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TECHNICAL

A STUDY IN CEREAL RUSTS

PHYSIOLOGICAL RACES

BY

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LETTER OF TRANSMITTAL

February 9, 1914

A. F. WOODS,

Director, Minnesota Agricultural Experiment Station,

University Farm, St. Paul.

Dear Sir:

I herewith transmit a paper, prepared by E. C. Stakman, entitled, "A Study in Cereal Rusts: Physiological Races." It contains facts of scientific interest and of practical value in the cereal rust problem. I therefore recommend its publication as a technical bulletin of this Station.

Very truly yours,

E. M. FREEMAN,

Chief of Division of Botany and Plant Pathology

TABLE OF CONTENTS

A STUDY IN CEREAL RUSTS

PHYSIOLOGICAL RACES

PART I. BIOLOGIC FORMS

HISTORICAL

Cereal rusts were known as destructive plant pests by the ancients. Observations were made on the effect of weather and location on their prevalence. The comparative susceptibility of the various cereals received some attention. Theophrastus speaks of the varying susceptibility of different cereals, as does Pliny, who says that barley is less likely to rust than are other grains. In modern times many observations have been made, but many of these were only incidental.

Dietel (1887) calls attention to a certain amount of morphological and physiological variation in rust fungi, but does not definitely establish the fact that there are distinct biologic forms. Previous to his time *Puccinia graminis* Pers. was considered a single species found on cereals and various grasses. However, in 1894 Eriksson (1894-1) showed that, although the morphology of the fungus on the different cereal hosts varied but slightly, there was a distinct specialization in parasitism. He therefore divided the different species of rusts into subdivisions which he termed "formae speciales." *Puccinia graminis*, the only species extensively used in the present investigation, he divided into five formae speciales, two of which he mentioned as being sharply demarcated and the remainder as being probably also distinct.

All of these forms of *Puccinia graminis* were capable of producing aecidia on various species of Berberis. It therefore occurred to Eriksson that perhaps his formæ speciales would be equalized when grown upon the alternate host. This, however, he found not to be the case. He concluded that the forms were physiologically distinct even when grown upon Berberis. In fact he was led to believe that they became more firmly fixed after a period on the barberry.

This discovery stimulated much research concerning the physiological relationships of the Uredineae. In the United States Hitchcock and Carleton (1894) made observations at about the same time that

Eriksson was making his in Sweden. Their conclusions pointed to the fact that there was not much danger of rust from one cereal infecting another. The same phenomenon was observed in rusts on a wide variety of hosts. Magnus (1894 and 1895), Rostrup (1894), Klebahn (1896), Dietel (1899), Ward (1901), Bandi (1903), and others firmly established the fact that this specialization of parasitism was quite common in the various rusts. To the formae speciales of Eriksson various names were applied. Schroeter (see Magnus 1894, p. 360) called them Schwester-Arten, and Rostrup (1894, p. 40) called them biologische Arten, while Hitchcock and Carleton (1894) referred to them as physiological races.

Neger (1902) found evidences of a similar condition among the Erysiphaceæ. Marchal (1902) conducted a large number of cross-inoculations with *Erysiphe graminis* and concluded that there was a fairly large number of "races specialisées." These did not differ essentially in any morphological character. He showed (1903) and Salmon (1903-2) later showed that the ascospores behaved in the same way with regard to this specialization of parasitism as did the conidia. Reed (1905) has shown that there may be more than one physiological race upon a single genus of host plant.

Magnus was one of the first to try to explain the phenomenon of specialization of parasitism. He distinguishes (1894, p. 366) between "Gewohnheitsrassen" or adaptive races and biologic forms. The former name he applies to such forms as merely show difference in infection power, while those which are fixed he calls biologic forms. Long association with one host plant, he says, may bring the development from Gewohnheitsrassen to biologic forms. This he showed (1895) to be true not only of rust fungi but of others as well. Dietel (1899) expressed a somewhat similar opinion, his idea being that formerly a given species attacked a variety of hosts, but that it became more and more specialized to form first Gewohnheitsrassen and then biologic forms. Eriksson (1902, p. 657) says that rust forms adapt themselves. Where a certain host is present in large numbers, and climatic conditions are favorable, changes take place in favor of the new host. These changes are expressed not only in the vitality of the fungus but also in a higher degree of systematic firmness. The new rust form, he says, becomes separated from its sister forms of parallel origin and becomes "scharf fixiert." His conclusion is, "Das Phänomen der Spezialisierung steht nicht länger da als der Exponent eines dem Schmarotzer innewohnenden, launenhaften und unerklärlichen Triebes, neue Formen zu produzieren. Dieser Trieb wird durch die umgebenden Verhältnisse—die vegetative Unterlage und das Klima,—unter denen der Parasit lebt, in eine bestimmte Richtung geleitet." This, Eriksson (1902, pp. 606 and 654) thinks, accounts for the fact

that the specialization has taken a different course in Sweden from that it has followed in the United States. The most widely grown crops would naturally be the ones on which the particular biologic forms adapted to them would attain their highest development. Therefore, the fact that a rust shows particular relationships in one country does not by any means preclude the possibility of a quite different set of relationships in another country.

The idea that the fungus changes its habits as a result of environment is substantiated by many observations. Perhaps an extreme case of such a tendency is found in the life history of *Puccinia graminis* in Australia. McAlpine (1906, p. 21) states that the teleutospores seem unable to infect the barberry in Australia, and, according to his observations, the fungus is quite rapidly being reduced to a reproduction by uredospores only. This is accounted for by the absence of the barberry.

Eriksson (1896, p. 339) shows that closely related host forms are somewhat similar in their relation to rust. He says, however, that the taxonomic relationship of host plants does not entirely determine the specialization of the rust form. Ward (1901) in his work on the rust (*Puccinia dispersa*) of the bromes states that the closeness of relationship of hosts is the determining factor in the ability of the rust to pass successfully from one host plant to another. Freeman (1902) also concluded that the farther removed a species of Bromus was taxonomically from the plant serving as a host for the rust the less probability there was of infection. Ward showed further (1903) that some forms of bromes might act as bridging species in enabling the rust to pass indirectly from one group of bromes to another, although direct transfer was impossible. Salmon (1904) showed that the same thing was true of *Erysiphe graminis* D. C. Freeman and Johnson (1911) have found that barley can act as a bridging form enabling *Puccinia graminis* to increase its range of infection power. Salmon (1904 and 1905) showed that the range of infection possibility of *Erysiphe graminis* forms may also be increased under certain cultural conditions. By injuring leaves and subjecting plants to heat and anesthetics he was able to infect normally immune forms.

The conception of a biologic form, then, is that it represents a tendency toward adaptation. This tendency may be due to various causes, the evidence being that it depends largely on the availability of host species. Hitchcock and Carleton (1894), Carleton (1899), and Freeman and Johnson (1911) investigated quite thoroughly the matter of biologic forms of *Puccinia graminis* in the United States. Freeman and Johnson (1911, p. 27) give the following as the biologic forms of this rust in the United States:

P. graminis tritici (stem rust of wheat) on wheat and barley.

P. graminis hordei (stem rust of barley) on barley, wheat, and rye.

P. graminis secalis (stem rust of rye) on rye and barley.

P. graminis avenae (stem rust of oats) on oats.

If these forms are merely adaptations it ought to be possible to change their parasitic tendencies by restricting or changing their environment. It ought to be possible to break down the biologic forms under conditions abnormal for host or parasite. Various methods have been tried in attempting to break down the specialization of parasitism of different fungi. Salmon's work along this line on the Erysiphaceae has already been mentioned. Ray (1903) states that by subjecting maize to ether vapor and then inoculating it with spores of *Ustilago zeae,* the resulting infection was much more virulent than that on plants not so treated.

Comparatively little work of this nature has been done with *Puccinia graminis.* The fact that physiological races of rusts behave differently under different conditions has been known for some time. Much of the information was, however, gathered from incidental observations. In breeding wheats for the purpose of obtaining rust-resistant forms it would be very helpful to be able to correlate certain characters with rust resistance. For this reason this phase of the question, from both practical and scientific points of view, is of much importance. The same is true of physiological races. It is important to know if they can be broken down by means of a high degree of soil-fertilization, if they become generalized by growing on the alternate host, and if they adapt themselves readily to new hosts. The present investigation was therefore undertaken with the object of determining the possibility of developing and breaking down physiological races and of obtaining definite information concerning the factors influencing varying resistance in immune or semi-immune varieties of wheat.

EXPERIMENTAL

METHODS

The rusts used in making inoculations were obtained originally from their respective hosts in the fields at University Farm, St. Paul, Minnesota. They were then artificially transferred to plants growing in the greenhouse. Transfers were made to new plants about once every three weeks until the rust had been confined to its own host for at least twelve successive transfer generations. In nearly all the experiments with biologic forms the rust had been confined to its own host for at least twenty generations, thus giving assurance that it was the particular biologic form desired.

The seeds of the host plants were planted in rich loam soil in four-

inch clay pots. Only ten plants were left in each pot and the first leaf of each was inoculated when six or seven days old. The plants were trimmed whenever necessary so as to leave only the one inoculated leaf on each plant. Fresh, viable uredospores were used for inoculations except where otherwise specified. The spores were put on the leaves with a flat inoculating needle which had been previously moistened in order that the spores might better adhere to the leaf surface. The pots were then placed in shallow pans filled with water, or on wet sand, and covered with bell jars for forty-eight hours. In nearly all cases a fine film of moisture covered the leaves during a considerable part of the time that they were under the jars. This, together with a moderate temperature, made the conditions for infection ideal. After the removal of the bell jars the plants were kept on greenhouse benches in such locations as to reduce to the minimum the danger of accidental infection.

The grains used were the following, the numbers, except where specified, being Grain Investigation numbers of the United States Department of Agriculture:

Fife wheat, Minnesota No. 163

Velvet Blue Stem wheat, Minnesota No. 169

Minnesota No. 188 wheat, a cross between White Fife and Ladoga Fife

Manchuria barley, Minnesota No. 105

Early Gothland oats, Minnesota No. 295

Swedish rye, Minnesota No. 2

Kubanka 1516—Nos. 8 and 9 pedigreed—Dickinson, N. Dak., 1910

Kubanka 2094

Arnautka 288

Arnautka 1431

Iumillo 1736 (1736-II-3 selected at Amarillo, Tex., 1910)

Einkorn (*Triticum monococcum*) 2433—Nos. 4, 6, 7, and 8 pedigreed—Dickinson, N. Dak., 1910

Emmer (*Triticum dicoccum*) 1522

Khapli (an Indian Emmer)

Of these the Kubankas, Arnautka, and Iumillo are varieties of Triticum durum. The durums generally have the reputation of being more resistant to rusts than are the ordinary wheats (Carleton 1905, p. 9). Einkorn, also, is quite resistant while the emmers vary greatly, one used in this work, G. I. No. 1522, not being very resistant. Khapli is an emmer obtained from India by E. C. Johnson, formerly Cereal Pathologist in the office of Grain Investigations, United States Department of Agriculture. It has no Grain Investigation number. It is the most resistant of all the forms used.

EXPERIMENTS WITH PUCCINIA GRAMINIS HORDEI

GENERAL STATEMENT

Freeman and Johnson (1911, p. 20) state that the barley stem rust is more versatile than any of the other biologic forms of the cereal rusts, and, further, that the range of infection of a given form is increased after having been transferred to barley. The results of the author, in general, agree with those of Freeman and Johnson, although the percentages of infection are in some cases quite different.

INOCULATIONS ON RYE

In the first series 20 leaves were inoculated, after which the pots were kept under bell jars for 48 hours. In 8 days very distinct signs of infection began to appear and on the tenth day distinct pustules were quite numerous. Eventually 16 leaves out of 18 showed distinct pustules. Two of the leaves had been killed. Very evidently not all of the mycelial wefts developed pustules, since pustules were often intermingled with typical, yellowish rust flecks and green islands. The pustules were in all cases small, most of them less than a millimeter in size, but they were fairly numerous. There were some fairly large areas in which no pustules developed, but which contained a large number of green islands.

In the second series 40 leaves were inoculated, 10 of which eventually produced pustules. Of the remaining 30 leaves 29 were very strongly flecked in such a manner as to show conclusively that infection had taken place although no pustules had been formed.

The results of both series indicate that rye is easily infected by the stem rust from barley. Out of 58 leaves, 26 were infected, indicating greater ease of infection than in case of the strain used by Freeman and Johnson (1911, p. 19) in their experiments. The 29 leaves which are indicated as having been strongly flecked would at first sight have been counted as having pustules. However, close examination showed that there were no ruptured pustules.

Although a fairly high percentage of successful infections can be obtained, rye is by no means a congenial host for *Puccinia graminis* from barley. This is shown by the fact that the pustules are always small. Long areas of the leaf may be killed and in this area there may be many unruptured pustules. Often an area extending across the entire leaf and one centimeter or more in length may be completely killed. (See Plate I, A.) This may not contain a single pustule, but close examination reveals the fact that there are many green islands, some of which have a yellowish tinge in the center. These latter look very much like unruptured pustules. Histological examination reveals the fact that the mycelium has spread, the host cells have died, and wefts of mycelium have formed, either directly under, or a slight

distance below, the epiderm. These wefts may send up a few hyphae which resemble those normally producing spores. In some cases a few small, abortive spores are formed, while in other cases, none are produced.

This phenomenon very closely parallels that occurring in some of the resistant forms of wheat. Attention will be called to this more in detail later on. It will be sufficient here to emphasize the fact that rye, when inoculated with *Puccinia graminis* spores from rye, does not show any of these dead areas (see Plate I, B), the leaves remaining green and producing pustules vigorously. When the spores, however, are taken from barley, infection takes place, but there is not such a perfect relationship set up between host and parasite as to enable both to live and thrive for a long time.

INOCULATIONS ON OATS

The first attempt to infect oats with stem rust from barley failed absolutely. For this reason the greatest precautions were taken to furnish optimum conditions for germination of spores and development of the mycelium. Of 104 leaves inoculated at various times, not one produced pustules. Of this number 8 were slightly flecked, but showed no tendency to form pustules. In fact, the flecks were extremely small and very few occurred on each leaf. In some cases there was but a single fleck. These might quite easily have escaped detection on some of the leaves had they not been invariably situated in the inoculated area. Their position in this area indicates quite strongly that they were true rust flecks. In these areas the tissues of the leaves seemed to have been killed outright. However, the mycelium must have been very restricted in its development since the diameter of a spot never exceeded one millimeter.

Inoculation after the use of anesthetics—

In the first trial two pots of oats were exposed to the fumes of ether for 15 minutes. Immediately after the exposure they were inoculated with fresh spores of *Puccinia graminis* from barley and then placed under a bell jar in a shallow pan of water. Two pots were exposed to chloroform fumes for an equal length of time and inoculated. At the same time 25 leaves were inoculated under normal conditions, as a check.

Of those leaves exposed to ether, 4 out of a total of 20 developed slight flecks but no pustules; neither were there any unruptured pustules, which are commonly found when infection is not normal. Of those exposed to chloroform 2 out of 19 developed pustules in 12 days. These pustules were very small, but were unquestionably pustules of stem rust. A few of the leaves were so indistinctly flecked that it was doubtful if they were true rust flecks.

In the second trial plants were again exposed to ether and chloroform, but the time was increased from 15 to 20 minutes. An attempt was made to insure conditions for successful infection. It was observed that a film of moisture formed on the leaves at night and the temperature was moderate, insuring successful infection if that was ordinarily possible.

Of the 20 leaves exposed to ether, 11 became very distinctly flecked, but there were no evidences whatever of successful pustule formation. In the chloroform series one leaf out of 20 produced a small pustule and 8 became clearly flecked. There were 20 check leaves, and, of these, 4 became flecked. The flecking was, however, rather indistinct.

Out of a total of 193 inoculations only 3 resulted in pustule-formation, and these only after exposure to chloroform. Of the remainder, 31 were flecked, but this flecking was not always sharp on the control plants. In the other cases, however, it was distinct. The spots were always less than one millimeter in diameter, and usually much smaller. It appeared that this small area of the leaf had been killed, thus preventing the further spread of the mycelium and precluding the possibility of pustule formation. In no case was there any indication whatever that large areas of the leaf were involved, as was the case when rye was inoculated with the rust from barley. In this connection it may be mentioned that Freeman and Johnson (1911, p. 19) were able to get 7 successful infections out of a total of 35, but the pustules were always very small. It may be that the difference is due to the use of various strains of the rust. The fact that there are strains of the same biologic form seems to be quite definitely indicated. In any case this seems to furnish an example of rather extreme incompatibility between host and parasite. In some cases it would seem that the infection threads of the fungus are checked almost immediately; or that they may gain a temporary foothold, only to kill the cells upon which they depend for nourishment and then develop but little further.

INOCULATIONS ON WHEAT

There is no question but that the stem rust from barley, in this country, usually passes to wheat with practically the same degree of readiness with which it passes to barley. In the trials made with this form this appeared to be the case whether susceptible or resistant forms of wheat were used. Inoculations on Arnautka, Khapli, and emmer show that the barley rust is quite as capable as is the wheat rust of attacking these varieties.

Pritchard (1911-1, pp. 181, 182) cites evidence tending to show that the forms on wheat and barley in North Dakota are distinct. This is not the case, however, with the strains used in the experiments in Minnesota.

SUMMARY OF INOCULATIONS WITH PUCCINIA GRAMINIS HORDEI

Wheat and barley are not included since in nearly every case 100 per cent of infection results. The denominator in each case represents the total number of leaves inoculated, and the numerator, the number of leaves which developed pustules.

Barley rust to:

Oats $\frac{0}{104}$; 8 slightly flecked

Oats after exposure to ether $\frac{0}{40}$; 15 flecked

Oats after exposure to chloroform $\frac{3}{39}$; 8 flecked

Rye $\frac{26}{58}$; 29 very strongly flecked

It will thus be seen that barley rust does not find either rye or oats a congenial host. It was transferred to rye much more easily in these experiments than in those reported by other investigators in this country. Without exposing the host plants to anesthetics, however, no successful infection of oats was obtained. By the use of ether and chloroform the possibility of infection was somewhat increased, as evidenced by the formation of pustules on a small percentage of leaves and the increased percentage of flecked leaves. In addition to this, the flecks were much more distinct than those on the check plants.

EXPERIMENTS WITH PUCCINIA GRAMINIS AVENAE

GENERAL STATEMENT

This rust in this country is supposed to infect no cereal except oats, although it is capable of infecting a number of grasses. Carleton (1899, p. 60) was unable to transfer it successfully to wheat, barley, or rye. Eriksson (1902, p. 601) mentions it as being found on oats and 18 species of grasses in Sweden. Freeman and Johnson (1911, p. 22), however, find that it can be transferred to barley also, and they report that Derr succeeded in obtaining direct transfers from oats to wheat and rye.

INOCULATIONS ON RYE

Since Derr, according to Freeman and Johnson (1911, p. 22), obtained but one successful infection by inoculating rye with stem rust of oats, a large number of trials were made with this form, especially since the same authors (l.c. p. 23) assert that under favorable conditions these can undoubtedly be made.

Inoculations under ordinary conditions—

The results of inoculations made under average conditions show clearly that it is possible to transfer the rust from oats to rye. Although the percentage of entirely successful infections was small, the

appearance of inoculated plants would lead one to believe that the rust transfers with greater ease than it really does. Out of a total of 55 leaves inoculated only 3 produced pustules. These pustules, although small, were very distinct. Of the remaining leaves, 13 were clearly flecked. It is the appearance of these so-called flecked leaves which is often misleading. When looking at them from a distance they sometimes appear to be quite badly rusted. Closer examination, however, shows that there are no pustules. Sometimes the flecks are so distinct as to suggest the appearance of unruptured pustules.

The effect of high fertilization—

A rich loam was well mixed with rich barnyard manure. Rye was planted in two pots and the leaves inoculated in the usual manner. Evidences of infection appeared at the usual time. It was found that the severity of infection was much greater than that on plants grown in unfertilized soil. Of a total of 18 leaves 9 developed pustules and the other 9 were distinctly flecked. The areas of infection on these plants were much larger than on those grown in ordinary soil. The leaf tissues were very clearly killed, sometimes in areas a centimeter long, and in these areas small pustules appeared. The appearance was very characteristic of semi-normal infection. The mycelium spread fairly well, but the host cells were killed and only small pustules were developed.

The effect of anesthetics—

Plants were inoculated after exposure to ether, chloroform, and nitrous oxide for periods ranging in different experiments from 5 to 15 minutes. The difference between the check plants and those exposed to anesthetics for 10 minutes was fairly distinct. This was not apparent so much in the number of pustules or flecks, but rather in the sharpness of the flecks. The following were the results:

After exposure to ether $\frac{7}{6\,4}$; 26 flecked

After exposure to chloroform $\frac{4}{4\,6}$; 13 flecked

After exposure to nitrous oxide $\frac{3}{2\,5}$; 6 flecked

It will thus be observed that in point of numbers the difference between the success of these infections and those on the check plants was not great. However, a real difference in severity of infection, although not remarkable, did exist. On the other hand the plants in highly fertilized soil developed a larger number of pustules and also a more typical infection.

Effect of leaf injury—

A number of leaves were injured, just previous to inoculation, by being punctured in many places with a sterilized needle point. Spores were then placed on this injured area in large numbers. The epidermis

was stripped from others and the inoculations then made. The results were not different from those obtained on check plants. Only one pustule developed on one leaf out of a total of 28. Six of the remaining leaves were flecked, but the flecks remained small. In no case did the virulence of infection equal that on plants grown in highly fertilized soil.

The stem rust of oats, then, can be transferred directly to rye. This was accomplished most easily when the plants were grown in heavily manured soil. Exposing them to various anesthetics before inoculation seems to increase the virulence of infection slightly, while leaf injury had no apparent effect. In the various trials, under different conditions, 27 out of 236 leaves developed pustules and 73 were flecked. Under conditions which might exist in the field 12 out of 73 leaves produced pustules and 22 were flecked.

INOCULATIONS ON WHEAT

The stem rust of oats can be transferred to wheat only with great difficulty. Carleton (1899, p. 60) did not succeed in obtaining infection in his experiments. Freeman and Johnson (1911, p. 22) made 100 inoculations but none was successful. They report, however, that Derr was able to make transfers.

Inoculations under ordinary conditions—

The fact that the oat rust is transferred with great difficulty to wheat became apparent very soon. Out of 108 inoculations only one was entirely successful, although 5 leaves became slightly flecked. Very evidently the infection threads, after having grown among the tissues of the leaf, must have died, since only very minute flecks developed, and these in only a few cases.

Effect of anesthetics—

Plants were inoculated after exposure to ether and chloroform for periods of from 1 to 15 minutes. The best results were obtained after exposure to ether for 5 minutes. In this case 3 leaves out of 50 developed pustules while all the other leaves were flecked. These pustules and flecks appeared 10 days after inoculation, this being slightly longer than a normal incubation period. None of the check plants showed any distinct signs of successful infection, while the flecks on the plants which had been exposed to ether were very conspicuous, forming a sharp contrast with the check plants. These flecks were scattered all along the line of inoculation and had the appearance of young, unruptured pustules.

This series offered the best evidence that anesthetics may have some influence in rendering a plant more susceptible to a rust than it would otherwise be. In subsequent trials, exposing the plants for a

greater length of time, no pustules were developed on any of the 40 leaves inoculated. Nine of these leaves were, however, quite strongly flecked. The use of chloroform did not result in the formation of pustules, although there was a distinct advantage over the check plants both in number and definiteness of flecks. In all, 40 plants were inoculated and of these 19 became distinctly flecked. After the use of ether, then, 3 out of 90 leaves were successfully infected, and 56 were sharply flecked, and these, added to the totals after the use of chloroform, give 3 pustules and 75 infected leaves which developed no pustules out of 130 trials.

Effect of manure—

Wheat was planted in two pots containing a rich loam very heavily fertilized with rich barnyard manure. Of the 20 leaves inoculated, 6 developed pustules and the rest were strongly flecked. In this case, however, there was a possibility that the pustules developed as a result of accidental infection. The flecks were apparently due to the artificial inoculation, since such flecks have never been observed after direct inoculation with spores from either wheat or barley, or after the inoculation of wheat with wheat or barley rust. It is therefore quite certain that the pustles, which were normal, were the result of accidental infection, while the flecks, which were exactly like those developed on a semi-immune form, were the result of artificial inoculation.

Effect of leaf injury—

In one experiment, 16 leaves were pricked full of holes in an area of one centimeter or more. They were then inoculated, and 4 became flecked, but no pustules developed. In another experiment the epiderm was stripped from 29 leaves immediately before inoculation. Although 10 became flecked, the flecks were extremely minute and no pustules were developed. Histological examination showed that the spores had sent out germ tubes in large numbers. These tubes grew among the host cells, but true infection did not take place. Sections of these plants were made and examined. It was clearly evident that leaf injury did not increase the chances for infection. The hyphae did not develop better than did those in normally inoculated plants.

Summary of inoculations on wheat—

Out of a total number of 283 leaves inoculated under varying conditions only 4 developed pustules and 113 became flecked, showing that, although the rust of oats can sometimes develop on wheat, it can seldom attain to pustule formation. The severity of infection, always very slight, can be increased somewhat by exposing plants to be inoculated to anesthetics. The experiment with high fertilization of

soil seems to indicate that here, as well as in the case of rye, inoculated with oat rust, the mycelium is enabled to develop more extensively and possibly produce more spores than when ordinary soil is used.

INOCULATIONS ON BARLEY

The results of this series of inoculations were very similiar to those obtained by other investigators. The behavior of the rust was typical of its usual behavior on an uncongenial host plant. The flecks were small in nearly all cases and were especially sharp when plants had been exposed to anesthetics.

Inoculations made under ordinary conditions—

These were not especially successful. No pustules were developed on 50 inoculated leaves but 10 of the leaves became flecked.

Inoculations after exposure to anesthetics—

Ether and chloroform were used. The period of exposure varied at different times from 5 to 15 minutes. Plants exposed only 5 minutes did not appear different from the check plants. After exposure for 15 minutes the flecking was more distinct than on the check plants. Only one pustule developed in the entire number of trials, this being after exposure to ether. There were 47 leaves inoculated after exposure to ether, and of these one developed a pustule and 15 were flecked. The flecks in this case had every appearance of unruptured pustules. No pustules were developed after exposure to chloroform, but 7 leaves out of 50 became flecked. Of 147 leaves inoculated 32 were flecked, but only one showed a pustule.

SUMMARY OF INOCULATIONS MADE WITH PUCCINIA GRAMINIS AVENAE

Oat rust to:

Wheat $\frac{1}{108}$; 5 flecked

Wheat after exposure to ether $\frac{3}{90}$; 56 flecked

Wheat after exposure to chloroform $\frac{0}{40}$; 19 flecked

Wheat after leaf injury $\frac{0}{45}$; 14 flecked

Wheat plants grown in manured soil $\frac{6}{20}$?

Barley $\frac{0}{50}$; 10 flecked

Barley after exposure to ether $\frac{1}{47}$; 15 flecked

Barley after exposure to chloroform $\frac{0}{50}$; 7 flecked

Rye $\frac{3}{55}$; 13 flecked

Rye after exposure to ether $\frac{7}{64}$; 26 flecked

Rye after exposure to chloroform $\frac{4}{46}$; 13 flecked

Rye after exposure to nitrous oxide $\frac{3}{25}$; 6 flecked

Rye after leaf injury $\frac{0}{45}$; 14 flecked

Rye grown in highly fertilized soil $\frac{9}{18}$; 9 flecked

The rust can be transferred successfully from oats to any one of the other cereals. It infects rye much more easily than wheat or barley. Anesthetics help to break down the barriers as does a high degree of soil fertilization. It should be mentioned that this fact seems due, not to any new ability the rust fungus has of attacking an uncongenial host, but to an increased capacity for development. Here again evidence of the possible nature of resistance is offered by the inoculated plants. The flecks, whether large or small, consist in many cases of dead tissue of the host plant. Histological examination shows that the fungus gains entrance but cannot develop to any extent in these areas which are killed by the fungus itself.

Experiments with Puccinia graminis secalis

These experiments were not very extensive and were made with the idea of determining rather the character than the number of successful infections.

INOCULATIONS ON WHEAT

In all 70 leaves were inoculated. None of them produced pustules, but 18 became flecked. The flecks were distinct but in no case were they large. There seemed to be much less dead tissue than is the case in many of the other forms, as for instance rye inoculated with barley rust. In fact the flecks were hardly noticeable unless one looked very carefully for them. Apparently they were due to the death of host-plant cells in the inoculated area.

INOCULATIONS ON OATS

In addition to a small number of control inoculations, some were made after exposure to ether and others after exposure to chloroform. The anesthetics apparently made no particular difference in the success of the attempts, although there were more flecked leaves after exposure to chloroform. Nothing out of the ordinary appeared so only the summaries are given:

After chloroform, 5 min. $\frac{0}{17}$; 5 slightly flecked

After ether, 5 min. $\frac{0}{20}$; 2 slightly flecked

Under ordinary conditions $\frac{0}{50}$; 1 slightly flecked

INOCULATIONS ON BARLEY

After exposure to ether for 5 minutes, 16 out of 21 inoculated leaves became very distinctly flecked. There were many of these flecks all along the line of inoculation. They were very suggestive of the flecking in other forms in which the mycelium was known to have spread to some extent. However, no pustules were developed. Judging from the experience with other forms, if these plants had been highly fertilized they might have developed pustules. Of the check

plants, only 4 out of 40 became flecked. No pustules were developed.

INOCULATIONS ON EINKORN

Einkorn was inoculated with rye rust both after exposure to ether for 15 minutes and without having been so exposed. The length of time in ether fumes was 15 minutes. No pustules developed on any of the leaves, 20 being used in each series.

EXPERIMENTS WITH PUCCINIA GRAMINIS TRITICI

INOCULATIONS ON BARLEY

It has long been a well-established fact that stem rust from wheat can easily attack barley. The infection in trials made by the writer was found to be normal and practically as virulent as if spores had been taken directly from barley. In the one series tried especially for the purpose 30 out of 30 leaves became characteristically infected. There is no evidence whatever of uncongeniality between host and parasite.

INOCULATIONS ON RYE

Inoculation under ordinary conditions—

In the control experiments 6 out of 30 leaves developed pustules and 20 were strongly flecked. In fact, they were so strongly flecked that flecking hardly expresses properly the appearance developed. Long areas on the leaf were killed outright. In these dead areas very small green islands were often found, some of which contained unruptured pustules. All the pustules were very minute, and some had ruptured the epiderm. It was another very good example of semicompatibility between host and fungus. The host leaves did not suffer very great injury; the fungus was enabled to spread to a certain extent but succeeded in producing only small pustules.

Inoculation after exposure to ether for five minutes—

The results here were very striking. Fifty leaves were inoculated, 6 of which produced pustules, while every leaf was infected with the mycelium of the rust fungus. The character of the infection was much the same as was that on the check plants. The areas were perhaps more extended. On some leaves there were dead areas 3 centimeters long, while ordinarily they were not so long on the check plants.

INOCULATIONS ON OATS

Inoculations under ordinary conditions—

Direct inoculation of oats by spores from wheat has not met with success on the part of either Carleton (1899, p. 54), who, however, reports a doubtful case, or Freeman and Johnson (1911, p. 18), who cite Derr as authority for the statement that this direct transfer can be made.

Attempts to transfer directly from wheat were unsuccessful. Not a large number were made, but of the 46 attempts no leaves produced pustules and only 2 of them developed flecks. The flecks were very small and not very distinct. In this connection it may be mentioned that when oat leaves were inoculated with aecidiospores derived from wheat teleutospores one leaf out of 56 developed pustules. This appears to indicate that direct transfer can be made. The success of the inoculation was probably not due to the fact that the rust had passed through the barberry stage. This seems especially true since the other cereals behaved in the same way toward the aecidiospores as they did toward the corresponding uredospores.

Inoculation after exposure to ether—

Only 30 leaves were inoculated after having been in ether fumes for from 3 to 5 minutes. No pustules were developed, but 10 of the leaves were flecked. There was a perceptible difference between the check plants and those of this series, although it cannot be said that the results were very striking.

INOCULATIONS ON OATS AND RYE AFTER THE USE OF BARLEY AS A BRIDGING FORM

The work done on bridging species has already been mentioned. Since it is almost impossible to infect oats directly with wheat aecidiospores or uredospores, attempt was made to transfer aecidiospores developed from wheat teleutospores first to barley and then to infect oats with the resulting uredospores. The attempt was made with fourth- and fifth-generation uredospores from barley, these uredospores having been derived from wheat-rust aecidiospores. Successful infection took place in one out of 39 attempts, 4 leaves being distinctly flecked.

The rye plants are possibly slightly more severely attacked if the rust is transferred first to barley, 16 out of 19 of the plants inoculated becoming infected.

SUMMARY OF EXPERIMENTS WITH PUCCINIA GRAMINIS TRITICI

Wheat rust to:

Barley $\frac{3}{3}\frac{0}{0}$

Rye $\frac{6}{3}\frac{}{0}$; **20 flecked**

Rye after exposure to ether $\frac{6}{5}\frac{}{0}$; 43 flecked

Rye after barberry and 4 generations on barley $\frac{1}{1}\frac{6}{9}$

Oats, uredospores $\frac{0}{4}\frac{}{6}$; **2 flecked**

Oats, aecidiospores $\frac{2}{5}\frac{}{6}$

Oats after exposure to ether $\frac{0}{3}\frac{}{0}$; 10 flecked

Oats after barberry and 4 generations on barley $\frac{1}{3}\frac{}{9}$; **4 flecked**

Effect of the Aecidial Stage on Biologic Forms

General statement

Eriksson (1894, pp. 292-309) was one of the first to suggest the possibility of breaking down biologic forms by means of the aecidial generation. He came to the conclusion, however, that this could not be done, that, as far as the cereal rusts are concerned, they behave in exactly the same manner when transferred through barberry as they do in the uredinial stage. Salmon (1903, p. 159) and Marchal (1903, p. 280) showed that with biologic forms of the Erysiphaceae the relations were exactly the same whether ascospores or conidia were used. There is not an exact parallelism between the two since the cereal rusts are heteroecious. However, the cases are somewhat similar since a sexual fusion intervenes in both. Arthur (1910, pp. 227-228) concludes that although there is distinct specialization of parasitism in *Puccinia poculiformis* (Jacq.) Wettst. (*Puccinia graminis* Pers.) on various grasses, this specialization breaks down in the aecidial stage. The barberry would, then, serve as a bridging form between the various grasses. Jaczewski (1910, pp. 356-357), on the other hand, does not find this to be the case with biologic forms of *Puccinia graminis* on cereals and grasses in Russia. His experiments support Eriksson's claim that the barberry does not change the physiological specialization of the various strains when they produce aecidia.

Experiments in 1912

In connection with the experiments described by the writer it should be mentioned that the aecidia used in one set of experiments were developed in the field but there was very slight chance for accidental infection. Wheat straw very badly affected with rust in the teleutospore stage was tied around barberry bushes. There was no other rusted straw of any kind within considerable distance, so that there was little chance of accidental infection. These aecidiospores which were developed from wheat rust were transferred to wheat, barley, oats, rye, and einkorn. The following were the results:

Results of Inoculations of Cereals with Aecidiospores of Puccinia graminis tritici Developed in the Field

Grain	Trials		Total
	I	II	
Wheat	21/31	29/30	50/61
Barley	12/27	23/30	35/57
Oats	0/26	1*/30	1/56
Rye	4/26	11/28	15/54
Einkorn	18/30	24/30	42/60

*Somewhat doubtful.

It will be noticed that the percentages of infection are practically the same as are those developed from uredospore inoculation. This is true also of the character of the infection.

On the rye plants, for instance, there was the same characteristic spotting and the same small, abortive pustules. The pustules on rye were all very small and there was no observable increase in virulence. The same thing is true of einkorn. On oats but one rather doubtful pustule developed, indicating that the aecidial stage in no way broke down the barriers in this case.

EXPERIMENTS IN 1913

In the fall of 1912 the barberry bushes in the rust plat were surrounded with badly rusted wheat straw, the rust being in the teleutospore stage. In the spring of 1913 the aecidiospores were used in inoculating the four common cereals and einkorn. The results were surprising since it was supposed that the aecidia had been developed from the wheat-rust teleutospore sporidia. The results of the various trials are given below:

RESULTS OF INOCULATIONS OF CEREALS WITH AECIDIOSPORES FROM FIELD BARBERRIES

Grain	TRIALS			Total
	I	II	III	
Wheat	8/40	2/25	3/30	13/95
Oats	0/40	0/30	0/30	0/100
Barley	32/40	16/27	18/30	66/97
Rye	40/40	7/13	8/27	55/80
Einkorn	12/40	2/7	....	14/47

It will readily be seen that there would naturally be much doubt as to the origin of these aecidia from wheat-rust teleutospore sporidia. These aecidiospores were being used in spraying wheats in the rust nursery for the purpose of developing a rust epidemic. The results were very discouraging. There was a great deal of *Agropyron repens,* badly affected with the teleutospore stage of *Puccinia graminis,* near the barberry bushes. Experiments were therefore started to determine whether or not the aecidia on barberries might not have been developed from this source rather than from the wheat-rust material. This view seemed all the more reasonable when it was observed that the *Agropyron repens* was very severely affected with the uredospore stage of rust, while the wheat, although it had been thoroughly and persistently sprayed with water containing the aecidiospores, had only a few scattered pustules of rust.

Barberry bushes which had been in the cellar all winter were set out in the field and covered with a heavy muslin cage. Badly rusted straw of wheat was tied around one bush and that of *Agropyron repens* around another. None of the check plants developed any aecidia while those surrounded with straw were very badly affected. The *Agropyron repens* material produced mature aecidia 10 days earlier than the wheat material and the aecidia were also developed in greater abundance.

RESULTS OF INOCULATIONS WITH AECIDIOSPORES AND UREDOSPORES FROM WHEAT AND AGROPYRON REPENS

Grain	A. repens aecidia	A. repens uredospores	Wheat aecidia	Wheat uredospores
Wheat	$\frac{6}{27}$	$\frac{3}{27}$	$\frac{25}{30}$	$\frac{30}{30}$
Oats	$\frac{0}{29}$	$\frac{0}{30}$	$\frac{0}{30}$	$\frac{0}{46}$
Barley	$\frac{12}{30}$	$\frac{27}{27}$	$\frac{24}{30}$	$\frac{30}{30}$
Rye	$\frac{12}{24}$	$\frac{27}{28}$	$\frac{7}{30}$	$\frac{6}{30}$

The field barberries were very probably infected with the rust from *Agropyron repens,* as will be readily observed by referring to the two tables. Further, the various biologic forms do not show any apparent change as a result of having been transferred to barberry, thus confirming the results obtained in previous experiments.

These inoculations, although not extensive, show quite clearly that *Puccinia graminis tritici* and *Puccinia graminis* from *Agropyron repens* do not seem to develop any greater range of infection possibility for cereals after having lived for a time on the alternate host—the barberry. The incubation period, even on wheat, barley, and einkorn, was a little longer in these experiments than that of uredo-developed mycelium.

ADAPTATION OF BIOLOGIC FORMS TO NEW HOSTS

Magnus (1894, p. 362) was one of the first to suggest that a particular biologic form might, by constant association with one host, change its physiological capabilities to such an extent as to make a new race out of it. This view was also expressed by Dietel (1899, pp. 81 and 113) who gave it as his opinion that a given rust formerly attacked a number of plants but by long association with one form became narrowed to this form more closely, possibly retaining also a somewhat weakened capability of attacking other forms. These authors distinguish between adaptation races (Gewohnheitsrassen) and true biologic forms, the tendency being, under favorable conditions,

for the former to develop into the latter. Eriksson (1902, p. 657) also expresses this view in a somewhat modified form. Ward (1902-1) shows that adaptation of *Puccinia dispersa* takes place. Klebahn (1904, pp. 152-167) cites numerous experiments to show that this may be the case. Miss Gibson (1904, pp. 184-191) grew a number of successive generations of rust on resistant host varieties, but, in that time, found little adaptational tendency. Massee (1904, p. 17) explains the resistance and susceptibility of various plants to parasitic fungi on the ground of the presence or absence of chemotactic substances in the host. He contends that saprophytes can be educated to become parasites. This would also apply in large measure to biologic forms. Salmon (1905, p. 183) grew *Erysiphe graminis* from wheat on *Hordeum sylvaticum* for five generations and found no diminution in the power to infect the original host. Freeman and Johnson (1911, p. 28) conclude that "the host plants exercise a strong influence, not only on the physiological and biological relationships, but in some cases even on the morphology of the host."

An attempt was made to test this matter. The object was to determine the change, if any, in the physiology of the rust as evidenced by its power of infection and in its morphology as evidenced by changes in spore dimensions. For this purpose *Puccinia graminis tritici* was grown on Minnesota No. 163, a susceptible wheat, and on einkorn 2433. The einkorn, in the first few trials, was apparently one of the most resistant of the Triticums to the wheat stem rust. The rust was transferred to einkorn and grown on this host through successive generations for 19 months, transfers being made, on an average, once every 3 weeks. After one year on einkorn the rust seemed to be much more virulent than the original wheat rust had been. Einkorn plants were inoculated with this einkorn rust and with uredospores from wheat. In both cases there was 100 per cent of infection, but the virulence of the infection was quite different. On the leaves inoculated with rust from einkorn, leaf areas one to two centimeters long were affected, being well covered with pustules shedding spores in great abundance. Individual pustules were from one to three millimeters long and nearly as broad, giving every indication of a severe rust attack. On the plants inoculated with wheat rust, on the other hand, the pustules were always smaller, although fairly numerous. Few of them were as much as one millimeter long and many of them did not rupture at all.

Some wheat plants were inoculated with rust from einkorn and others with the rust taken directly from wheat. Here again there was a considerable difference in virulence of infection. The pustules developed from einkorn-rust inoculations were fairly large and numerous. Some of them were two millimeters long, although the average length was less than this. The infection on wheat inoculated with wheat rust was

much more severe. Areas of the leaf two centimeters in length were often almost covered with pustules, some of which attained a length of seven millimeters.

A number of other trials were made, the last after the rust had been confined to einkorn for 17 months. In one experiment the results were just the opposite of what were expected. This is explained by the fact that lack of greenhouse space necessitated keeping the einkorn plants inoculated with einkorn rust in a draught of cold air. There were only a few wheat uredospores available in inoculating the wheat, so, in this experiment, einkorn-by-einkorn inoculations were not so successful as einkorn-by-wheat. In subsequent trials, however, under uniform conditions, the results first described were substantiated.

The conclusion, then, is justified that by confining *Puccinia graminis tritici* to einkorn for successive generations throughout a year or more the rust adapts itself somewhat to its new host and loses, at least to a slight degree, its power to infect the original host. It would no doubt require a very long period of time to fix this character in the plant to such a degree as to make it a new biologic form. But there is very evidently such an adaptational tendency (see Plates II and III). It must be noted, however, that this new character is not so firmly fixed that it cannot be overbalanced by environmental factors. The experimental production of new forms is apparently possible, but a long period of time is required.

The change in the fungus manifests itself not merely in the parasitic tendency toward the host but in the morphology as well. Wheat and einkorn were inoculated with spores from the same plant. The uredospores after growing for a year on wheat averaged $37.85 \times 22.76\,\mu$ while those grown on einkorn for a year measured $33.58 \times 21.79\,\mu$. When einkorn was inoculated with *Puccinia graminis tritici* aecidiospores, the resulting uredospores were more nearly identical with the wheat-rust spores in length. The width, however, remained practically the same. The average size of these spores was $35.92 \times 21.69\,\mu$.

SUMMARY OF PART I

1. Direct transfers of *Puccinia graminis* have been made from oats to both wheat and rye. The rusts from oats and barley used in these experiments could be transferred to rye more easily than those used by Carleton or those used by Freeman and Johnson. The barley rust, however, did not prove as versatile as the strain used by Freeman and Johnson.

2. The use of anesthetics has some effect in rendering an immune plant slightly more susceptible to the rust; leaf injury apparently had no effect.

3. High fertilization, by increasing the virulence of the attack

on semi-immune forms, may have some influence in breaking down biologic forms.

4. There appears to be a physiological and even a slight morphological change in the rust fungus when grown continuously on a semi-immune host. The physiological change manifests itself as an adaptation to the new host, which, however, is very gradual.

5. There are indications that biologic forms of cereal rusts, at least *Puccinia graminis tritici,* do not lose their specialization tendencies when grown on barberry.

6. The degree of incompatibility of host and parasite varies greatly. In semi-compatible forms, fairly large leaf areas are sometimes killed, indicating a killing of host cells by fungus and consequent death of the mycelium itself. In this respect they resemble very closely some of the rust resistant forms of wheat. The biologic forms of rusts, therefore, with susceptible and immune varieties of host plants, throw light on the question of the nature of resistance to *Puccinia graminis.*

PART II. RUST-RESISTANT VARIETIES OF WHEAT

HISTORICAL

It has long been a matter of common observation that some wheats are more resistant than others to the attacks of *Puccinia graminis* and other rusts. Among the earlier observers Henslow (1841, p. 3), La Cour (1863, p. 326) and Little (1883, p. 634) note that some wheats are less injured by rust than are others. Bolley (1889, p. 16) observes that those varieties least susceptible to rust are "hardy, stiff-strawed wheats, having smooth, fibrous leaves." Anderson (1890, p. 84) says that hard, flinty wheats are more rust-resistant than others. He thinks it may be due to the large amount of silica in the hard wheats. Cobb (1892) advanced his "mechanical theory" to explain resistance. It was due, according to his idea, to morphological characters of the host, namely, thick cuticle, waxy coating, and small stomata. Hitchcock and Carleton (1893, p. 12) also correlated resistance with morphological characters, asserting that resistant forms had stiff, upright leaves with a thick epidermis. Eriksson (1895, p. 199) and many others since have shown, however, that a wheat resistant to one species of rust is not necessarily resistant to another species, thus indicating a rather delicate relationship as the basis of resistance.

Eriksson and Henning (1896, pp. 332-365) were unable to substantiate Cobb's mechanical theory, since morphological characters

could not always be correlated with resistance. They suggest that resistance is of a complex chemico-physiological nature and is inherent and fairly constant within the plant. Ward (1902) decided in connection with *Puccinia dispersa* on the bromes that resistance was independent of any recognizable morphological character and suggested that the problem was much the same as that dealing with the factors governing fertility and sterility of stigmas to pollen. Biffen (1907, p. 128) concludes that resistance to *Puccinia glumarum* is independent of any discernible morphological character. He reasserts this principle in a later work (1912, pp. 421-429). Bolley (1908), although not positive, inclines to the view that disease-resistance is physiological rather than morphological in its nature. Cook and Taubenhaus (1912) show that various vegetable acids are toxic to parasitic fungi and that the amount of some of these acids present depends on the stage of ripening of the fruit. Jones, Giddings, and Lutman (1912, p. 83) conclude that the resistance of potato tubers and leaves to *Phytophthora infestans* is due to something either largely or wholly within the tissues. The consensus of opinion among the more recent investigators seems to be that there is a very delicate balance maintained between host and parasite. This balance is dependent, to a certain extent, on the environmental conditions under which host plants are grown. This, however, as well as other phases of the problem, will be discussed more fully under the various sections into which the question naturally subdivides itself.

FORMS WHICH ARE RESISTANT

Attention has frequently been called by various observers to the fact that freedom from disease does not necessarily indicate resistance. Varieties which mature early may escape the disease, and various other factors, mainly ecological, may influence the degree of resistance. Careful experiments, however, have shown that some varieties of Triticum durum are really resistant. The resistance of durum wheats varies with locality according to Bolley's observations (1906, p. 662). Consequently to determine absolute resistance the plants should be grown under controlled conditions. Further, they should be subjected to conditions favorable for infection.

Field Observations

Field observations were made on various forms which were grown in a rust plat. An epidemic was induced by spraying frequently with water containing a large number of spores of *Puccinia graminis tritici.* Frequent observations were made on the amount of rust. Final notes were taken at ripening time. The percentage indicates in each case the estimated percentage of resistance, assuming absolutely immune forms to have a resistance of 100 per cent.

ESTIMATED PERCENTAGES OF RUST ON VARIETIES GROWN IN 1911 AND 1912

1911		1912	
Variety	Per Cent	Variety	Per Cent
Einkorn 2433			
Dickinson 1910 Ped. No. 4..	92	Minnesota No. 169.....	15
No. 6	90	Iumillo 1736	98
No. 7	93	Minnesota No. 188.....	30
No. 8	95	Einkorn 2433	96
Iumillo 1736	95	Khapli	95
Kubanka 1516 No. 8.....	15	Minnesota No. 169	35
Kubanka 1516 No. 9.....	10	Arnautka 288	97
Minnesota No. 163.....	10	Arnautka 1431	67

It will be noticed that in 1912 the resistance was slightly greater than in 1911. This may be accounted for by the fact that in 1911 the grains were sown late, giving the rust ample opportunity to develop fully.

EXPERIMENTAL

GREENHOUSE TRIALS

The varieties tested for resistance in the greenhouse were: Minnesota No. 163, Minnesota No. 169, Kubanka 1516, Nos. 8 and 9, Kubanka 2094, Iumillo 1736, einkorn 2433, Nos. 4, 6, 7, and 8, emmer 1522, Arnautka 288, and Khapli. Of these, Minnesota No. 163, Minnesota No. 169, Kubanka 1516 did not prove resistant. The behavior of einkorn has already been discussed under adaptation of biologic forms. Very careful observations were made on the others to determine as accurately as possible their comparative resistance. The inoculations were made with fresh, viable spores and the plants put under bell jars 48 hours after inoculation.

The incubation period varies with temperature conditions, both high and low temperatures lengthening the period very perceptibly. No experiments with this particular object in view were made, but numerous observations brought the fact out very clearly. Under the same conditions on Minnesota No. 163 the incubation period is shorter than on any of the resistant forms. With an average temperature of about 65 degrees Fahrenheit and a variation of from 40 to 75 degrees and a relative humidity of about 55 per cent, pustules appear on Minnesota No. 163 in 7 or 8 days. In case of Iumillo the period is usually about 2 days longer, although considerable variation was found. Emmer has an incubation period of about 11 days, Arnautka, 12 days, and Khapli, 14 days. Arranged in order of their susceptibility these varieties are as follows:

Variety	Incubation Period
Minnesota No. 163	7 days
Iumillo 1736	9 days
Emmer 1522	11 days
Arnautka 288	12 days
Khapli	14 days

It will thus be seen that the incubation period is longer on the more resistant varieties. These figures are, of course, not absolute, but vary with the temperature, and, to some extent, with soil conditions. All however, vary in nearly, but not absolutely, the same proportion.

The character of infection is distinctly different on the different varieties. It is quite noticeable that the same phenomena are observed as appear on various biologic forms. On Minnesota No. 163 the pustules are large, varying from 2 to 6 millimeters in length. They rupture the epiderm very readily and shed spores in great abundance. Very rarely are small, unruptured pustules developed. The host tissues nearly always remain fairly healthy, a yellowing which gradually appears furnishing the external evidence that the fungous hyphae are in the plant. On einkorn and Iumillo, which in the greenhouse are only fairly resistant, the pustules are usually somewhat smaller. There is a tendency in these two forms toward the development of small dead areas. These areas are either very distinctly yellowed or sometimes killed outright. The general appearance is, however, usually not sharply different from that of infected Minnesota No. 163, except in degree of infection as evidenced by smaller pustules on einkorn and Iumillo. On emmer there were often long infected, yellow areas in which there was a fairly large number of very small pustules, usually less than one millimeter in length, many of which never ruptured. Then again fairly large areas of host tissue were practically killed and only a few small green islands developed. On both Arnautka and Khapli, areas from one to two centimeters long were killed, the leaf appearing white and dead (see Plate VI, A). In these areas there was often a moderately large number of "green islands" with very small, unruptured pustules in the centers. When the pustules did rupture they were always very small, seldom, if ever, exceeding one millimeter in length and often being mere dots. The large areas involved can be explained rather on the basis of multiple infection than on the basis of the spreading of the mycelium from a few infections. Histological examination of diseased areas verifies this supposition.

The spores on the resistant varieties were smaller than on Minnesota No. 163. Spores of Minnesota No. 163 averaged 35.38 x 21.39 μ. while those of emmer were 33.04 x 21.30 μ. The Khapli spores were smallest, being only 29.69 x 20.68 μ. It was found that spores from different pustules varied somewhat in average size. Therefore spores

from a number of pustules were measured in determining the averages. The fact that pustules are produced only with difficulty and that the spores are smaller on resistant varieties would seem to indicate that the fungus is not vigorous and cannot develop extensively although it may gain entrance into the leaf tissues.

COMPARATIVE VIRULENCE OF AECIDIAL AND LONG-TIME UREDOSPORE INOCULATIONS ON RESISTANT FORMS

Various observers have remarked on the reinvigorating power of the aecidial stage of *Puccinia graminis.* Plowright (1882, p. 234) was of the opinion that much more damage was done by aecidial infections than by infection by uredospores which had been reproduced for a number of successive generations. Bolley (1889, p. 13) states that the aecidium, being a sexual product, should be considered as functionally reinvigorating. He also reasserts this principle in a later work (1909, p. 182). Arthur (1902, pp. 68 and 69 and 1903, p. 17) observes that primary uredospores have a greater disturbing effect on the host than do long-time uredospores. Freeman and Johnson, on the other hand, cite experiments (1911, p. 33) to show that when the aecidial and teleutospore stages were excluded for 52 generations the fungus still retained its power of infection. The fact that sexuality in the rusts has been definitely established would make it seem reasonable to suppose that there would be a reinvigorating power. However, Barclay (1892, pp. 8 and 40) states that in India there are no barberries for "enormous distances" from fields of wheat in which *Puccinia graminis* is quite destructive. McAlpine (1906, p. 58) points out that *Puccinia graminis* probably causes no more damage in any country in the world than it does in Australia where barberries are practically absent and aecidia have never been found. The rust is quite serious in South Africa, but, according to Pole Evans (1911), the aecidial stage is absent.

Comparative trials were made with aecidiospores, primary uredospores, and long-time uredospores. The varieties used were not in all cases the most resistant, since no seed of some of the more resistant forms was available when the aecidia appeared. Trials were made on Minnesota No. 163, Kubanka 1516, Iumillo 1736, and einkorn 2433. The two last-mentioned forms are fairly resistant. The long-time uredospores used represented the twenty-fourth generation on wheat. A number of trials were made and the results were not always uniform. The incubation period of the fungus when developed from primary uredospore or from aecidiospore inoculations was slightly longer than when developed from long-time uredospores. The pustules developed from long-time uredospores were apparently smaller and more numerous, while those from aecidiospores and primary uredospores averaged

somewhat larger, were deeper brown, and seemed to be shedding spores in greater abundance. On einkorn, in one experiment, the results were directly opposed to this. The differences were not especially striking, the aecidial infections being perhaps slightly more virulent. There is considerable evidence that the virulence of the rust attack when carried by aecidiospores or primary uredospores is exceptionally virulent. The results of these experiments, however, would not justify such a conclusion in this particular case.

METABOLISM OF THE HOST AND RUST RESISTANCE

There seems to be no question but that weather and soil conditions, determining the metabolism of the host plants, exert an influence on the prevalence of rust in the field. Little (1883, p. 634) states that weather is the determining factor and adds that high manuring, especially with nitrogenous manures, predisposes wheat plants to rust. Bolley (1889), Anderson (1890), and many others since have held that this is the case. Bolley suggests as a possible cause the increased evaporation and consequent raising of the relative humidity. Jones (1905) shows that Phytophthora rot of potatoes tends to be more serious after a heavy application of nitrogenous fertilizers to the land. Miss Gibson (1904) concluded as the result of experiments with the chrysanthemum rust that in an almost immune form normal development of rust does not depend on the state of health of the plant, but that a luxuriant state of growth favors the development of the fungus. Hennings (1903, pp. 41-45), on the other hand, states that in observing plants infected with perennial smuts and with rusts he found that the disease disappeared when the plants were placed under the most favorable cultural conditions. This is not in accord with Arthur's generalization (1903, p. 13) that "so intimate is the association of host and parasite that as a rule the vigor of the parasite is directly proportional to the vigor of the host." Apparent discrepancies may, however, be explained by the fact that different plants and different parasites react quite differently. As far as the rusts of cereals are concerned, Arthur's generalization would seem to be correct. Biffen (1912, pp. 421-429) shows that *Puccinia glumarum* is most virulent when a complete fertilizer is used and that the virulence decreases with the decrease in amount of fertilizer.

Less work has been done to determine the exact manner in which these causes operate. De Bary (1887, p. 359) says, "The physiological reason for these predispositions cannot in most cases be exactly stated; but it may be said in general terms to lie in the material composition of the host, and therefore to be indirectly dependent on the nature of its food." Marshall Ward (1902, p. 145), in experiments with *Puccinia dispersa* on bromes, tried the effect of mineral starvation and concluded

that "lack of minerals in no way secured immunity from infection, although seedlings deficient in phosphorus or nitrogen tended to show retardation of infection." The well-nourished plants produced more spores than the underfed ones. This seems to be due not so much to the presence or absence of any particular chemicals and a direct effect on the fungus but rather to the effect on the host. However, attempts have been made to prevent diseases, among them rusts of cereals, by adding various substances to the soil. Anderson (1890, p. 84) recommends the use of salt, iron sulfate, and lime as tending to decrease the amount of rust. Galloway (1893, p. 208) tried the effect of flowers of sulfur, potassium sulfid, ammonium carbonate, potassium bichromate, calcium hydroxid, and iron sulfate when applied to the soil, but found them of no particular value in preventing rust. Laurent (1902, pp. 1040-1042) concludes that potatoes can be immunized against *Phytophthora infestans* by treating the soil with copper sulfate. Marchal (1902, pp. 1067 and 1068) tried the effect of copper sulfate and iron sulfate of various strengths when added to Sach's solution, on the severity of attack of *Bremia lactucae* on lettuce. He found that by adding 4 or 5 parts of copper sulfate to 10,000 parts of Sach's solution he was able to render the plants practically resistant to the fungus, yet leave the vegetation normal. In experiments attempting to immunize cereals to rusts he was unsuccessful. Massee (1903, p. 142) prevented the development of fungi on tomatoes and cucumbers by watering them with copper sulfate solution. This did not give the desired results with Oidium on barley. He states that not all plants can be treated in this way without endangering their health. Chemical analysis of treated and untreated tomatoes showed that there was no more copper sulfate in treated and therefore immune plants than there was in those which had received no treatment. Massee suggests therefore that the copper sulfate reacts with certain substances in the soil, thus indirectly conferring immunity. Freeman and Johnson (1911, pp. 69-70) call attention to the complexity of the problem and the need for differentiating results.

EFFECT OF WATER CONTENT OF SOIL

Statements to the effect that low-lying, wet soils predispose cereals to rust are frequently made in the literature of the subject. Reference has already been made to some of these. It was observed very frequently in experiments mentioned in this paper that when the relative humidity was high infection was not only surer to result but that it was also more severe. It was therefore thought worth while to determine whether a high water content of the soil would act as a predisposing factor.

The varieties used were: Einkorn 2433, Kubanka 1516, Iumillo

1736, and Minnesota No. 163. There was no difference in the amount of water until the plants germinated. Immediately after germination, however, the soil in one series was kept very wet while that in another series was kept as dry as was possible without endangering the life of the plants. The soil in the wet series had a water content of 31.35 per cent, while that of the dry series was 6.16 per cent at the conclusion of the experiment. Repeated trials were made with substantially the same results.

The number of einkorn and Iumillo leaves which became infected in the wet series was smaller than in the dry series. The percentages for the others were practically the same. In virulence of infection, however, there was considerable difference. The varieties also reacted somewhat differently so each will be considered separately.

On Kubanka the virulence of infection, especially in the early stages, is very markedly inferior on the plants in the wet series. The pustules during the early stages are often small and on some plants do not appear at all, the leaf merely becoming yellow. Later the plants in dry soil were often completely covered with large, vigorous pustules while those in wet soil, although producing a moderately large number of pustules, were not nearly so badly affected. In both series there were many secondary infections along the leaf. There was a distinct tendency in the wet series toward leaf-yellowing. It was at first thought that the mycelium might be spreading through the tissues. Histological examination, however, failed to confirm this supposition. Apparently it was only a slight chlorotic condition due to excessive water content. The infection was unquestionably more severe on plants grown in dry soil (see Plate IV).

On einkorn the differences were not so sharp, although there appeared to be a slightly more severe infection on plants in the dry series than on those in the wet series. The rust appeared at about the same time, the virulence of infection being at first quite distinctly greater on the dry-soil plants. Later this difference was not quite so marked, although still apparent.

The sharpest difference was on Iumillo. Only a few leaves in the wet series were really badly infected, while those of the dry series showed a surprisingly virulent attack. There is no question but that the infections secured on plants in these dry series were more severe than were those on any other Iumillo plants inoculated during the various trials with this variety. It is not often that a really vigorous development of the fungus occurs on Iumillo, but when the water content of the soil is very low, the infection at times shows surprising virulence (see Plate V).

The results on Minnesota No. 163 varied more than those of the other forms. The pustules in nearly every case were large and vigor-

ous with but little difference between the two series. There seemed to be a tendency for the mycelium to spread more in the plants grown in wet soil, but the pustules were not larger than those on dry-soil plants. Whatever difference there was appeared as a slightly greater virulence on the wet-soil plants.

It will be noticed that Iumillo and Kubanka, drought-resisting plants, were more severely attacked when grown in dry soil. Minnesota No. 163, on the other hand, a mesophyte, did not show so much difference. It is probable then, that, conditions having been favorable for a rust infection, the water relation in the soil which is most favorable for the host plant's development is also the most favorable for the development of the rust. It seems probable also that in at least some forms it is not the water content of the soil which predisposes grains growing in low places to rust but rather the increased relative humidity which enables the rust spores to germinate and infect the plants. The temperature in such places also probably exerts an influence. This is pointed out by Freeman and Johnson (1911, p. 65) in connection with the rust epidemic of 1904.

EFFECT OF FERTILIZERS

In the first series the varieties used were: Minnesota No. 163, Arnautka 288, Khapli, emmer 1522, Iumillo 1736, einkorn 2433, and Kubanka 2094. Some were planted in ordinary rich loam, others in rich loam plus fresh barnyard manure, and a third series in rich loam to which barnyard manure and bone meal had been added. Especial care was taken to keep all plants under the same conditions of temperature, moisture, and light both before and after inoculation.

On the wheat plants which were grown on very heavily fertilized soil the infection was clearly more severe. The infected areas were very large as were also the individual pustules. The most severely attacked plants were in one pot which had been fertilized with manure and bone meal. Aside from this one pot, however, there was but little variation among the fertilized pots. The infection on the check plants remained inferior, although it was very vigorous.

Similar results were obtained from einkorn and Iumillo plants. In the case of these two forms the plants in the manure-and-bone series developed the worst rust attack, the manure was next, and the plants in ordinary soil were more lightly attacked. It should be remarked that the differences were not strikingly sharp, although they were quite apparent. Emmer and Arnautka gave no distinct results. There was a great deal of variation in the individual pots and no one series stood out clearly from the other two.

The Kubanka and Khapli plants showed some differences. The character of infection is very different from that of the other forms.

In the case of the former the plants grown in pots to which barnyard mauure had been added were most severely attacked, while there was little difference between those grown in loam and those manured with both barnyard manure and bone meal. Khapli plants grown in soil fertilized with both manure and bone were fairly successfully infected. Areas of the leaf, one centimeter in length, were sometimes infected, and pustules as big as a pin head were developed. There was little difference between the other pots, the infection being somewhat milder than in the heavily fertilized ones

It is quite probable that there was available in the rich loam very nearly all of the plant food the plants were capable of utilizing. This would account for the fact that the differences were not always greater. On the whole it might be concluded that very heavy fertilization is somewhat conducive to increased severity of attack on very resistant varieties as well as on susceptible forms.

Since the check plants in the trials just described were grown under such favorable conditions, it was determined to grow the checks in poorer soil in the next series. Therefore they were planted in moderately fine sand (S) (see table below) to which but a very slight amount of leaf-mold had been added. Nitrogen (N) was added to another series in the form of calcium nitrate, to a third was added calcium phosphate (P), and the fourth received both calcium phosphate and calcium nitrate (P and N). The salts were applied in water. The plants were watered three times with distilled water containing the proper salt or salts, at the rate of 3 grams per 500 cc. The same varieties were used as were used in the preceding series.

Observations on results gave the following order of virulence, the first being most virulent and the others arranged on the same basis. Two observers took notes with the following results:

EFFECT OF FERTILIZERS ON VIRULENCE OF RUST ATTACK

Grain	Order of Virulence			
	1	2	3	4
Einkorn	N	P and N	P	S
Emmer	P and N	N	P	S
Kubanka	P and N	N	P	S
Khapli	P and N	N	P	S
Arnautka	N	S	P	P and N
Iumillo	N	P	S	P and N

The somewhat conflicting results suggested the desirability of another trial. Four series were arranged as follows: Pure sand (S), ordinary field soil (O), sand plus nitrogen (N), and sand plus phosphorus (P). The nitrogen and phosphorus were added as in the preceding experiment. Three persons working independently made ob-

servations, but there was no great difference of opinion in any case. The order in which different observers placed them was sometimes different, showing that there was sometimes little choice among the various pots. The results are given in the following table:

EFFECT OF FERTILIZERS ON VIRULENCE OF RUST ATTACK

Grain	Order of Virulence				Remarks
	1	2	3	4	
Emmer	N	P	S	O	
Kubanka	S	P	N	O	
Iumillo	N	O	P	S	
Khapli	S	N	P	O	
Arnautka	P	S	N	O	
Wheat	S	N	P	O	
Einkorn	N	P	S	O	
Einkorn	P	N	S	O	Little difference between N and P
Emmer	N	S	P	O	Little difference between P and S
Kubanka	S	P	N	O	Distinct
Iumillo	N	P	O	S	Little difference between P and N
Khapli	S	N	P	O	Little difference between P and N
Arnautka	P	N	S	O	Little difference between N and S
Wheat	N	S	P	O	Little difference between S and P

In this experiment the two most resistant forms available for study were watched very carefully. Both Kubanka 2094 and Khapli proved to be very resistant even when very highly fertilized. It is a rather striking fact that in both cases plants grown in sand showed a slightly more virulent infection. The differences were not great. In fact, it was often hard to decide which plants were most severely affected. It was observed that plants which had been under the most favorable conditions for infection were most severely attacked regardless of the fertilizer used. The difference in conditions was due to the fact that there were not enough bell jars to cover all the plants, so some were placed in tubs containing water on the bottom. They were then covered with pieces of glass. The films of moisture were not so persistent here as under the jars, and the difference in the amount of rust was quite marked. This was taken into account in determining results and making comparisons.

If these plants had been allowed to grow longer it is quite probable that those fertilized with nitrogen would have become more severely rusted while those fertilized with phosphorus would have been slightly less severely affected. This would seem to be due not to the specific action of the chemicals on the rust fungus but rather to their effect on the general condition of the plant, and, in the field, on the

immediate atmospheric conditions. The direct effect of chemicals in the soil on the amount of rust on resistant varieties is not great, only a slight quantitative difference being apparent.

In order to determine by more accurate methods whether or not there was a direct effect of substances in the soil on the amount of rust (1) fungicides were put into nutrient media, and (2) certain nutrient salts were used in amounts varying from deficiency to excess.

Sach's modified culture solution was used and to this one per cent of agar was added. The series was arranged as follows, the amounts of nitrate and phosphate being the variables:

I. Potassium nitrate; 2 grams per 1,000 cc.
II. Calcium phosphate; 3 grams per 1,000 cc.
III. Potassium nitrate; .05 grams per 1,000 cc.
IV. Calcium phosphate; .075 grams per 1,000 cc.

Minnesota No. 163 wheat was used. After inoculation plants were all placed under bell jars and kept under uniform conditions. Arranged according to virulence, the most severely affected being placed first, they would be arranged as follows: III, II, IV, I.

This was tried a second time with exactly the same results. The plants appeared about the same, all growing fairly well in the agar. All were well infected, producing a fairly large number of large, healthy pustules. They were kept three weeks after inoculation and by this time there was not much difference between II, IV, and I, but III was still much more virulently attacked. It seems, therefore, that an excess of nitrogen does not necessarily, in itself, cause an increase in the amount of rust and an excess of phosphorus does not affect it very appreciably.

The effect of excluding nitrogen and phosphorus was next tried. Sach's modified medium plus one per cent of agar was again used and in I no potassium nitrate was added while calcium phosphate was excluded from II. The plants in I were lighter colored from the first than either those in II or the checks. They were inoculated six days after planting. A good, vigorous infection resulted, the plants in I being slightly more severely attacked than those in II. The leaves of I began to turn yellow after three weeks, and the rust did not spread farther. The check plants were more severely attacked than those in either I or II. Here again, however, the differences were not very great. There was a slight quantitative difference but qualitatively there was scarcely any difference. This is in keeping with Ward's conclusions reached after his work on mineral starvation, reference to which has already been made.

An attempt was then made to determine whether it was possible to confer immunity by means of various salts. Copper sulfate, copper carbonate and iron sulfate were used in strengths varying from 1 to

10,000 to 1 to 2,000. They were added to Sach's medium in these proportions. Minnesota No. 163 was used for all the trials. Copper sulfate could not be added in larger amounts than 1 to 5,000, since it stunted the plants when more was added. Copper carbonate could not well be used in greater concentration than 1 to 2,000. Iron sulfate did not dwarf the plants when used at the rate of 1 to 2,000. None of the solutions used diminished the amount of rust very appreciably when used in such concentration as to permit of normal development of the host plant. There was a slightly smaller amount of rust on plants grown in the medium to which copper sulfate had been added in amounts of 1 to 4,000 and 1 to 2,000. However, a very good infection was secured on all of them, even on those which never grew to a greater height than one inch. Neither was there any less mildew on any of the plants. None of these substances, apparently, can immunize wheat against rust, even when used in such concentration as to dwarf the plants to one-sixth their normal size.

These experiments show that in the case of *Puccinia graminis*, since it is a very highly specialized, obligate parasite, there is a very intimate relationship between host and parasite, and whatever is conducive to the health of host is ordinarily conducive to the vigorous development of the parasite also. This applies not only to susceptible forms but also to forms uncongenial to a biologic form and to resistant varieties.

THE NATURE OF RESISTANCE

The work of Cobb, Eriksson, Ward, and others on the nature of resistance has already been mentioned. The theory which Ward deduced from his extensive work on parasitism was that there are enzymes or toxins and antitoxins produced by host or parasite or both. His work on "A Lily Disease" (1888) showed that in all probability Botrytis secretes an enzyme which enables it to live more successfully on the host. Pfeffer had already given the name chemotaxis to the attraction certain substances seemed to have for certain growing plant parts. Miyoshi (1894, p. 21) claimed to have been able to observe a very definite chemotropism when a Tradescantia leaf was injected with a wheat-leaf decoction and then inoculated with *Uredo linearis* (*Puccinia graminis*). The same author decided (1895) that a large number of fungi responded to chemical attraction. Massee (1904, p. 7) attached a great deal of significance to chemotaxis. He asserted that infection depended on the presence in the plant cell of positive chemotactic substances and further that "in the future we shall be justified in defining an immune plant as an individual in which the positive chemotactic substance, necessary for facilitating the entrance of the germ-tubes of a given parasitic fungus into its tissues, is absent." On

the other hand Errera (1892, p. 373) contends that so-called chemotropism is in many cases merely positive or negative hydrotropism. Fulton (1906, pp. 81-107) says that there is no definite chemotropic response on the part of fungi. Nutrient solutions cause marked growth, in his opinion, but cause no definite turning of hyphae in their direction. Hydrotropism, however, was observable.

The behavior of the germ tube of *Puccinia graminis* and its entrance into the host plant has been described and figured by various authors. Ward (1881-1, p. 217) figures it as forming a slight swelling and then growing directly into the tissues of the host. Bolley (1889, p. 14) shows the germ tube growing directly through the stomatal opening and branching out between the mesophyll cells. This has never been seen by the writer. An appressorium always formed in all cases of infection observed. Pole Evans (1907, p. 445) describes and figures normal infection quite completely.

A considerable amount of work has also been done on determining the fate of germ tubes when they are produced on immune host plants. Klebahn noted (1896, p. 263) that sporidia of *Puccinia convallariae-digraphidis* could bore through the epidermal walls of *Polygonatum multiflorum,* an uncongenial host, but that the germ tubes developed no further. He concludes that infection is of the nature of a conflict between host and parasite. Ward (1901 and 1902) showed in connection with *Puccinia dispersa* on bromes that the germ tube might enter and cause normal infection, the mycelium might develop and never produce pustules, or the tissues of the host plant might be killed very early, thus precluding the possibility of much development on the part of the fungus. The same author further shows (1904, p. 29) that in normal infection of a susceptible species of Bromus the host cells retain life for a surprisingly long time. Miss Gibson (1904) examined the leaves of a large number of plants, widely separated taxonomically, which had been inoculated with spores of *Uredo chrysanthemi.* She found that the germ tubes might enter the plant tissues very readily but never formed any haustoria and consequently no pustules. Furthermore the hyphae usually died when they came in contact with a cell. On resistant varieties of Chrysanthemum it was found that haustoria might develop, but areas of host tissue in the neighborhood of the hyphae were killed, thus preventing the further spread of the fungous mycelium. Her conclusion is to the effect that when the germ tube of a uredine fungus enters any but its proper host plant a struggle goes on, resulting in the death of the host locally and of the parasite. The closer the relationship between the plant and the proper host of the rust the longer and more extensive will be the struggle. Salmon (1905) found that when barley was inoculated with spores of *Erysiphe graminis* from wheat incipient haustoria might be formed

in the cells, but that they became disorganized within a very few days. He attributed this to defective symbiotic relations between host and parasite. Miss Marryat (1907) showed that *Puccinia glumarum* when grown on a semi-immune host plant killed local areas of the host, sent out but few haustoria, and never developed any but small or abortive pustules.

It is a matter of common observation that in dealing either with cereals uncongenial to a given biologic form of *Puccinia graminis* or with varieties of wheat resistant to *Puccinia graminis tritici* flecks are often visible after inoculation, but no pustules, or only small ones, are produced. Examples of this are shown in Plate I, A and Plate VI, A. All degrees of this killing can be observed. The more readily the rust infects a plant the less likely are these dead areas to appear. When *Puccinia graminis tritici* is put on Minnesota No. 163 wheat, pustules are formed in great abundance, but the leaf tissues remain alive for a long time. When, on the other hand, resistant forms such as Kubanka 2094 or Khapli are inoculated, areas of the leaf are killed outright; and if pustules are formed at all they are very small. In extreme cases of incompatibility such as is found between *Puccinia graminis avenae* and wheat, the leaf area involved is usually so small that no indications of it can be seen with the unaided eye.

Histological Details of Infection

In order to determine the behavior of germ tubes in susceptible and nearly immune forms, leaves of Minnesota No. 163 and of Khapli were inoculated with *Puccinia graminis tritici.* Minnesota No. 163 was also inoculated with *Puccinia graminis avenae.* They were then placed in a pan of water under bell jars for 48 hours. Leaves were selected and killed every 24 hours, beginning with the first day.

For killing, aceto-alcohol, medium chromo-acetic acid, and Flemming's weaker killing-fluid were used. The leaves were embedded in the usual manner and sectioned from five to ten microns thick. For staining, Haidenhain's iron-alum haematoxylin and orange G, Erlich's haematoxylin, Gram's stain and eosin, Delafield's haematoxylin used according to Durand's method, fuchsin and methyl green and the safranin, gentian violet, orange G. combination, used according to Harper's modification of Flemming's method, were used. The last named gave the best results.

Infection of Minnesota No. 163

At the end of 24 hours many of the spores have usually germinated, sending out long germ tubes, although some apparently germinate later. Two tubes may be sent out from the same spore, but usually one develops more vigorously than the other. The tube usually follows the epiderm closely; swellings are often found above

the wall between two epidermal cells (Plate VII, 3). In these swellings, which appear very much like very young appressoria, the protoplasm often aggregates more densely than in the other parts of the tube. The germ tube may often follow the epiderm for considerable distances, sometimes for the length of 15 epidermal cells, before a definite appressorium is formed. Nearly always when a stoma is reached the tube forms a very definite swelling (Plate VII, 1) which constitutes an appressorium. The protoplasmic contents of practically the entire germ tube are concentrated in this appressorium. It dips down into the stomatal opening and a fine protoplasmic process is sent through to the substomatal space. Here a definite swelling takes place, forming the substomatal vesicle (Plate VII, 4 and 5). Apparently, in some cases, the vesicle develops no further. In the great majority of cases, however, the protoplasm aggregates in it and infection-threads are sent out. Very often these infection-threads follow closely along under the epiderm cells and send small knoblike or sometimes flattened haustoria into the host cells. Branching then takes place among the cells of the leaf, many haustoria being sent out and the hyphae developing very rapidly. The threads may grow directly across the substomatal space and branch between the mesophyll cells (Plate VII, 6). This does not seem to be the usual method, however. Nuclear division takes place rapidly to keep pace with the growth of the hyphae. Frequently a number of nuclei are found in a single cell. The entire hypha often retains its protoplasm for a considerable length of time. Ordinarily the tip only remains densely protoplasmic while the rest of the hypha becomes much vacuolated. Very long hyphae are often found growing very vigorously but apparently not sending out haustoria. They seem to be in the nature of distributive filaments. The fungus does not seem to spread very far from the point of infection. When large areas of the leaf are involved, a number of points of entry can nearly always be found. On the fourth day an infected area is usually well filled with much branched hyphae. About this time wefts begin to be formed, the protoplasm aggregating in the tips of the hyphae. A dense mass of filaments is formed just beneath the epiderm and the epidermal cells are wedged apart. The large, upright fungus cells, which show the binucleate condition very clearly, begin to form uredospores. This is well under way on the fifth day and by the sixth or seventh day the epiderm has been completely ruptured while spores are being shed in great profusion. It is interesting to note that the host cells, which are often half filled with large knoblike, filamentous or coiled haustoria, are usually still quite healthy at the time pustule formation begins.

The whole appearance of both fungus and host during the first few days after infection indicates a fairly perfect relation between

the two. The fungus flourishes vigorously while for a considerable length of time the host cells, even in the infected area, are apparently quite healthy. In no case does there seem to be an extensive killing of host tissue.

INFECTION OF KHAPLI

Spore germination of course occurs normally. The germ tubes grow along the surface of the host epiderm cells in the same manner as do those on Minnesota No. 163. The formation of appressoria takes place in an entirely normal manner. The stimulus causing entry into the stomatal slit is present, the method of entrance being substantially the same as in Minnesota No. 163. Apparently the vesicle sometimes fails to send out infection threads but merely remains directly beneath the stomatal slit and becomes vacuolated. It may send out numerous, short, club-shaped branches all of which soon become vacuolated and never send out any haustoria into the host cells. From the beginning of growth in the host it is easily discernible that the vigor of the hyphae is not nearly so great as is the case with those growing in Minnesota No. 163. There is a greater tendency for the tips of infection threads to round up, become vacuolated, and never develop further.

Fairly successful infection, however, may take place. Infection threads may be sent out just under the epiderm or directly across the substomatal space (see Plate VIII, 3 and 5). Haustoria, attached to the hypha by delicate sterigmata, are sent into the cells and the hypha grows fairly well. Sometimes many incipient infection threads are formed from a single vesicle, only one developing (Plate VIII, 5). Shortly after infection threads are sent out the vesicle usually dies.

The infection threads are not always successful in sending haustoria into the host cells. When the hypha comes into contact with the cells the protoplast of the latter often shrinks back from the wall, the nucleus shows definite signs of disintegration, the chloroplasts are apparently lost, and the entire cell dies. The hypha may die also, or it may grow and kill other cells. However, it usually eventually succumbs. Typical examples of this will be seen on Plate VIII, 6 and 7. It will be noticed that at 6 the cell on which the hypha is abutting is apparently dead, the chloroplasts have disappeared, and the nucleus is disintegrating. At 7 this has taken place in only a part of the cell. It sometimes happens that one branch of a hypha is fairly successful while another may never develop to any extent at all.

Whether or not the host cells are killed within a short time after the hyphae come in contact with them, infection does not appear to be normal. The hyphae may grow fairly well, but never as vigorously as in Minnesota No. 163. Haustoria may be sent out in fair abundance

(Plate IX, 1, 2, and 4). The hyphal tips may branch very profusely but they become vaculoated very early in most cases and appear unthrifty. It requires 8 or 9 days for the stage of infection to be reached on Khapli that is reached in 3 or 4 days in susceptible varieties. Short, thick, or rounded hyphal segments are quite common, those at the end of a branch often containing as many as 5 or 6 nuclei, some of which appear to be distintegrating.

The tips of hyphae naturally die when a group of host cells among which they are growing is killed. However, they may disintegrate without having first killed host cells (Plate IX, 1, 2, 3, and 4). There may be many variations. The hyphae may not send out haustoria and die in consequence, or, even if they do send them into the cells, death may occur. Branches of hyphae which have sent haustoria into host cells frequently become vacuolated and gradually die, or the protoplasmic contents may change to granular, deep-staining masses. The whole appearance suggests fungous hyphae growing in an unfavorable nutrient solution.

In about 11 or 12 days a distinct tendency toward the formation of hyphal wefts can be observed. These vary greatly both in size and position. They may be mere aggregations directly under the epiderm or deeper down in the tissues; or they may begin to wedge the epiderm cells apart after the manner of young pustules. Often small, unruptured pustules are formed in which there is a fairly large number of abortive spores. The pustules may rupture the epiderm but they are always extremely small, and as a rule the spores are small. The average size of a large number of spores measured was $29.69 \times 20.68\ \mu$, whereas the average size of the wheat rust spores which were used in making the inoculations was $35.38 \times 21.39\ \mu$.

After about 20 days practically all the host cells and a large number of the hyphae in an infected region are dead. Haustoria may be present in the host cells in fairly large numbers, but most of them are dead, their protoplasmic contents having broken up into granular, deep-staining masses. The nuclei of the host cells are often disintegrating also. The infection by this time, and usually earlier, has completely run its course. Comparatively very few spores have been produced, and, under natural conditions, secondary infections would probably not occur to any extent.

THE COURSE OF INFECTION IN OTHER RESISTANT FORMS

Substantially the same sequence of events occurs in other resistant forms, such as Arnautka 288, Kubanka 2094, emmer 1522, einkorn 2433 (sometimes), and in such cases as the infection of rye by barley rust. The differences seem to be in degree rather than in kind. In the cases of emmer and einkorn the killing of host cells is rarely found but

unsuccessful attempts to form pustules are often noticed. The sequence of events in Arnautka 288 and Kubanka 2094 is quite similar to that in Khapli. These are semi-immune forms in which the contest between host and parasite is somewhat prolonged. Fatalities occur on both sides but not in sufficient number to render infection absolutely unsuccessful. It is, therefore, essential to note the sequence in such extreme cases of almost total immunity as are furnished by wheat when inoculated with *Puccinia graminis avenae.*

MINNESOTA NO. 163 INOCULATED WITH PUCCINIA GRAMINIS AVENAE

It will be recalled that very rarely indeed does successful infection follow inoculation of wheat with *Puccinia graminis avenae.* Long germ tubes are sent out by spores germinating on the surface of the leaf. These follow the epiderm, dipping into depressions in an entirely normal manner. Appressoria are found, a small neck grows through the stomatal slit and the substomatal vesicle is formed. This vesicle sometimes sends out only very small knoblike branches which soon die, or branches may be sent out and very few hausteria produced.

The vesicle often sends out many short, knoblike branches, appearing almost like elongated amoebae. These do not appear vigorous after a few days. The epiderm cells in the immediate vicinity appeared to be killed. If definite infection threads are sent out they never grow very long but kill two or three host cells and then stop growing.

The difference between conditions in such a case as this and such a one as Khapli is apparently in degree only. Whereas in Khapli the fungus might develop to a certain extent, thus involving fairly large leaf areas, in such an extreme case of immunity as is presented by wheat to oat rust only a few host cells are involved and the contest between host and parasite is short and decisive, only a very few host cells being killed; and the hyphae seldom develop sufficiently to give any external evidence that the germ tube has even entered.

As to the fundamental causes for these facts, only speculation is possible. It would seem reasonable to suppose either that there was a lack of an attracting substance or the presence of a deleterious substance. Massee's chemotaxis theory has already been mentioned. Chemotaxis or its absence would hardly explain the phenomena mentioned, since the fungus succeeds in effecting an entrance into even the most immune forms. The evidence would rather seem to favor the view that the whole problem is one of toxins in host or parasite or, very probably, in both. In some cases the host is apparently hypersusceptible, while further study may prove that there is in other cases a real resistance. Careful biochemical investigation alone can settle this question definitely.

Certainly, however, there can be no question of a certain antagonism between host and parasite when one observes phenomena such as are illustrated in Plate VIII, 6. This is in remarkably sharp contrast with the apparent congeniality exhibited in such cases as are shown at 6, 7, and 8 in Plate VII. Antagonism would seem to be explicable at present only by the toxin or enzyme theory. The recent work of Bolley (1908 and 1909), Pole Evans (1909 and 1911), McAlpine (1910), Freeman and Johnson (1911), and Biffen (1912) indicates clearly that immunity and resistance are concepts which, from the very nature of their variability and sometimes apparent capriciousness, must be cautiously discussed. At least one substance, commonly found in plants, has been found by Cook and Taubenhaus (1911, pp. 40 and 43) to be toxic to certain rusts of the genus Uromyces.

Whatever the immediate instruments governing congeniality or antagonism, the fundamental facts brought out quite clearly in the results described in the present investigation have a bearing on the practical and theoretical questions involved in the problem of preventing cereal rusts and the breeding of rust-resisting varieties. External morphology as pointed out by Ward (1902-1) for brome rusts, Salmon (1905-2) for mildews, and Biffen (1907) for yellow rust is also of very slight importance in the immunity of cereal varieties to stem rust. No observed facts in intimate histology, moreover, give any clue to resistance. In the absence of biochemical information concerning the activities of invading hyphae and invaded host tissues, actual performance alone can be depended upon as a safe criterion in the development of resistant forms or immune varieties. This is all the more true since Pole Evans (1911) has found that a hybrid wheat produced by crossing rust-immune and rust-susceptible wheats may rust quite badly and be capable of causing infection of the immune parent and a more severe attack of rust on the susceptible parent variety than rust from that variety itself will cause. The production of flecks and dead areas on an inoculated plant is a character of possible use in indicating at least a semi-immunity.

There does not seem to be any obvious constant correlation between immunity and other observable characters, as for instance drought-resistance. Although the immune varieties of wheat used in this investigation are drought-resistant, it is also a well-established fact that other drought-resistant wheats such as Kubanka 1516 are very susceptible. Moreover, the antagonism exhibited by Minnesota No. 163 wheat toward *Puccinia graminis avenae* and by rye toward *Puccinia graminis hordei* does not differ fundamentally from that exhibited by Khapli toward *Puccinia graminis tritici* and in the first two cases a correlation with drought-resistance is out of the question.

That the important scientific questions involved in the specialization of biologic forms and that of rust-resistant varieties of wheat are essentially the same seems obvious. The same phenomena can be observed in both; there are various degrees of resistance and susceptibility in both and a thorough investigation with refined biochemical methods will probably not only prove the similarity, but show the real reason for resistance and susceptibility.

SUMMARY OF PART II

1. In making inoculations in the greenhouse on wheats resistant to *Puccinia graminis tritici* it was found that only two, Khapli and Kubanka 2094, especially the former, possessed a very marked degree of real resistance, although a number of others were fairly resistant in the field.

2. It was observed that the more resistant a form proved, the more pronounced was the tendency of the rust to kill small areas of the leaf. The pustules developed in these areas were always very small.

3. The length of the incubation period of the rust is correlated to a certain extent with the degree of immunity, the most nearly immune forms, as a rule, having the longest incubation period.

4. On the most resistant varieties, such as Khapli, the spores are often small in size and sometimes abortive.

5. Infection secured on partially resistant varieties as a result of inoculations with aecidiospores and primary uredospores proved only slightly more virulent than did that secured by means of inoculation with long-time uredospores.

6. Drought-resistant durum wheats grown in very dry soil rusted more severely than those grown in soil with a higher moisture content. Minnesota No. 163 did not show much difference, the plants in wet soil being slightly more severely attacked. The conditions normal for the host plant are probably also the conditions under which the rust develops best.

7. It was found that in general the absence or presence, in excessive amounts, of various nutrient substances, such as nitrogen and phosphorus salts, did not directly affect the immunity or susceptibility of wheats. Conditions favoring a normal development of the host were conducive to vigorous development of the rust. The action of fertilizers, either natural or artificial, is probably indirect. Temperature conditions and relative humidity of the atmosphere are probably more important than soil conditions.

8. The addition of copper sulfate, copper carbonate, and iron sulfate to nutrient media in which plants inoculated with rust were grown did not markedly diminish the amount of rust when they were

used in such concentration as to permit of the normal development of the host plants.

9. A careful comparison of the sequence of infection in such a susceptible form as Minnesota No. 163 with that in such an immune form as Khapli reveals the fact that the fungus gains entrance in the same manner in both cases. The rust mycelium is able to grow luxuriantly in Minnesota No. 163 and produce spores in great abundance. In Khapli, however, it does not thrive. The reason seems to be a physiological incompatibility as evidenced by the killing of host cells by the fungus and the more or less sudden death of the fungus itself. Infection may occur and pustules may be developed, but it is evident that the fungus is not in a congenial environment. The conditions seem to be essentially similar when examination is made of a cereal almost completely immune to a biologic form, such as Minnesota No. 163, inoculated with *Puccinia graminis avenae*. Here, however, the host cells and rust hyphae are killed earlier and the leaf area involved is consequently smaller. This, however, requires further study.

10. The question as to the immediate instruments of immunity can probably only be answered by means of biochemical investigations. In the meantime, morphological and histological characters being clearly of minor importance in determining immunity, only the performance of a supposedly resistant variety under varying conditions can be depended on for a criterion of its value in this respect.

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BIBLIOGRAPHY

Anderson, H. C. S. Rust on Wheat Experiments and Their Object. Agr. Gaz. New South Wales I:1. 1890.

Arthur, J. C. The Aecidium as a Device to Restore Vigor to the Fungus. Proc. Soc. Prom. Agr. Sci. **1902**: 65-69. 1902.

Problems in the Study of Plant Rusts. Bull. Torrey Club **30**: 1. 1903.

Barclay, A. Rust and Mildew in India. Jour. Bot. **30**: 8 and 20. 1892.

Bary, A. de. Ueber einige Schlerotinien und Schlerotinien Krankheiten. Bot. Zeit. **44**: 377. 1886.

Comparative Morphology and Biology of the Fungi, Mycetozoa and Bacteria: 359. 1887.

Bandi, W. Beiträge zur Biologie der Uredineen. Hedw. **42**: 118. 1903.

Biffen, R. A. Studies in the Inheritance of Disease Resistance. Jour. Agr. Sci. **2**: 109-128. 1907.

Studies in the Inheritance of Disease Resistance II. Jour. Agr. Sci. **4**: 421-429. 1912.

Bolley, H. L. Wheat Rust. Indiana Agr. Exp. Sta. Bull. 26. 1889.

New Work upon Wheat Rust. Sci. (n.s.) **22**: 50. 1905.

Observations Regarding the Constancy of Mutants and Questions Regarding the Origin of Disease Resistance in Plants. Am. Nat. **42**: 171-183. 1908.

Some Results and Observations Noted in Breeding Cereals in Especially Prepared Disease Garden. Rept. A. B. A. **5**: 177-182. 1909.

——— and Pritchard, F. J. Rust Problems. Facts, Observations and Theories; Possible Means of Control. N. Dak. Agr. Exp. Sta. Bull. 68. 1906.

Büsgen, M. Ueber einige Eigenschaften der Keimlinge parasitischer Pilze. Bot. Zeit. **51**[1]: 53. 1893.

Butler, E. J. The Bearing of Mendelism on the Susceptibility of Wheat to Rust. Jour. Agr. Sci. **1**: 361-363. 1905.

Carleton, M. A. Cereal Rusts of the United States. U. S. Dept. Agr. Div. Veg. Phys. and Path. Bull. 16. 1899.

Macaroni Wheats. U. S. Dept. Agr. B. P. I., Bull. **3**: 61. 1901.

Investigations of Rusts. U. S. Dept. Agr. B. P. I. Bull. 63. 1904.

Lessons from the Grain-Rust Epidemic of 1904. U. S. Dept. Agr. Farmers' Bull. 219. 1905.

Cobb, N. A. Contributions to an Economic Knowledge of the Australian Rusts. Agr. Gaz. New South Wales **3**: 53. 1890.

Contributions to an Economic Knowledge of Australian Rusts. Agr. Gaz. New South Wales **3**: 181-212. 1892.

Cook, M. T. and Taubenhaus, J. J. The Relation of Parasitic Fungi to the Contents of the Cells of the Host Plants I. The Toxicity of Tannin. Del. Coll. Agr. Exp. Sta. Bull. 91: 40-43. 1911.

The Relation of Parasitic Fungi to the Contents of the Cells of the Host Plants II. The Toxicity of Vegetable Acids and the Oxidizing Enzyme. Del. Coll. Agr. Exp. Sta. Bull. 97. 1912.

Dietel, P. Beiträge zur Morphologie und Biologie der Uredineen. Bot. Centralb. **32**: 54. 1887.

Waren die Rostpilze in frühern Zeiten plurivor? Bot. Centralb. **79:** 81 and 113. 1899.

Eriksson, Jakob. Ueber die Specialisirung des Parasitismus bei den Getreiderostpilzen. Ber. Deutsch. Bot. Gesells. **12:** 331. 1894.

Die Hauptresultate einer neuen Untersuchung über die Getreideroste. Zeits. Pflanzenk. **4:** 70-71. 1894. (2)

Ist die verschiedene Widerstandsfähigkeit der Weitzensorten gegen Rost Konstant oder nicht. Zeits. Pflanzenk. **5:** 198-200. 1895.

Vie latente et plasmatique de certaines Uredinees. Compt. Rend. Acad. Sci. **124:** 475-477. 1897.

Ueber die Spezialisierung des Getreideschwarzrostes in Schweden und in anderen Ländern. Centralb. Bakt. II. **9:** 590. 1902.

Der Malvenrost (Puccinia malvacearum Mont.) Seine Verbreitung, Natur und Entwickelungsgeschichte. Kungl. Svensk. Vetensk. Hand. **47,** No. 2. 1911.

——— and Henning, Ernst. Die Getreideroste: 327-336. 1896.

Errera, L. On the Cause of Physiological Action at a Distance. Ann. Botany **6:** 373. 1892.

Evans, I. B. Pole. The Cereal Rusts. I. The Development of their Uredo mycelia. Ann. Botany **21:** 441-462. 1907.

Report of the Plant Pathologist. Transvaal Dept. Agr. Ann. Rept. **1908:** 120-134. 1908. (Exp. Sta. Rec. **21:** 640. 1909.)

South African Cereal Rusts with Observations on the Problem of Breeding Rust-Resistant Wheats. Jour. Agr. Sci. **4:** 95-104. 1911.

Frank, B. Ueber einige neue und weniger bekannte Pflanzenkrankheiten. Ber. Deutsch. Bot. Gesells. **1:** 30. 1883.

Freeman, E. M. Experiments on the Brown Rust of Bromes (Puccinia dispersa). Ann. Botany **16:** 487-494. 1902.

——— and Johnson, E. C. The Rusts of Grains in the United States. U. S. Dept. Agr. B. P. I., Bull. 216. 1911.

Fulton, Harry R. Chemotropism of Fungi. Bot. Gaz. **41:** 81-107. 1906.

Fuschini, C. The Use of Iron Sulphate for the Control of the Rust of Plants. Revista (Conegliano), IV. 17: 443-446. 1911. (Internat. Inst Agr., Bull. Agr. Intelligence and Plant Diseases. **2:** 2600. 1911.)

Galloway, B. T. Experiments in the Treatment of Rusts Affecting Wheat and Other Cereals. Jour. Myc. 7: 208. 1893.

Gibson, Miss C. M. Notes on Infection Experiments with Various Uredineae. New Phytol. **3:** 184-191. 1904.

Hennings, P. Einige Beobachtungen über das Gesunden pilzkranker Pflanzen bei veränderten Kulturverhältnissen. Zeits. Pflanzenk. **13:** 41-45. 1903.

Henslow, J. S. Report on Diseases of Wheat. Jour. Roy. Agr. Soc. Eng. **2:** 1. 1841.

Hitchcock, A. S., and Carleton, M. A. Preliminary Report on Rusts of Grain. Kans. Exp. Sta. Bull. 38. 1893.

Second Report on Rusts of Grain. Kans. Exp. Sta. Bull. 46. 1894.

Jaczewski, A. von. Studien über das Verhalten des Schwarzrostes des Getreides in Russland. Zeits. Pflanzenk. **20:** 321-359. 1910.

Johnson, A. G. Further Notes on Timothy Rust. Proc. Ind. Acad. Sci. **1909:** 417-418. 1909 (Exp. Sta. Rec. **28:** 53. 1913.)

Jones, L. R. Disease Resistance of Potatoes. U. S. Dept. Agr., B. P. I. Bull. 87. 1905.

Investigations of the Potato Fungus Phytophthora Infestans. U. S. Dept. Agr. B. P. I. Bull. 245. 1912.

Klebahn, H. Kulturversuche mit heteröischen Rostpilzen. Zeits. Pflanzenk. **6**: 261-270. 1896.

Die Wirtswechselnden Rostpilze. 1904.

La Cour. Sygdomme i Kornet og Midlerne derimod. Tidsskr. for Landøk Kjøbenhavn 1863. (Eriksson and Henning. Die Getreideroste: 326.)

Laurent, Emile. De l'action interne du sulfate de cuivre dans la resistance de la pomme de terre au Phytophthora infestans. Compt. Rend. Acad. Sci. **135**: 1040-1042. 1902.

Little, W. C. Report on Wheat Mildew. Jour. Roy. Agr. Soc. England. **19**: 634. 1883.

Magnus, P. Die Systematische Unterscheidung nächst verwandter parasitischer Pilze. Hedw. **33**: 362. 1894.

Eine Bemerkung zu E. Fischer's erfolgreichen Infectionen einiger Centaurea-Arten durch die Puccinia auf Carex Montana. Bot. Centralb. **63**: 39. 1895.

Marchal, E. De la Specialization du parasitisme chez l'Erisyphe graminis. Compt. Rend. Acad. Sci. **135**: 210-212. 1902.

De l'immunisation de la Laitue contre le Meunier. Compt. Rend. Acad. Sci. **135**: 1067-68. 1902.

De la Specialization du parasitisme chez l'Erisyphe graminis D. C. Compt. Rend. Acad. Sci. **136**: 1280, 1281. 1903.

Marryat, Dorothea C. E. Notes on the Infection and Histology of Two Wheats Immune to the Attacks of Puccinia glumarum, Yellow Rust (with Plate II). Jour. Agr. Sci. **2**: 127-137. 1907.

Massee, George. On a Method for Rendering Cucumber and Tomato Plants Immune Against Fungus Parasites. Jour. Roy. Hort. Soc. **28**: 142. 1903.

On the Origin of Parasitism in Fungi. Phil. Trans. Roy. Soc. London. B. **197**: 7. 1904. (Pub. 1905.)

McAlpine, D. The Life History of the Rust of Wheat. Dept. Agr. Victoria. Bull. 14. 1891.

Ueber die Verwendung geschrumpften Körner von rostigem Weizen als Saatgut. Zeits. Pflanzenk. **2**: 193. 1892.

Rust and Smut Resistance in Wheat and Smut Experiments with Oats and Maize. Jour. Dept. Agr. Victoria. **8**: 284-289. 1910.

Miyoshi, Manchu. Ueber Chemotropismus der Pilze 1. Bot. Zeit. **52**[1]: 1-27. 1894.

Die Durchbohrung von Membranen durch Pilzfäden. Jahrb. Wiss. Bot. **28**: 269-289. 1895.

Neger, F. W. Beiträge zur Biologie der Erysipheen, 2 Mitteilung. Flora **90**: 221-271. 1902.

Nordhausen, M. Beiträge zur Biologie parasitärer Pilze. Jahrb. Wiss. Bot. **33**: 1. 1899.

Orton, W. A. The Development of Farm Crops Resistant to Disease. Yearbook U. S. Dept. Agr. **1908**: 453-464. 1908.

Pfeffer, W. Locomotorische Richtungsbewegungen durch chemische Reize. Ber. Deutsch. Bot. Gesells. **1**: 524-533. 1883.

Plinius, Secundus. Naturalis Historia. The Natural History of Pliny. **4**: 54-59. 1900. [Translation by Bostack and Riley, London, 1900.]

Plowright, C. B. The Connection of Wheat Mildew with the Barberry. Gard. Chron. **18:** 234. 1882.

Pritchard, F. J. A Preliminary Report on the Yearly Origin and Dissemination of Puccinia graminis. Bot. Gaz. **52:** 169-192. 1911. (1911-1.)

The Wintering of Puccinia Graminis Tritici E. and H. and the Infection of Wheat through the Seed. Phytopath. **1:** 150-154. 1911. (1911-2.)

Ray, J. Etude biologique sur le parasitisme: Ustilago maydis. Compt. Rend. Acad. Sci. **136:** 567-570. 1903.

Reed, George M. Infection Experiments with Erysiphe graminis. Trans. Wis. Acad. **15**[1]**:** 135-162. 1905.

Infection Experiments with the Mildew on Cucurbits, Erysiphe cichoracearum, D. C. Trans. Wis. Acad. **15**[2]**:** 527-547. 1907.

The Development of Disease Resistant Plants. Mo. St. Bd. Hort. Ann. Rept. **1908:** 284-296. 1908.

Infection Experiments with the Powdery Mildew of Wheat. Phytopath. **2:** 81-87. 1912.

Reinhardt, M. O. Das Wachsthum der Pilzhyphen. Jahrb. Wiss. Bot. **23:** 479-563. 1892.

Rostrup, E. Mykologiske Meddelelser. Bot. Tidssk. **19:** 36-51. 1894.

Salmon, E. S. On Specialization of Parasitism in the Erysiphaceae. Beihefte Bot. Centralb. **14:** 261. 1903 (1).

Infection Powers of Ascospores in Erysiphaceae. Jour. Bot. **41:** 159. 1903 (2).

On Erysiphe graminis D. C. and Its Adaptive Parasitism within the Genus Bromus. Ann. Myc. **2:** 255-267; 307-343. 1904 (1).

Cultural Experiments with "Biologic Forms" of Erysiphaceae. Phil. Trans. Roy. Soc. London. B. **197:** 107-122. 1904 (2).

On Specialization of Parasitism in the Erysiphaceae III. Ann. Myc. **3:** 172-184. 1905.

Further Cultural Experiments with Biologic Forms of the Erysiphaceae. Ann. Botany **19:** 125-198. 1905 (1).

On the Stages of Development Reached by Certain Biologic Forms of Erysiphe in Cases of Non-Infection. New Phytol. **4:** 217-222. 1905 (2).

Sawer, E. R. Cereals in South Africa. Cedara Memoirs on South African Agriculture. Pietermaritsburg, 1909. (Exp. Sta. Rec. **23:** 31-33. 1910.)

Stahl, E. Zur Biologie der Myxomyceten. Bot. Zeit. **42:** 161-176; 187-191. 1884.

Strebel. Ueber Kultur und Schätzung verschiedener Halmfrüchte. Bidermanns Centralb. Agr. Chem. **1885:** 189. (Eriksson and Henning, Die Getreideroste: 327.)

Sydow, P. and H. Monographia Uredinarum. **1:** 692-698. 1904.

Theophrastus Eresius. Theophrasti Historia Plantarum. Lib. **VIII** (Amsterdam 1644).

Tischler, G. Untersuchungen über die Beeinflussung der Euphorbia Cyparissias durch Uromyces Pisi. Flora **104:** 1-64. 1911. (Bot. Centralb. **120:** 546. 1912.)

Ward, H. M. Illustrations of the Structure and Life History of Puccinia graminis. Ann. Botany **2:** 217. 1888 (1).

A Lily Disease. Ann. Botany **2:** 319. 1888 (2).

The Relations between Host and Parasite in Certain Epidemic Diseases of Plants. Proc. Roy. Soc. London. 47: 393. 1890.

The Bromes and Their Rust Fungus, Puccinia dispersa. Ann. Botany **15:** 560. 1901.

On the Relations between Host and Parasite in the Bromes and Their Brown Rust, Puccinia dispersa Erikss. Ann. Botany **16:** 233-315. 1902 (1).

Experiments on the Effect of Mineral Starvation on the Parasitism of the Uredine Fungus, Puccinia dispersa, on Species of Bromus. Proc. Roy. Soc. London. **71:** 138. 1902. (Pub. 1903) (2).

Further Observations on the Brown Rust of the Bromes, Puccinia dispersa (Erikss.), and Its Adaptive Parasitism. Ann. Myc. **1:** 132-51. 1903.

On the Histology of Uredo dispersa, Erikss., and the "Myoplasm" Hypothesis. Phil. Trans. Roy. Soc. London. B. **196:** 29. 1904. Recent Researches on the Parasitism of Fungi. Ann. Botany **19:** 1-50. 1905.

Weevers, T. Betrachtungen und Untersuchung über die Nekrobiose und die letale Chloroformeinwirkung. Rec. Trav. bot. neerl. **9:** 236-276. 1912. (Bot. Centralb. **120:** 648. 1912.)

EXPLANATION OF PLATES

The drawings were all made with the aid of the camera lucida. The Zeiss 3 mm. N. A. 1.3 homogeneous oil immersion and compensating ocular No. 4 were used for all except Figures 8 and 10, Plate IX, where compensating ocular No. 6 was substituted.

Plate I A. Rye after inoculation with *Puccinia graminis hordei,* showing dead leaf areas and a very few minute pustules.
B. Normal but rather light infection on rye by *P. graminis secalis,* showing absence of dead areas.

Plate II A. Einkorn 2433 inoculated with *P. graminis* originally obtained from wheat and confined to Einkorn for twenty-five successive generations.
B. Einkorn 2433 inoculated with *P. graminis* from wheat.

Plate III A. Wheat, Minnesota No. 163, inoculated with rust which had been confined to Einkorn for twenty-five generations.
B. Normal infection of wheat following inoculation with *P. graminis tritici* from wheat.

Plate IV A. Kubanka 1516 grown in very wet soil after inoculation with *P. graminis.*
B. Kubanka grown in very dry soil after inoculation.

Plate V A. Iumillo 1736 grown in very wet soil after inoculation with *P. graminis tritici.*
B. The same variety inoculated under same condition but grown in very dry soil.

Plate VI A. Khapli, showing characteristic infection following heavy inoculation with *P. graminis.*
B. Normal infection on Minnesota No. 163.

Plate VII. Infection of Minnesota No. 163 wheat by *P. graminis tritici.*
1, 2 and 3—48 hours after inoculation.
4 and 5—72 hours after inoculation.
6 to 9—5 days after inoculation.
1. Surface view showing appressorium forming over a stoma.
2. Appressorium being formed directly, without germ tube development.
3. Germ tube apparently passing over stoma and forming a swelling—an unusual occurrence.
4. Part of a germ tube, appressorium, and neck connecting the appressorium with the substomatal vesicle which has been cut at one side.
5. Substomatal vesicle, cut at one side, beginning to branch.
6. Infection thread growing from vesicle directly across substomatal space; remains of appressorium outside. (Section slightly torn.)
7. Part of an infection thread showing haustorium in epidermal cell.
8 and 9. Later stages showing development of long hyphae.

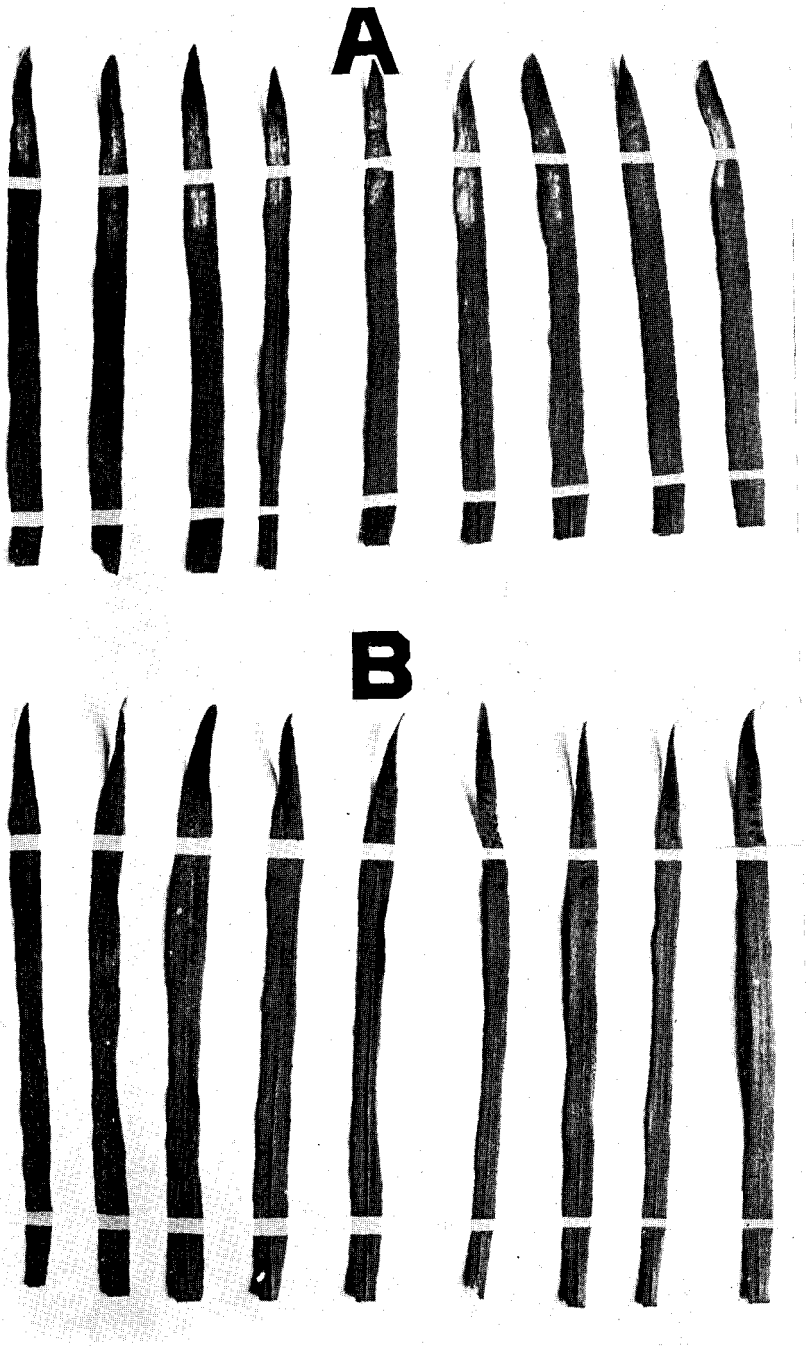
Plate VIII. Infection of Khapli with *P. graminis tritici.*
1 and 2—72 hours after inoculation.
3 and 4—6 days after inoculation.
5, 6 and 7—8 days after inoculation.

1. Part of a germ tube and an appressorium.
2. Germ tube, appressorium, and substomatal vesicle into which the nuclei have passed.
3. Infection thread, with a few short branches, killing the host cell. The protoplast has shrunk and the nucleus is disintegrating.
4. Infection thread in contact with a cell which it is apparently killing.
5. Substomatal vesicle and a number of somewhat abortive infection threads. In the epidermal cell on the left two haustoria, deeply stained, and possibly dead. Below, fairly successful infection.
6. Empty appressorium and vesicle. On the left infection threads, one of which has sent a haustorium into an epidermal cell.
7. Infection threads growing toward leaf tissues.

Plate IX. Same as Plate VIII, except Figs. 6 to 10, which are of Arnautka.
1 to 5—10 days after inoculation.
6 to 10—23 days after inoculation.

1. Short hyphal segment containing four nuclei. Haustorium in epidermal cell. Hyphae sent out from segment disintegrating.
2. Disintegrating hypha with haustorium in host cell.
3. Long hyphae, the tips of some becoming much vacuolated and apparently dying.
4. Typical appearance of hyphae under epiderm, showing haustoria and some dying hyphal tips.
5. Hyphae deeper down in leaf tissues showing tendency to aggregate.
6. Dead host cells and practically dead hyphae.
7. Hyphae typical of those in subepidermal wefts showing knoblike branches which are often quite characteristic.
8. Small, partly ruptured pustule.
9. Single uredospore.
10. Subepidermal weft showing unsuccessful attempt at pustule formation and a number of abortive spores.

PLATE I



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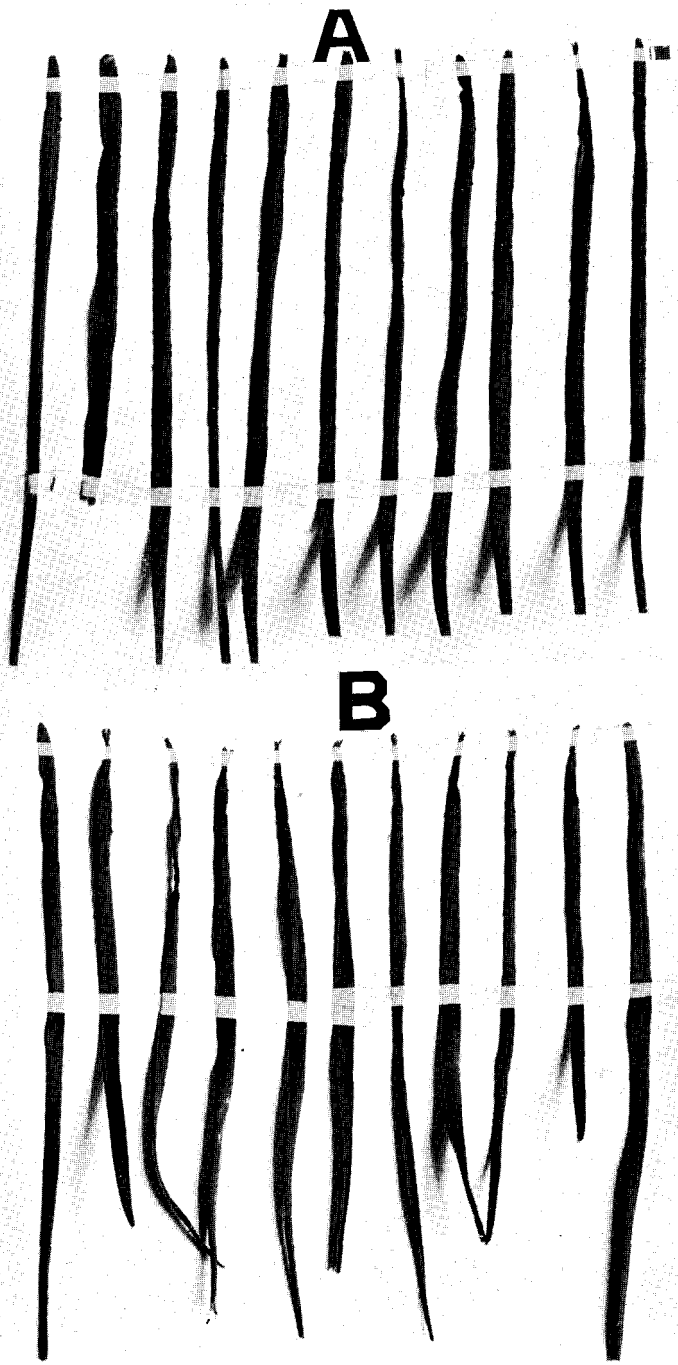

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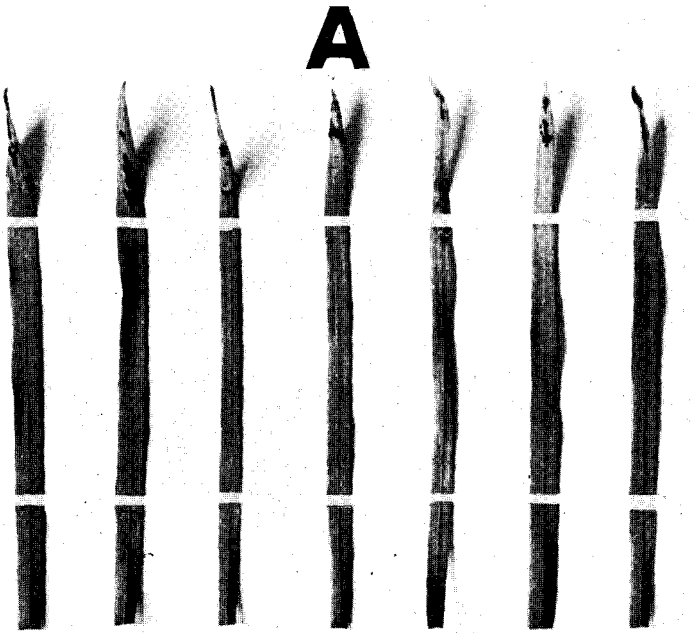

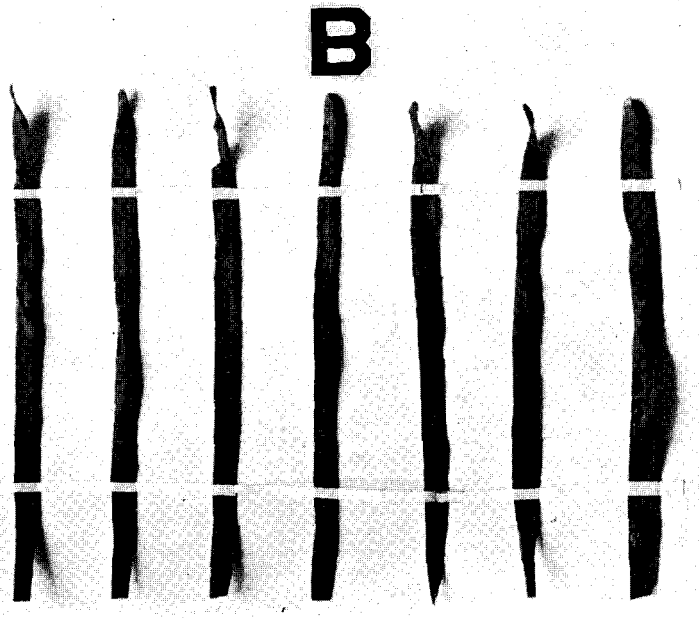

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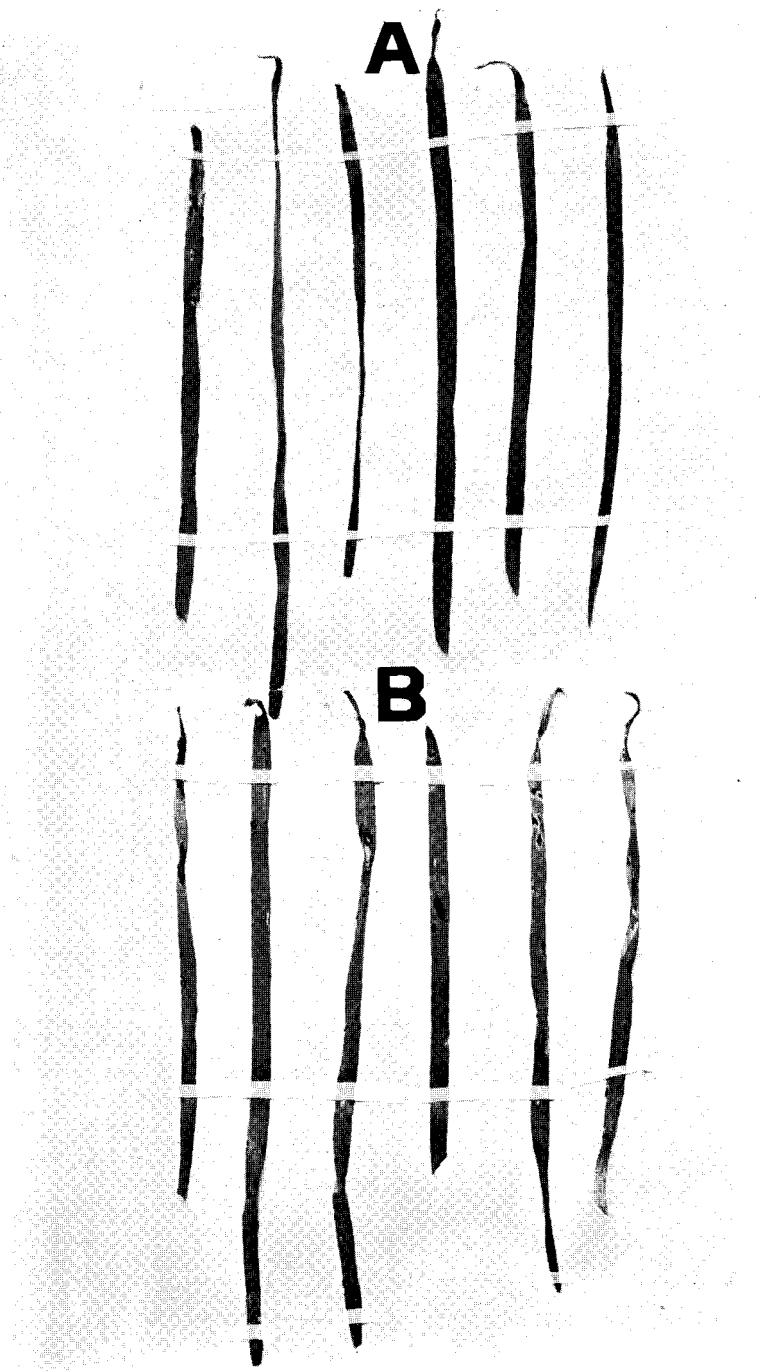

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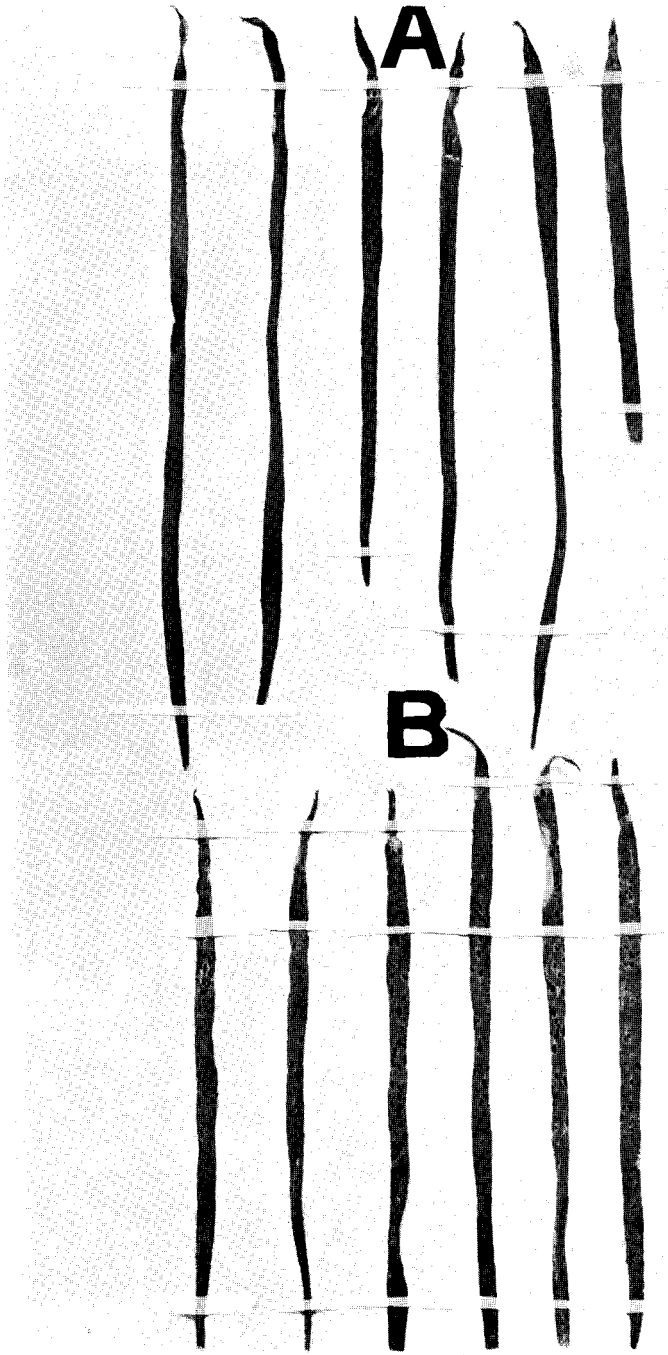

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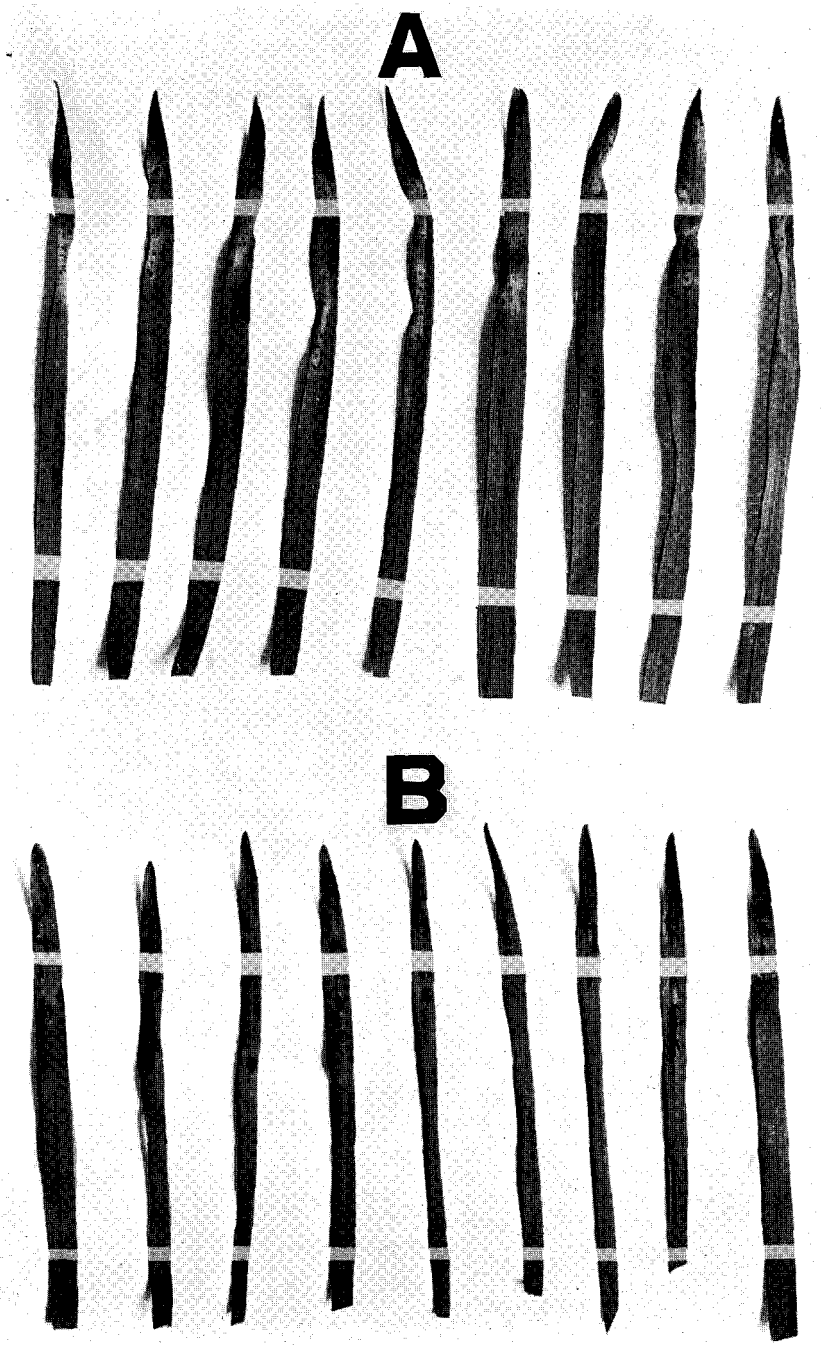

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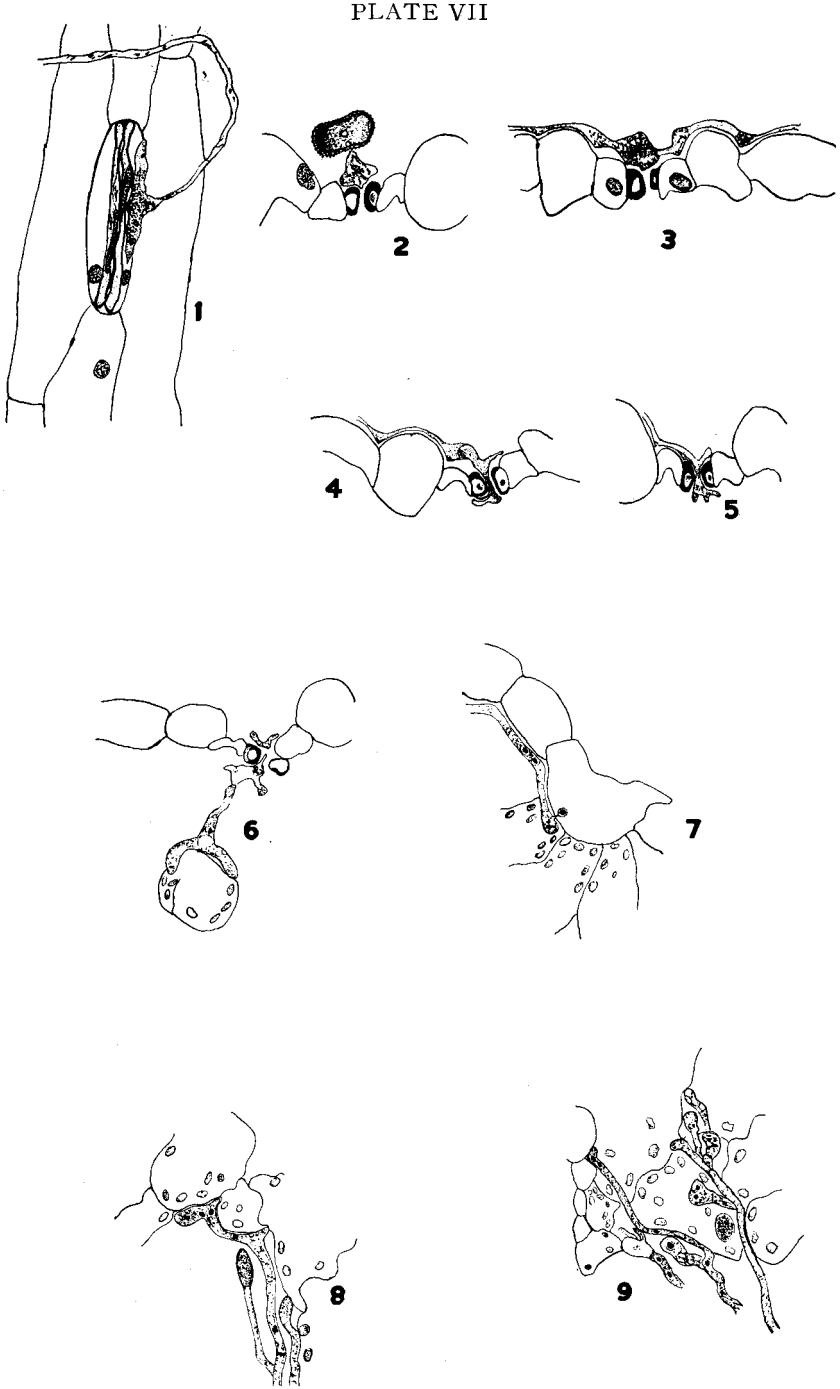

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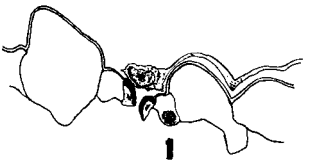
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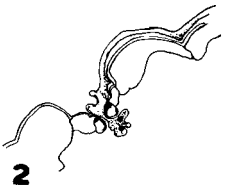
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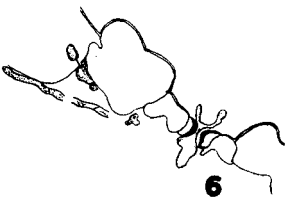
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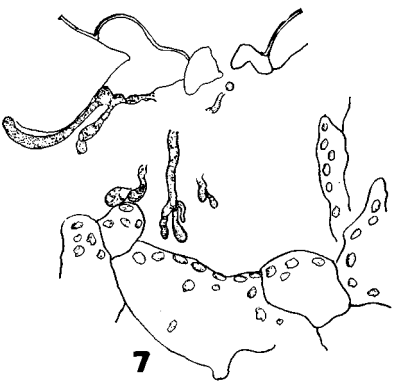
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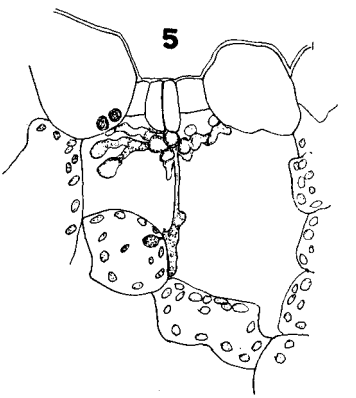
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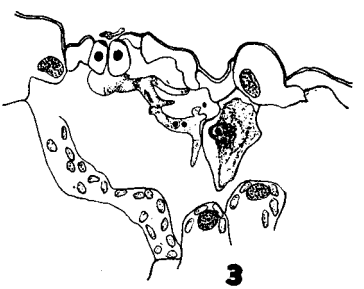
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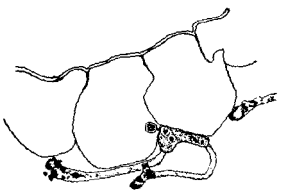
PLATE IX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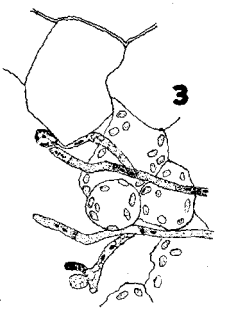
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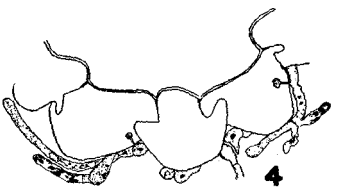
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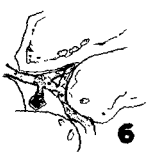
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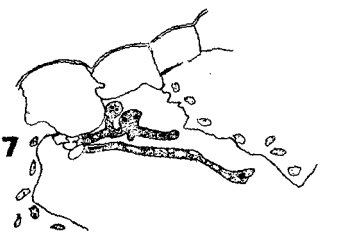
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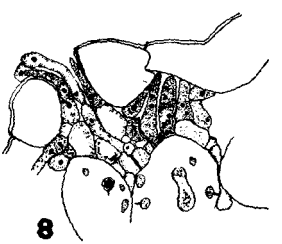
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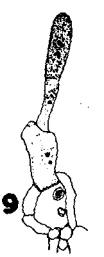
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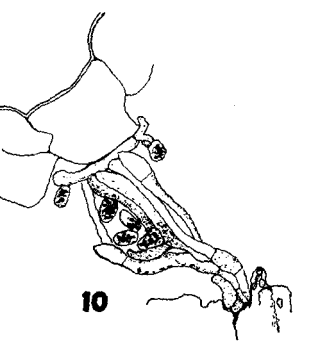
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