A BACTERIAL DISEASE OF WHEAT IN THE PUNJAB.

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A bacterial disease of wheat in the Punjab due to *Ps. tritici*, n. sp. was described by Hutchinson (1917) from Pusa. Hutchinson, however, was not fully able to prove its pathogenicity by means of inoculation experiments, because, as he mentioned, "the disease does not appear in the field until the crop is nearing maturity which in the Punjab is at too late a season of the year to make it possible to bring fresh material from thence to Pusa and there carry out the field experiments in the same year, the wheat growing season in the Bihar being several weeks earlier than in the former locality."

The disease is characterised by a curling of the stalk of the inflorescence. The head becomes covered with a bright yellowish slimy mass of bacteria. The photograph in Fig. 1 shows several such heads of wheat plants. If the atmosphere remains humid, the slimy yellow mass of bacteria may be seen trickling down but in a dry atmosphere, it becomes at first sticky and later the exposed portions become dry and hard. Milne, then Economic Botanist to the Government of the Punjab, pointed out to Hutchinson that the incidence of the disease depended upon mechanical injury to the plant caused by eel-worms and thought that without such intervention, the disease never appeared in the field. In view of the well-known intermediary action of such organisms as eel-worms and beetles in connection with other bacillary diseases of plants, Hutchinson considered Milne's suggestion to be a plausible one. Later Milne (1919) published a bulletin on "Earcockle in wheat" where he described the disease caused by the nematode, *Tylenchus scandens*, and gave drawings showing distortions in the ear caused by them. In these nematode infections, Milne found bacterial growth along with gall formations in the ear caused by the nematodes. These galls contained eggs of *Tylenchus*. Fahmy and Mikhail (1925) in Egypt found that inoculations with bacteria alone were not successful and Carne (1926) in Western Australia found that bacteria were carried into the plants by eel-worms. The author examined a large number of specimens of what he thought to be the bacterial disease of wheat as described by Hutchinson and also specimens of earcockle disease of Milne, during the last 5 years from different places in the Punjab. He also examined specimens of earcockle material collected and very kindly

sent to him by Mr. Milne. From the result of examinations of different specimens as well as from cultural studies, the author found that the two diseases were quite different and the outward symptoms, though similar to some extent, were not quite the same. In the bacterial disease proper though nematodes may sometimes be found near the growing regions, galls are never seen as the grains do not develop at all, whereas, in earcockle, a certain number of grains always developed normally while others turned into galls. These galls are not very readily distinguishable at the beginning, but later as they increase in size and turn black, they become very prominent.

That the nematodes (*Tylenchus scandens*) infect the wheat plants in the soil at the time of germination of the seeds, produce distortion of the ear and form galls, has been proved by Milne by means of inoculation experiments in the field and in many of these cases, gummy bacterial exudation appeared in later stages of infection. Hutchinson could not carry on field experiments for reasons stated already and though he was able to get slimy growths of bacteria in plants growing under bell-jars in a very humid atmosphere, he never succeeded in producing the characteristic distortions. The author carried out inoculation experiments in the field for the last three years and after two seasons of failure, he succeeded last season in infecting wheat-plants in the field with bacteria from culture and producing the typical distorted ears. The photograph in Fig. 2 shows a typical infected head produced by artificial inoculation. The photograph in Fig. 3 shows the same after removal of the sheath. Here the curling of the stalk of the inflorescence is clearly seen.

In the third week of March, 1930, the author first noticed several fields of wheat in Multan (Punjab), affected by what he thought to be a bacterial disease. A large number of diseased specimens were brought to Lahore for further studies. Pure cultures of *Ps. tritici* were obtained and their physiological characteristics studied. Though late in the season, wheat plants were grown in the laboratory, in pots and also inside large test-tubes on moist cotton wool soaked in culture solution (Knop's). These plants when 2″–4″ high were sprayed with 24 hours old suspension of bacteria. In the test-tubes, the sprayed plants showed growth of bacteria on the surface of the leaves, but not in the pots unless these were covered by bell-jars. The plants in the test-tubes, as they outgrew the tubes, were placed under bell-jars to keep them under humid conditions, for in dry air, the bacterial masses soon dried up. No further results were obtained as these plants never produced any ears and under such abnormal humid conditions, the plants did not survive long. The same sort of result was obtained from the plants growing in pots; if the experimental plants were kept covered by bell-jars

to keep them moist, these did not survive long, and when kept uncovered, the bacterial suspensions dried up soon without producing any infection. Under field conditions, however, the wheat plots being frequently irrigated, the atmosphere near the ground level is more or less in a humid condition.

As no definite results were obtained, the experiments were repeated the following season but without any further success. No field experiments could be carried on, for, by the time the diseased specimens were received and the bacteria isolated, it became almost time for harvesting the wheat in the Lahore district. As there are no culture stations in India, where type cultures could be stored during the period that the wheat is not in the field and as in this laboratory no arrangements could be made to keep on cultures during the long summer vacation, the author had to depend on fresh isolations every year. This year, however, diseased specimens were received from Multan in early March, and it being an abnormally cold year in Lahore, there were many wheat fields, where plants which had been sown late were only a few inches in height and had not produced any inflorescence. In the College Botanical Garden four such plots were found and a large number of plants were inoculated with cultures of bacteria isolated from the Multan material. For this, 24 hours old cultures on sterilised potato blocks were selected, the bacterial mass scraped with an aseptic scalpel, and suspension in distilled water of a pale-yellow colour was made. The experimental plants were sprayed with this suspension by means of a mouth-blown sprayer. The control plants were sprayed with distilled water only. After 24 hours all the plants were sprayed with distilled water for three days successively.

In another set of experiments, a suspension of the yellowish bacterial mass from the scrapings of the diseased specimens from Multan was made and a number of plants were similarly sprayed.

The sprayed plants were examined in the field every alternate day. After a fortnight, in both sets, several plants, when they developed ears, were found to have distorted and curling stalks. No grains were produced in the ears. Thus, for the first time typical distortions were produced by artificial inoculation. But as the atmospheric temperature was rather high by this time, no slimy yellowish bacterial growth on the surface was noticed. When however two such plants were uprooted and put in a beaker of water in the laboratory and were covered by tall bell-jars, they at once developed slimy, yellowish bacterial growth. But in such humid atmosphere as when covered by a bell-jar and also due to the power of these bacteria to break down protein with the production of alkaline reaction which not only favours its own growth but those of other organisms, many saprophytic organisms soon appeared and destroyed these plants. A large number of hand

sections of the distorted stalks in the inoculated plants were cut and when properly stained and mounted, most of the cells were found to be filled up by bacterial masses. Though a large number of disections of the growing tips were made to find out if there were any chance infections by nematodes, in no cases, however, nematodes were seen. This shows definitely that this disease of the wheat could be due to an infection of bacteria which when sprayed over wheat plants can cause infection and produce symptoms of the disease without the intervention of the nematodes. If, however, nematodes be present in the soil, they will no doubt help in spreading the disease.

In a real earcockle disease caused by the nematodes, *Tylenchus scandens*, which produce galls instead of grains, if a later infection of bacteria (*Ps. tritici*) occurs, distortion and also bacterial growth on the surface of the ear may take place. Nematode infection always takes place in the soil, usually at the time of germination of the seeds, but bacterial infection may be brought about at any stage of the growth of the wheat plant. The infection in the latter case may spread from one plant to another, by simply rubbing as when the wind blows. If, however, infection takes place after the ears have developed, no distortion takes place. The photograph in Fig. 4 shows a case of late bacterial infection. Here instead the stalk of the inflorescence showing distortion, the individual flower stalks are a bit elongated and, as inoculation in this particular case was done the inflorescence was coming out, the ovules were formed, but these contained no grains in most cases. Many of them were full of bacterial masses.

Hutchinson mentions that it is a disease of the badly drained areas where, "in all probability not only is cultivation and drying out of the soil imperfectly carried out, but where the higher humidity of the air during the growing season of the crop would tend to ensure the deposition of dew in sufficient quantity to promote the growth of the organism in the plant."

Though the above statement by Hutchinson is mainly true, the disease however is not confined to badly drained plots but also appears in not ill-drained soils when temperature and humidity conditions remain favourable. That is why the disease does not appear regularly in those plots.

To find out the effect of temperature and moisture on the growth of the organism under cultural conditions, cultures were incubated at different temperatures and grown under different humid conditions—sterilised potato blocks on which the organisms grew well, were inoculated and also cultures on potato-extract agar were made and incubated at 5°, 10°, 15°, 20°, 22·5°, 25°, 30° and 35° C. Best growth occurred at 20° and 22·5° C. At 25° C, the growth was not so good and at 30° C. very slow growth took place. Also

at 15° C. very little growth took place. There was absolutely no growth at 35° C. and also at the low temperatures of 10° C. and 5° C. In all those cases where no growth took place, the effect of exposure to high and low temperatures was different. After 10 days of no growth, the cultures from 5°, 10° and 35° C. were transferred to an incubator at 22·5° C. Those cultures which had been incubated at 35° C. for 10 days previously, did not show any revival of growth but the cultures which had been exposed to low temperatures previously revived and grew well. This shows that the organisms are very sensitive to high temperatures. Even at 30° C. where the organism grew very slowly, if incubated for a longer period, say for a fortnight, no sub-cultures could be obtained, the organisms having all died out at that temperature when exposed for such a length of time. The thermal death point of these organisms for a 10 minutes' exposure lies between 48° C.—50° C. Cultures exposed to low temperatures for even three weeks at 5° and 10° C. revived when placed at 22·5° C.

Effect of humidity on growth was also studied. Blocks of sterilised potatoes in watch glasses, after inoculation, were placed inside large Petri dishes containing 25 cc. of concentrated H_2SO_4; H_2SO_4 and water in the proportions 3 : 1, 2 : 2 and 1 : 3 and pure water. These were all incubated at 22·5° C.—the temperature at which the organisms were found to grow best. It was noticed after a week's incubation that in the first two sets of Petri dishes containing H_2SO_4 (concentrated) and H_2SO_4 and water (3 : 1), no growth took place. In the other two sets of acid and water mixtures fair growth took place, but best growth was obtained in the set containing pure water, showing that these organisms grow best in a saturated atmosphere and that is really found in the field.

The above observations only give a clue to the limits of constant temperature to which the cultures are exposed, for it is realised that the disease in the field is subject to varying temperatures of day and night and varying humidity due to rain and dew and besides the plant tissue may act as a protection against the bacterial mass.

Cross-inoculation experiments with a few other Graminaceous plants were tried, but these were not successful. However, it has been found that all varieties of wheat are not equally susceptible to this disease. In the inoculation experiments by means of spraying, four different types of wheat were tried, but typical infections were produced in "Lyallpur 9" variety only.

The cultural behaviour of the organism agreed mainly with those by Hutchinson and hence is not described again and his nomenclature *Ps. tritici* is accepted.

Summary.

Two similar diseases of wheat in the Punjab, *viz.*, one due to bacteria—*Ps. tritici* and another due to nematodes—*Tylenchus scandens* were described by Hutchinson (1917) and Milne (1919) respectively. Sometimes the two organisms are found together and Milne had suggested that the nematodes were the cause of the disease and that the bacteria were only secondary organisms getting in through the punctures made by the nematodes. As Hutchinson was unsuccessful in producing the disease by means of artificial inoculations, and in view of the well-known intermediary action of eel-worms and beetles, etc., in connection with other bacillary diseases, he thought Milne's suggestion, a plausible one. Workers in Egypt and Australia also failed to produce the disease by inoculating with bacteria alone. It has now been shown that though the symptoms of the two diseases are somewhat similar, really they are not the same. In a typical bacterial disease no grains are formed, more distortions are produced and the ears are covered with thick yellowish slimy masses of bacteria, whereas in a typical earcockle disease, many grains develop normally while others form galls. These galls contain eggs of *Tylenchus* and turn black when mature. Curling and distortions are not so pronounced and sometimes along with the earcockle bacterial masses may be seen.

It has now been shown for the first time by means of successful inoculation experiments, producing distortions, that bacteria alone can cause the disease and that the presence of nematodes is not necessary. The methods of infection in these two diseases also differ. In earcockle, infection always takes place in the soil, usually at the time of the germination of the seeds, whereas in the bacterial disease infection may take place at any stage of growth of the wheat plant, although late infection causes very little of distortion. It has been noticed that the disease becomes serious in an humid atmosphere with low temperature. The cultural characteristics of the organisms have been studied and have been found to be the same as described by Hutchinson and hence his nomenclature, *Ps. tritici* has been accepted.

In conclusion, the author expresses his indebtedness to Professor S. G. Paine of the Imperial College of Science and Technology, London, and to Mr. N. V. Joshi of the Imperial Institute of Agricultural Research, Pusa, for kindly reading the manuscript and suggesting improvements. He hopes to take up further investigation of the subject soon and this note represents the results of his observations and experiments done so far.

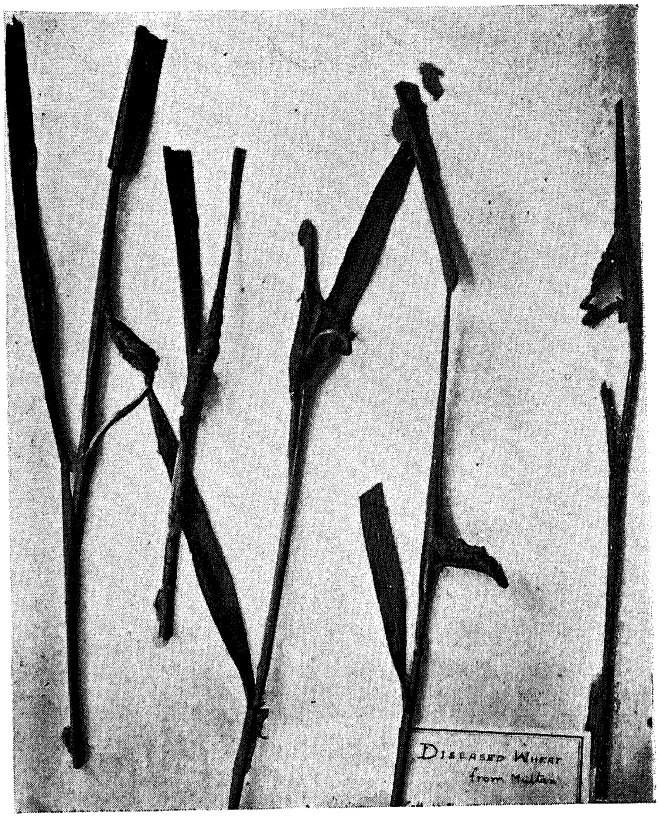

FIG. 1.

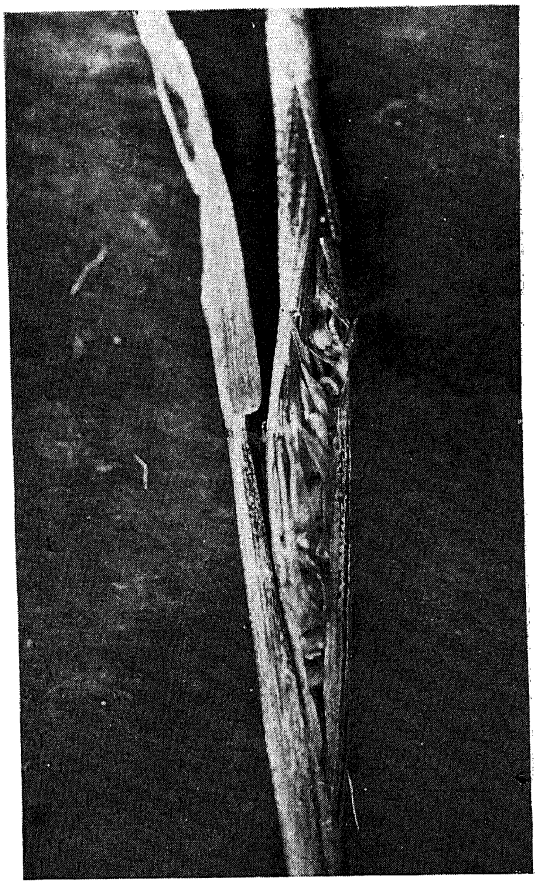

FIG. 2.

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EXPLANATION OF PLATE.

FIG. 1.—Photograph of wheat plants collected from Multan showing distorted ears due to bacterial infection. Slightly reduced.

FIG. 2.—Photograph of an inflorescence still enclosed by the sheath showing distortion produced by artificial inoculation. Slightly magnified.

FIG. 3.—Photograph of the inflorescence shown in Fig. 2. It shows characteristic distortions very clearly. Slightly magnified.

FIG. 4.—Photograph of the inflorescence artificially inoculated at a later stage of development. Note the grains are outwardly formed and very little distortion of the main stalk. Slightly magnified.