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The Development of the Stripe Rust Pathogen Puccinia glumarum in Wheat Leaves During the Incubation Period.

by M. Yu. Stepanova

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The morphological forms of the development of stripe rust in leaf tissues were investigated on artificially inoculated wheat leaves in different light conditions - in natural light and in conditions of darkness.

A variety of winter wheat DS-7083, which is susceptible to stripe rust, was used for the artificial inoculation. The inoculation was made with uredospores of P. glumarum from Leningrad Oblast (city - Pavlovsk). The experiments were conducted in 1955 in hothouse conditions in the city of Pushkin in April, October and November.

In the spring an artificial inoculation of the leaves with uredospores of P. glumarum was made twice because in the first inoculation of 21 April the infected condition of the leaves in the control amounted to a total of only 7.5% and to trace the development of the fungus in the leaf tissues was difficult. In the repeat inoculation of 30 April we received a higher percentage of leaf infection (80%).

A 100-% infection was received with the inoculation in October and it was possible to observe a clear picture of the penetration and development of the fungus P. glumarum in the tissues of the leaves.

The experiments with artificial inoculation under conditions of etiolation were conducted only at the late period, in November. They were conducted repeatedly because of a weak infection of the plants in the control (3-5%). A total of 100 plants were inoculated and 1,000 sections examined.

Method: The seed were preliminarily soaked in water for 24 hours. The number of plants in the pots was 5. The inoculation of the leaves with the uredospores was made in the sprout phase, on the 5th-8th day, at a temperature of 16-18°C. The tip of the leaf was inoculated 5 mm from the edge. After that the inoculated plants were placed in a moisture chamber for 24 hours. The viability of the uredospores was checked simultaneously with the inoculation. Within 24 hours after the inoculation we made longitudinal sections of a piece of the leaf, through the area of inoculation, by means of a freezing microtome. The size of the section was 5μ. The sections were examined by microscope with an enlargement of 1,250.

In the repeated germination of the uredospores we observed a difference in their ability to germinate. In identical conditions of temperature, moisture and light the uredospores sometimes germinated weakly or not at all. In experiments with inoculations, however, we noted that the spores from a series that do not germinate in water were able to cause a 100-% infection of plants. Therefore we have the right to believe that the viability of uredospores does not constitute a criterion for the outcome of experiments with artificial inoculation because their virulence can be much higher than the percentage of germination. Evidently, favorable conditions for their germination, which are still unknown, are created on the leaf (possibly the plant's emission of oxygen, which is necessary to the fungus, etc.)

Eriksson (1901), A. A. Yachevskiy (1909), and N. A. Naumov (1939), in their investigations, also refer to the "capricious", "sluggish" germination of P. glumarum uredospores. Allen (1955), in her experiments on the germination of rust-fungi uredospores, observed large fluctuations in their percentage of germination. She explains this phenomenon by an excretion by the uredospores, upon germination, of special substances - inhibitors, which suppress the germination of the spores. The inhibitors develop in insignificant quantities, but the more spores included in the experiments the more inhibitors are released. The inhibitors can develop both in the spores of fungi and in the seeds, and in the pollen grains of some plants.

The Development of P. glumarum in Conditions of Natural Light.

We present the results of our investigations below.

Within 1-2 days after the inoculation there are found on the surface of the sections, uredospores with brightly colored contents of a yellow color. Some of them had germinated, forming a growth mycelium; the latter usually penetrate through the stoma and forms beneath it a substomatal "sac". A similar occurrence is described in the works of Lindfors (1924) and Allen (1928). Some of the uredospores, when germinating, sometimes release their content outside (fig 1).

Both in the growth hypha and in the substomatal, the content is comprised of protoplasm that contains yellow or colorless inclusions (see figs. 2,3 and 5).

The infection's penetration occurs only through the stoma, as we repeatedly observed complete withering of the germinated uredospores' growth hyphae if the latter did not reach the stoma.

E. Goyman (1954) writes that the germ tubes of uredospores of P. coronata at first, evidently, wander along the surface of the leaf "aimlessly". Having found themselves near a stoma, the contents of the tip of the growth hypha enter into it. On the third day we noted in the tissues of the upper epidermis, chiefly near the stoma, the presence of short hyphae of the fungus, of various form and sizes, spread along the interstices of the tissue. They are colorless and have a scarcely noticeable membrane, sometimes binucleate. This form of hyphae was detected with a magnification of 1250 (Reichert microscope). The given magnification made it possible for us to detect the fungus at this period of its development. In one of the sections beneath the stoma we noted a mycelium in the form of a short hypha, which consists of round swellings of various form joined together. There was a plasmatic content in each of these segments and a pair of nuclei in one of them (fig 4).

In the period from 1-3 days after inoculation the mycelium developed very slowly, localising primarily around the stoma, and its deeper spread into the leaf tissue is extremely slow. The structure of the cells and chloroplasts is not disrupted. Lindfors (1924) notes that as early as the 3rd day after infection the fungus's plasma has a very thin membrane and a large quanitity of nuclei in the cells. We were unable to observe a profusion of nuclei neither in the short hyphae nor in the well developed mycelium. The number of nuclei did not exceed 2-4 in our investigations (staining according to Giemsa). However, the author mentioned above indicates that at a certain moment of the fungus's development the nuclei are poorly detected and prior to the formation of uredospores they are reduced to two in each cell in the mycelium.

Within 6-8 days we noted an insignificant chlorosis at the inoculation points on the leaves and a disruption of the cellular structure in the microscopic sections; the chloroplasts were light green and of a fine granular structure. We also noted a profuse mycelium in the interstices of the leaf's parenchyma. The mycelium was densely filled with a colorless plasmatic content. The mycelium sometimes carries 1-2 nuclei in its growing part (staining according to Giemsa, see fig 6).

A more intense growth of the rust occurs in the period of 6-8 days. At this time there is noted a profusion of mycelia in all the leaf's tissue layers along the interstices. Now and then short hyphae are encountered here.

At 10-12 days after inoculation we noted a strong chlorosis in the inoculation areas of the leaves and a disruption of the cellular structure with a destruction of the chloroplasts. The chloroplasts were small and of a yellowish brown color. Their distribution in the cells was without order. In this period of the fungus's development we usually note a contraction of the mycelium towards the stomata and an accumulation of the mycelium in the form of a "tangle", which indicates the initial formation of pustules (fig 7). The mycelium in the deeper layers of the leaf's parenchyma is reduced, forming fine threads that are barely noticeable against the background of the cellular walls (fig 10). In some microscopic sections we note the presence of septa and pairs of nuclei in the growing part of the mycelium (staining according to Giemsa, see fig. 8).

We noted the formation of pustules and the presence of chlorotic patches below the inoculation area on the 14th-15th day. In this period, in the areas of pustule formation the cellular structure was completely disrupted and only isolated cells retained their normal form. Chloroplasts are absent in the areas of pustule formation. The uredia are profuse; some of them break through the upper epidermis of the leaf. The formation of the uredia is observed primarily around the stomata. Prior to the formation of the pustules the mycelium is usually binucleate and forms the greatest number of septa (see fig 9). Similar findings are noted in the works of Kleban (1904), Lindfors (1924) and Allen (1928).

Working with a fixed material and with the use of dyes (hemotoxylin, gentian-violet, safranine), Allen detected a large number of nuclei in a mycelium: from 2-4 up to 40. Working with live material and using the

Giemsa staining method (nuclear), we at no time noted such a large quantity of nuclei, and only in rare instances did we detect one, two-four nuclei and then principally in the growing part of the mycelium, during the period of its more intense growth and development and later, before the start of spore formation. Farther in his work, Allen mentions the presence of haustoria; we noted no typical haustoria although we investigated a colossal amount of microscopic sections both on material of P. glumarum and on material of other rust fungi (P. triticina, P. graminis).

The Development of P. glumarum Under Conditions of Etiolation.

Method: After the planting of the seeds in pots they were placed into a darkened chamber and grown. The artificial inoculation of the etiolated leaves with uredospores was conducted in the sprout stages, on the 4th-5th day.

After inoculation the plants were placed in a moisture chamber for 24 hours. The control plants, which were inoculated the same, were grown in light.

The leaves were inoculated twice because of a very weak percentage of infection (2-5% in the control). Therefore it was difficult to follow the penetration of the infection into the tissue of the etiolated leaf. Microscopic sections of the artificially inoculated leaves and the control were examined daily from the moment of inoculation and until the end of the incubation period. A total of 300 microscopic sections were examined.

The results of our investigations are presented below.

Within 48 hours we noted in the etiolated plants a disruption of the cellular structure, an absence of chloroplasts and a presence of finely grained inclusions of a weak yellow color in the cells. In single sections we noted the presence of the fungus around the stoma in a form of short hyphae with a colorless content and a very thin membrane.

In subsequent examinations after 4-5-8-10-12-14-16 days we encountered no signs whatsoever of the fungus, whereas on the control we noted the development of small single pustules in the inoculation areas on the 12th day.

After the 16th day of etiolation the inoculated plants were brought out into the light. After a few days single pustules appeared on these plants.

Conclusions

1. It is confirmed that the penetration of the spore infection P. glumarum into the leaf occurs only through the stomata.

2. Within a day or two after inoculation the fungus penetrates into the tissue of the upper epidermis, forming a substomatal "sac", or growth hypha, which is sometimes binucleate.

3. The first four days the fungus develops very slowly in the leaf tissues in the form of short hyphae and localizes primarily around the stomata in the cells of the upper epidermis.

4. On the 6th-8th day there occurs a more intense growth of the rust and a spread along the entire tissue of the leaf. Within 11-14 days the formation of pustules is noted (at 16-18°C). In the places where pustulation occurs, there is a disruption of the leaf's cellular structure and complete destruction of the chloroplasts.

5. The thin walled, slightly septate mycelium of the rust fungus is easily discernable in its growing part in the plant tissue around the stomata prior to and at the moment of sporulation. The remaining portion is deprived of its contents and reduced to thin colorless hyphae that are weakly discernable in the tissue of the parenchyma.

6. Under conditions of etiolation also the inoculation of the leaf occurs, but the fungus, when penetrating into the tissue, forms only the short hyphae of the mycelium and on this the development evidently ceases. With a transfer of the etiolated plants into conditions of natural light, however, the development of the rust is resumed and the fungus is able to sporulate.

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