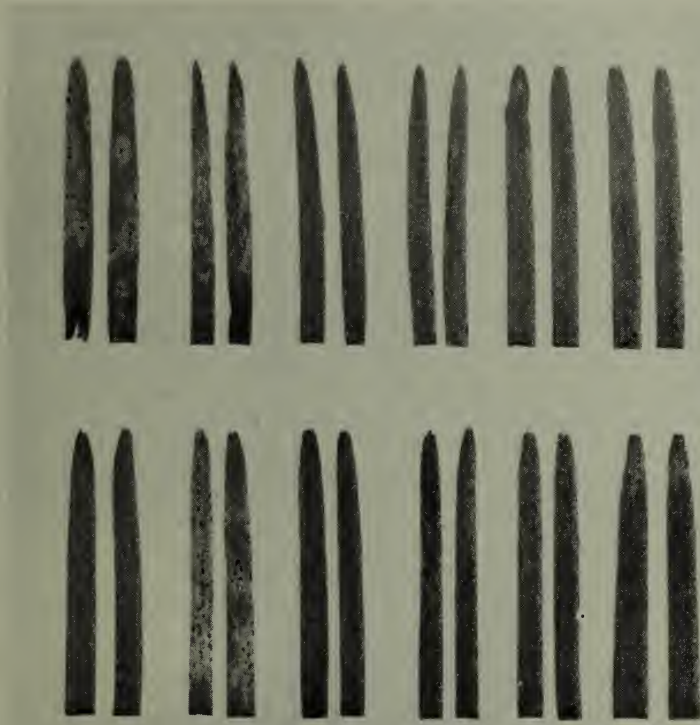


FIG. 26.—Reactions of the twelve differential wheat varieties to the *tritici x secalis* hybrid form 111. Top row, from left to right: Little Club, Marquis, Kanred, Kota, Arnautka, Mindum. Bottom row, from left to right: Speltz, Marz, Kubanka, Acme, Einkorn, Vernal, Khapli.

They have even suggested that they have accomplished here, the synthetic production of *Puccinia graminis hordei* F. and J. Evidently it must be left to future investigations to determine beyond doubt the nature of *tritici-secalis* hybrids and their importance in the origination of new strains of rust.

#### *Crosses between Puccinia graminis tritici and Puccinia graminis agrostidis*

Crosses between these two physiologic races have been reported by Stakman, Levine and Cotter (53). The parents used in their crosses were, *P. graminis tritici* form 36, and a field collection of *P. graminis agrostidis*, from Minnesota. Progeny was obtained only from the *tritici* side of the cross. The uredinial progeny was resolved into a considerable number of physiologic forms, many of which are well known *tritici* forms. Some of these forms may have originated as the result of an accidental selfing of the *tritici* parent. Some, however, produced decidedly unusual reactions on the twelve differential wheat varieties. One of these forms, form 72, is characterized by low virulence on both common and

durum varieties and high virulence on Einkorn and the emmers, including Khapli, which is resistant to practically all known *tritici* forms. None of the forms isolated from these crosses infected species of *Agrostis*. Although some of these forms may be hybrids, the evidence for their hybrid origin does not seem wholly conclusive.

Further evidence is available on hybridization between *P. graminis tritici* and *P. graminis agrostidis* through hitherto unpublished results obtained by Newton, Johnson and Brown. The *tritici* parent used in these crosses was physiologic form 9 which had proven homozygous for pathogenicity. This form had been selfed on several occasions, and in every instance its progeny was identified as form 9. Hence this form is peculiarly well adapted for hybridization experiments. Owing to the fact that it is homozygous it seems permissible to assume that any form, other than form 9, which might originate from the form 9 side of an inter-racial cross, would be of hybrid nature. The *agrostidis* parent was a field collection of teliospores on *Agrostis alba*, gathered in New Brunswick, Canada.

Considerable difficulties were experienced in obtaining aecial formation as a result of transfers of pycnial nectar from haploid pustules of one parent to haploid pustules of the other. In fact, the writers were for some time of the opinion that the two races were inter-sterile. In the seven crosses attempted, pycnial nectar was transferred from *agrostidis* pustules to 14 haploid *tritici* pustules. Aecia were formed in only three pustules, and in two of these the aecia evidently arose through a fortuitous selfing of form 9 as is indicated by the fact that the uredinial progeny of these aecia consisted in all cases of form 9.



FIG. 27.—Reactions of Little Club wheat and *Agrostis alba* to the F<sub>1</sub> hybrid form of a cross between *P. graminis tritici* and *P. graminis agrostidis*.

The aecia of the third pustule were evidently of hybrid origin as will be shown later. Pycnial nectar was also transferred from *tritici* pustules to 36 haploid *agrostidis* pustules. Three, only, of these pustules gave rise to aecia. The reactions of the progeny of these aecia showed that in all three cases the aecia were formed through a chance selfing of the *agrostidis* parent. In two of these pustules, the selfing may be accounted for by their coalescence which resulted in the formation of aecia at the point of contact. In the third pustule the agency that caused the selfing is unknown. Hence no aecia of hybrid origin were formed on the *agrostidis* side of these crosses and only one pustule of hybrid aecia was formed on the *tritici* side. These results agree with the experiences of Stakman, Levine and Cotter, who were unable to bring about aecial formation in haploid *agrostidis* pustules by means of transfers of pycnial nectar of *tritici* pustules.

As stated above, aecia of hybrid origin were formed in only one pustule on the *tritici* side of one of the crosses. Four uredinial cultures of mono-aecial origin were established from this pustule. These cultures proved identical in their reactions on the wheat differential hosts. Eleven of the twelve hosts were immune or highly resistant; the remaining variety, Little Club, was moderately resistant, the reactions varying from "1+" to "X=". Although a number of other varieties of common wheat have been tested, none have been found more susceptible than Little Club. *Agrostis alba* appears to possess about the same degree of resistance to the hybrid form as does Little Club. The reactions of these hosts are shown in fig. 27.

The similarity in pathogenicity of the *tritici-agrostidis* hybrid and the *tritici-secalis* hybrids mentioned above is so striking as to suggest a general tendency towards this type of hybrid in crosses of *P. graminis tritici* with other physiologic races of *P. graminis*. However, before such a statement can be made with any assurance more extensive work than has been achieved at the present time will have to be done.

#### THE PRESENT CONCEPTION OF PHYSIOLOGIC FORMS

Studies in hybridization among physiologic forms of *P. graminis tritici* have somewhat modified the physiologic form concept, at least as far as the rusts are concerned. There has been a tendency, especially among phytopathologists not fully conversant with physiologic-form work, to regard the physiologic form as an ultimate unit; in other words, to envisage it as a pure line. It is true that this conception has not been generally entertained by experimenters engaged in physiologic-form investigations, who have realized the possibility of hybridization between forms and perceived the consequent likelihood that the hybrids would be heterozygous. Nevertheless, in the absence of experimental proof of hybridization, the impression that physiologic forms were genetical unities became more or less prevalent.

The apparent inability of physiologic forms to change their pathogenic characters in the uredinial stage probably constitutes the chief reason for the belief in the genetical stability of these strains. Physiologic forms have been kept in continuous culture for years without any changes in their pathogenic characters being observed. Although it has recently been demonstrated that the infections of physiologic forms on their hosts may vary in response to environmental conditions, yet such changes in pathogenicity are merely fluctuations and have no bearing on the genetical stability of physiologic forms. So long as these forms remain in the uredinial stage, permanent changes in pathogenicity are rarely encountered. Even though, as recent work (53) has shown, mutations in pathogenicity occur, it has yet to be proved that such pathogenic changes are frequent enough to discount seriously the opinion generally held as to the parasitic constancy of physiologic forms.

The problem of the pathogenic stability of physiologic forms takes on a different aspect when these forms are allowed to complete their life cycle by infecting the barberry. Apparently the majority of physiologic forms are in a heterozygous condition, and segregation of the factors governing pathogenicity takes place during the germination of the teliospores. These factors recombine in various ways to initiate aeciospores that, in turn, give rise to a uredinial generation consisting frequently of several physiologic forms pathogenically different from the parent form. Only two homozygous forms have been discovered in all the selfing experiments which have, so far, been carried out.

When it is considered that almost all physiologic forms can, on selfing, break up into many physiologic forms and, furthermore, that the evidence available indicates that all the physiologic forms are inter-fertile and so may hybridize with one another, it becomes evident that there is practically no limit to the number of forms which may come into existence. The genetic complexity of *P. graminis tritici* becomes even more apparent when it is pointed out that the differential hosts in use at present are inadequate to differentiate the rust into all its genotypically different strains. An example may be given to illustrate this point. The writers have carried out inheritance studies on cultures of form 9 obtained from three different sources. The three cultures are identical in urediniospore colour and in their reactions on the differential wheat varieties. One culture, collected in the field and identified as form 9 is homozygous for both colour and pathogenicity. This culture, when selfed, produces a uredinial progeny consisting of form 9 only. The second culture of form 9 was secured as an  $F_1$  hybrid from a cross between the above-mentioned form 9 and form 15. Form 9 was dominant over form 15, thus producing a hybrid phenotypically identical with one parent but genotypically different from it. This form 9 hybrid produced, on selfing, a progeny consisting of forms 9, 15, 57 and 85 of normal (red) uredinial colour, and forms 15 and 85 of greyish-brown uredinial colour. The third culture of form 9 was obtained as an  $F_1$  hybrid between form 9a (orange) and form 52 (greyish-brown). On selfing, this hybrid form gave rise to uredinial progeny in four distinct colours: red, greyish-brown, orange, and white. This uredinial progeny was identified as one or another of three forms, namely, forms 9 and 15, and a hitherto undescribed form. This example shows clearly that the three cultures of form 9 were genotypically different in spite of the similarity of their reactions on the twelve differential hosts. It may be possible that host varieties which would differentiate these cultures could be found if a search were made for them; and, if three genotypically distinct cultures of form 9 could be differentiated in this manner, there is reason to believe that distinctive reactions could be found in other forms. Indeed, an increase in the number of differential hosts would, perhaps, materially increase the number of physiologic forms, which would, of course, mean a closer approximation to a condition in which each genotype is represented by a characteristic phenotype or, specifically, by a physiologic form. The closer the approximation to this ideal the greater will be the accuracy of genetical studies of physiologic forms.

It appears therefore, that most physiologic forms are not pure lines, but are, on the contrary, in a heterozygous condition, at least with reference to pathogenicity. As they are inter-fertile, hybridization may occur freely in regions where barberry is plentiful. In hybridizing forms, it is found that they appear for the most part to obey Mendelian laws of inheritance. Hence, there is a close analogy in genetical behaviour between these fungi and species of higher plants in which cross fertilization takes place.

Not only do the physiologic forms of *P. graminis tritici* hybridize among themselves, but they also are capable of hybridizing with some, at least, of the other physiologic races of *Puccinia graminis*. Crosses between these physiologic

racés, like inter-specific crosses in higher plants, are somewhat more difficult to accomplish than crosses within the race. There is some evidence that the  $F_1$  hybrids in inter-racial crosses tend to be pathogenically intermediate between the parent races, but the present state of knowledge concerning such hybrids does not warrant either a generalization as to their parasitic behaviour or a speculation on the genetical nature of their progeny.

The essential facts on which the present physiologic form concept is based have already been stated. Physiologic forms, as such, exist only in the uredinal stage. As they are dikaryotic, they may be conceived of as "dikaryotic clones," to borrow the expression used by Stakman, Levine and Cotter (53). Each of the conjugate nuclei is of a sex different from, and opposite to, that of the other; and, in heterozygous forms each nucleus is the bearer of different factors for pathogenicity. The pathogenicity of the dikaryophyte is determined by the factors borne in the two nuclei, and is probably, modified in many cases, by the influence of the cytoplasm, as has been shown elsewhere in this paper. The physiologic form is not to be considered as an ultimate genetical unit, as it has been shown that one of these strains may represent any one of several distinct genotypes. But it seems at least theoretically possible, that an increase in the number of differential hosts may approximate a condition in which each physiologic form corresponds to a distinct genotype.

## THE PRACTICAL SIGNIFICANCE OF PHYSIOLOGIC FORMS

Some phases of the practical significance of physiologic forms have already been discussed, *e.g.*, their value in epidemiological studies. Among the more important of their other uses may be mentioned their utilization in testing the purity or identity of wheat varieties and their use as a means of studying the genetics of rust resistance.

### Physiologic Forms as Determiners of the Purity of Wheat Varieties

It has been pointed out above that physiologic forms of wheat stem rust are determined by means of the rust reactions of certain wheat varieties. Conversely, known physiologic forms may be used to determine either the identity or the purity of wheat varieties.

A few years ago the authors had occasion to determine the identity of two selections of Marquis wheat. One of these was morphologically characteristic of Marquis. The other selection differed from Marquis sufficiently to cause some dispute among agronomists as to its morphological identity with Marquis. Both strains were infected by a number of physiologic forms known to produce typical reactions on Marquis. These forms included form 19 which produces a "2" infection and forms 21 and 36 which produce a "4" infection. One of the selections exhibited typical Marquis reactions to the three forms, the other selection was atypical in that, when infected by form 19, it produced a "4" reaction instead of the "2" type characteristic of Marquis. The latter strain, although differing morphologically but slightly from Marquis did not possess its physiological properties.

In the same manner, it is easy to determine the purity of a wheat variety. Johnston and Bower (22) have used a physiologic form to which the variety Kanred is resistant to determine the purity of Kanred wheat submitted for certification. In this instance, the percentage of susceptible seedlings served as an index of the impurity of the variety.

Not only may the amount of mechanical mixture in a variety be determined in this manner, but the amount of natural crossing to which it is subject may also be estimated by the same method. Goulden and Neatby (16), in fact,

made use of this method to determine the frequency of natural crossing in pure lines of Marquillo wheat. They grew pure lines of Marquillo adjacent to rows of Huron and Preston. Marquillo is resistant to form 21, but Huron and Preston are susceptible to it. As susceptibility is dominant in crosses between these two varieties and Marquillo, the  $F_1$  seedlings from the natural crosses are easily detected by their reaction to form 21.

### Physiologic Forms as a Means of Studying the Genetics of Rust Resistance

Soon after the discovery of physiologic specialization in wheat stem rust attempts were made through crosses between various varieties to combine in one wheat strain resistance to several physiologic forms. With this purpose in view, numerous crosses have been made by plant breeders, particularly in North America, and considerable information has been gathered on the genetics of rust resistance.

The earlier attempts to produce rust resistant varieties by means of crosses were made with a view of combining in one variety resistance to all the known physiologic forms or at least to those prevalent in the district for which the variety was designed. It was assumed that the seedling resistance was indicative of the resistance of the mature plant. But as the number of known physiologic forms increased the difficulty of combining resistance to all these forms with other desirable characters became more and more apparent. Indeed, these difficulties appeared at one time to form an almost insuperable obstacle to plant breeders. The solution of the rust problem was rendered more hopeful through the discovery of the fact that certain varieties such as Hope and Pentad exhibited a resistance in the mature plant stage which differed from and was inherited independently of any resistance they might possess in the seedling stage.

In a paper such as this it is not practicable to review all the literature which has accumulated on the subject of seedling resistance and its inheritance. A brief discussion of a few of the investigations may serve to give a general view of the manner in which physiologic forms have been used and are being used in the development of rust resistant plants and in studies on the genetics of rust resistance.

Puttick (43) was one of the first to use pure cultures of physiologic forms to study the genetics of rust resistance, in a cross designed to produce rust resistant varieties. He studied the reaction of the  $F_2$  generation of a cross between Mindum (*T. durum*) and Marquis (*T. vulgare*) to two physiologic forms of *P. graminis tritici*, namely, forms 1 and 19. Form 1 attacked Marquis heavily but Mindum weakly, while form 19, on the other hand, attacked Mindum heavily and Marquis weakly. On the  $F_2$  plants, all gradations between a high degree of resistance and complete susceptibility appeared. The fact that strains which were immune or highly resistant to both forms occurred in the  $F_2$  generation made it clear that a variety of wheat resistant to both forms could be produced by crossing. A single main pair of genetic factors was held accountable for the reaction of the  $F_2$  progeny to form 19, but modifying factors were assumed to account for the various grades of infection obtained.

Hayes and Aamodt (18) studied rust resistance in a cross between Marquis and Kota. The forms selected for this study were form 19, to which Marquis is resistant and Kota moderately susceptible, and form 27, to which Marquis is resistant and Kota immune. The reactions of the  $F_3$  generation to form 27 could be satisfactorily explained on the basis of two independently inherited factors for immunity and resistance contained in the Kota and Marquis parents, respectively, each factor being allelomorphic and dominant to a factor for susceptibility.



Goulden, Neatby, and Welsh (15) made a study of the inheritance of rust resistance in a cross between H-44-24 (a strain originating from a cross between Yaroslav emmer and Marquis) and Marquis. H-44-24 is moderately resistant to form 21 and highly resistant to form 36. Marquis is susceptible to both forms. In the  $F_1$  generation the moderate resistance of H-44-24 to form 21 was found to be recessive, but its high resistance to form 36 was dominant. To form 36, the  $F_3$  plants showed reactions varying from the resistance of H-44-24 to the susceptibility of Marquis. The results of the tests on 745  $F_3$  families indicated that two factors govern resistance to form 36. The  $F_3$  families were classified into three groups according to their reactions to this form: homozygous resistant; segregating; and homozygous susceptible. The actual numbers in these classes fitted closely to a 3:12:1 ratio, that is, three were homozygous resistant, 12 were segregating, and 1 was homozygous susceptible.

Another point of interest in their paper is the demonstration of the fact that in this cross it is impossible to develop a strain with seedling resistance to both form 21 and form 14. All lines highly resistant to form 14 were susceptible to form 21. Apparently the Marquis factor, or factors, for high resistance to form 14 were allelomorphic to the H-44-24 factors for moderate resistance to form 21. Hence it was impossible in this cross to combine the moderate resistance of H-44-24 to form 21 with the high resistance of Marquis to form 14.

Neatby (28) has thrown further light on the inheritance of rust resistance of seedlings in this cross by a study of the reactions of a number of  $F_5$  lines to 15 physiologic forms. The forms fell into three groups, as indicated in fig. 28. Two sets of factors were identified, one of which affected the reactions to forms of group II, and another which affected the reactions to the forms of groups I and III only. These two sets of factors are represented in the figure by the letters B and A respectively. The reactions to forms belonging to group II are mainly, if not entirely, governed by the B set of factors; but the reactions to the forms of group I are affected by both sets of factors; and the reactions to the forms of group III are apparently controlled by the A set.

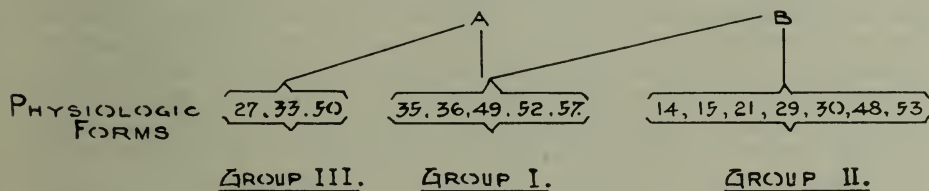


FIG. 28.—Diagrammatic representation of factor relations in the cross Marquis x H-44-24. (For explanation see text). Included by permission of Dr. K. W. Neatby.

The above discussion indicates the manner in which physiologic forms have been utilized in determining the inheritance of seedling resistance and suggests some of the difficulties encountered in developing adequate resistance of this type. So long as the plant breeder is working with seedling resistance, physiologic forms are indispensable to his work. The importance of physiologic forms in studies of the genetics of rust resistance has, however, been modified somewhat through the discovery of the existence in certain varieties of mature plant resistance which in many cases is inherited independently of seedling resistance. Plants possessing mature resistance appear to be equally resistant to the various physiologic forms irrespective of the pathogenic relations of the forms to seedlings of the same variety. It should not be overlooked, however, that the discovery of mature plant resistance and the proof that it was inherited independently of seedling resistance were accomplished through the instrumentality of physiologic forms.

### Physiologic Forms in Relation to Mature Plant Resistance

It has already been pointed out that in some varieties the rust reaction of the adult plant does not correspond to the reaction of the seedling. Similarly, in the progeny of a number of crosses, the inheritance of the rust reactions of mature plants differs from the inheritance of seedling reactions. In all such cases there is a progressive increase in rust resistance as the plant approaches maturity; and at maturity (*i.e.*, after heading) the plant appears to possess resistance to all physiologic forms irrespective of the infections produced by these forms on the seedlings.

The existence of mature plant resistance differing radically from seedling resistance is no longer open to doubt. Melchers and Parker (27) reported on a strain of Crimean Kansas which gave no evidence of resistance in the greenhouse but which was partially resistant in the field. Hursh (19) called attention to the difference between the rust reactions of Acme seedlings and the reactions of the same variety when approaching maturity in the field and attributed these differences to the greater amounts of sclerenchyma in the mature plants, which acted as a mechanical barrier to the growth of the rust. Goulden, Newton and Brown (17) studied the reactions of fourteen wheat varieties at two stages of maturity to sixteen physiologic forms of stem rust, and, on the basis of their results, they were able to divide the varieties into three groups: (1) Varieties showing no evidence of mature plant resistance, Garnet, Marquis, Quality and Khapli; (2) varieties showing various degrees of mature plant resistance, from a mere indication to a very pronounced evidence, Reward, Kota, Marquillo, Black Persian, Hope, H-44-24, Pentad and Acme; and (3) varieties showing no appreciable difference between the seedling and mature plant reactions but suspected of possessing considerable mature plant resistance owing to the very low percentage of rust which developed on them in the mature stage, Vernal emmer and Iumillo.

The phenomenon of mature plant resistance simplifies very greatly the task of developing rust resistant varieties. As the varieties or hybrid lines displaying this type of resistance are presumed to be equally resistant to the various physiologic forms, the plant breeder can leave the individual forms out of consideration and determine rust resistance to all the physiologic forms *en masse*. Instead of obtaining his data from infections with pure cultures of physiologic forms in the greenhouse, the geneticist can depend upon infections in the field. Although, in theory, natural infections should serve to determine adult plant resistance, yet as a precaution, the plants grown at this laboratory are exposed to an artificial epidemic of a large number of physiologic forms, as mature plant resistance has only been under observation for a comparatively short time.

Such field studies have elicited a certain amount of information on the genetics of mature plant resistance. Clark and Ausemus (1), in a study of a cross between the immune variety, Hope, and the susceptible variety, Marquis, conclude that two genetic factors operate in the inheritance of immunity, a character reported to be dominant. Neatby and Goulden (29) state that, in the cross Marquis x Hope, resistance is governed by two complementary factors. In the cross Marquis x H-44-24, they find that field resistance is governed by a single factor. They state further that "results obtained in crosses between Reward and Hope, and between Reward and H-44-24, reveal a factor in Reward capable of inhibiting the action of the Hope and H-44-24 factors for resistance. Results in these crosses also support the conclusion that the H-44-24 resistance is governed by a single factor pair, and the Hope resistance by at least two pairs."

The Mendelian inheritance of mature plant resistance provides not only an opportunity of working out the genetics of this kind of resistance but also offers the plant breeder some surety that he will be successful in combining rust resistance with other desirable characters, likewise inherited in a Mendelian manner. The fact that mature plant resistance to the various physiologic forms is inherited as a unit is also of the greatest importance.

Although varieties possessing adult resistance seem at present to offer the most immediate solution of the rust problem, yet owing to the difficulty of combining desirable milling and baking qualities with certain agronomic characters and rust resistance, it is possible that such varieties may not effect a permanent solution. In such an event the plant breeder might be forced to utilize for breeding purposes a variety like Iumillo which possesses a high degree of rust resistance (presumably physiologic) in all stages of growth. In a plant breeding program in which the source of resistance resides in a variety of this kind, it seems inevitable that physiologic forms of the most diverse types would have to be used to determine the rust resistance of the progeny. However remote such a contingency may appear it is one which should not be overlooked by the plant breeder.

### SUMMARY

1. Forty-one physiologic forms of *Puccinia graminis tritici* have been isolated from cereals and grasses in Canada during the period of 1919 to 1930. Up to 1926 all of these forms were identified as one or other of the forms found in the United States by Stakman and his colleagues, but since that year a number of forms different from those described by them have been isolated in Canada.
2. It has been shown that different physiologic forms predominate in different years. From 1919 to 1921, form 17 was the predominating one, form 21 occurred only rarely. From 1922 to 1929, the reverse was true, form 21 became one of the most prevalent forms while form 17 was rarely present. Other forms, such as form 24, appeared for a season or two and then disappeared for a period of years, or, again, as with form 11, they appeared so consistently year after year that they were regarded almost as permanent forms, and then without any apparent cause they disappeared not to reappear for a number of years. Since 1925, form 36 has been the predominating form.
3. A small number of physiologic forms is apparently responsible for most of the injury from stem rust in Canada. From 1919 to 1930, 2,171 isolations of wheat stem rust were made from cereals and grasses in Canada. Upon identification, forms 36 and 21 comprised over 50 per cent of the isolations, and these two forms together with three others, forms 38, 17 and 49, made up 75 per cent of them.
4. It has been shown that physiologic forms with long uredinial periods seem to have a much better chance of survival than have those with short uredinial periods. The physiologic forms which have occurred most frequently in Canada during the period 1919 to 1930, forms 36, 21, 38, 17, and 49, all have had relatively long uredinial periods, while the very rarely occurring forms, 83, 53, 48, and 33, all have had much shorter uredinial periods.
5. In Eastern Canada and British Columbia, where barberries are fairly common, a proportionately larger number of new physiologic forms have been isolated than in the great central wheat plain of the West, where there are practically no barberries. There seemed, also, to be some correlation between the introduction of new wheat varieties into a district and the occurrence there of new physiologic forms.

6. It has been shown that certain physiologic forms of *Puccinia graminis tritici* are peculiarly susceptible to changes in environmental conditions. Of the environmental factors thus far studied, temperature and light intensity appear to be the most influential in this respect. Hence it is important to keep these factors under control when physiologic forms are being identified. A temperature of 65°F. (18°C.), in conjunction with a moderate light intensity such as prevails in spring and autumn, has been found satisfactory for the determination of physiologic forms of *Puccinia graminis tritici*.
7. The stability of physiologic forms of rusts has been studied by many investigators. The available evidence indicates that physiologic forms are relatively stable and constant in pathogenic behaviour so long as they are perpetuated in the uredinal stage. The theory that they may rapidly change their pathogenicity by adaptation has not been supported by experiment. The evidence for changes brought about through mutation is more convincing. Such mutations as have been recorded, however, are retrogressive in the sense that the mutants have lost characters which might be considered valuable to the fungus in its struggle for existence.
8. It has been demonstrated by experimental methods that physiologic forms of *Puccinia graminis tritici* hybridize freely with each other. There is also strong circumstantial evidence that hybridization takes place in nature. The first-generation hybrid of two physiologic forms may be identical in pathogenicity with the one or the other of the parent forms, but more frequently it differs from both. Some hybrid forms are to a certain extent intermediate between the parent forms with respect to their infection types on the differential hosts. When the first-generation hybrids are selfed segregation and recombination of factors apparently takes place with the result that a number of pathogenically different rust strains occur in the second generation. In later generations, homozygous strains appear. The breeding behaviour of these rust strains, therefore, presents such a marked parallelism with the breeding behaviour of closely related varieties of higher plants as to leave no doubt but that Mendelian laws are operative in the inheritance of pathogenicity.
9. Not only may the physiologic forms of *Puccinia graminis tritici* hybridize with one another, but some, at least, of the physiologic races of *Puccinia graminis* are capable of inter-crossing. Crosses of the *tritici* race with the *secalis* and *agrostidis* races have shown that the two latter races are infertile with the *tritici* race; but such inter-racial crosses appear to be more difficult to accomplish than crosses among physiologic forms of the *tritici* race. Inter-racial hybrids thus far obtained are too few to permit of a generalization as to their parasitic capabilities. Some of the first-generation hybrid forms, however, are extremely low in virulence on all the hosts which have been tested.
10. Although Mendelian laws of inheritance appear to be operative in all the crosses investigated, there is also considerable evidence that in some crosses the cytoplasm of the parent forms influences the inheritance of pathogenicity.

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