

Notes on the Pathological Anatomy of Rice Grains Affected by *Helminthosporium Oryzae*.

By

Y. Nisikado and T. Nakayama.⁽²⁰⁰⁾

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I. Introduction.

The rice grains affected by *Helminthosporium oryzae* Breda de Haan (*Ophiobolus Miyabeanus* Ito et Kuribayashi) not only reduces the market value by their discoloured hulls and kernels, but it is also the most important cause of the primary outbreak of the Sesame Leaf Spot or Gomahagare disease when sown in the spring. As the seed disinfection being the most effective means in combatting the primary outbreak of the disease, numerous studies have been reported on this subject. The present study attempts to contribute toward the understanding of the condition and extent to which the causal fungus remain harbored in the rice grain from microscopic point of view. A particular reference is also made on the mode of entrance of the fungus into the seed grain. It is the hope of this study that these observations will not only add to the already numerous knowledge on the Gomahagare disease, but also help toward understanding more exactly on the nature of the present seed borne disease when seed disinfection is being attempted as a control measure.

II. Method of Study.

The fungus employed in the inoculation had a very profuse production of conidia when cultured in rice straw decoction agar medium—rice straw 100 g., cane sugar 20 g., agar 17 - 20 g., water 1000 cc. The rice plants used in the observation were cultivated under ordinary practice in the field; when approximately ten days prior to the emergence of the head, they were carefully transferred with the surrounding soil to the galvanized iron pots for inoculation. Each head of rice was covered with a sterilized paper bag before it emerged from the sheath, and at the time of inoculation by atomizing the suspension of fungus spores produced on the agar medium, the heads on the average were seven days after the blooming. The inoculated plants were placed in a high humidity chamber which was constructed as a small glass house.

The samples of grains were taken for fixation with the solution consisting of 50% alcohol 100 cc., formalin 6.5 cc. and acetic acid 2.5 cc. (RAULINS, 1933) at the intervals of 12, 24, 48 and 72 hours. Since the hulls were highly impregnated with silicious material, all samples containing hulls were treated with a dilute solution of hydrofluoric acid for several days. After a thorough washing in running water, and when all traces of hydrofluoric acid had been washed out, usual method was followed in the dehydration and imbedding in paraffin, and as a differentiating stain the Pianeze method as described by VAUGHAN (1914) was followed primarily. Sections of rice kernels were made by imbedding in celloidin, and were stained with the Thionin-orange G of STOUGHTON (1930) or fuchsin alone. Both the outer and the inner glumes were observed, but on account of their similar anatomy and response to the pathogen, the two were not distinguished in the present report.

III. Observations on the Glumes.

As the inoculated fungus spores germinate on the surface of the rice hull, the mycelium advances mainly along the depressed portion of the epidermis. The penetration into the host tissue occurred at first when the mycelium reached the base of the small hair which was in contact and surrounded by four epidermal cells as shown in Fig. 1. The hyphae as it attempts to push its way downward at the

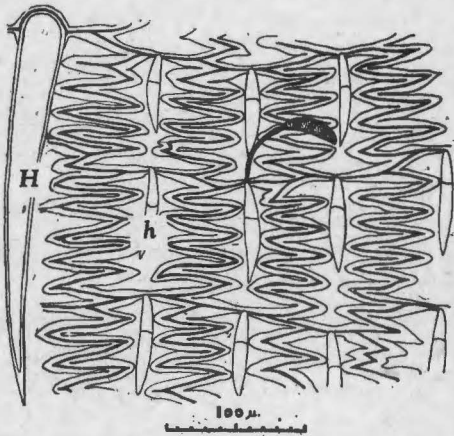


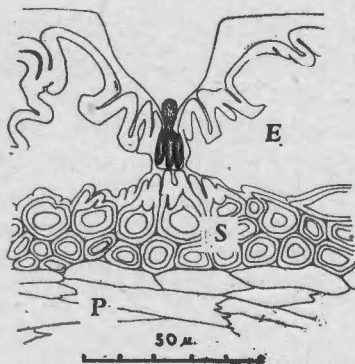
Fig. 1. General surface structure of the hull, showing the germinated spore of *Helminthosporium oryzae* invading the basal portion of the small hair. H, large hair; h, small hair.

depressed portion formed between the hair and the epidermal cell, breaks through the wall of the hair. The hyphae grows and finally occupies the lumen of the hair cell. (Fig. 2).

In most cases, the mycelium that gained entrance into the hair and occupied the lumen of the cell remains in this state for the rest of the period more or less in dormant condition. The diseased tissue in this condition apparently does not show any reaction toward the fungus; consequently there is no detectable lesion when observed by the naked eyes. This state of the disease has been noticed by the writers during the course of the experiments on the disinfection of rice grain affected by this fungus.

To further clarify this point a supplementary experiment was conducted by planting apparently normal seeds that were free from lesions when observed under the binocular microscope. The seeds were of the same lot as the ones used in the microscopic observation. Of the 50 seeds that were sown in the sterilized soil in the germinating dishes, 47 germinated and produced 29 diseased plants which is 61.7 per cent of the germinated group.

Fig. 2. Cross section of a hull showing the condition of the invaded hyphae of *Helminthosporium oryzae* in the basal portion of the small hair surrounded by epidermis E, sclerenchyma S, and parenchyma P.



The hyphae in the hair under certain conditions make further progress into the host tissues by invading into the epidermal cells situated laterally as shown in Fig. 3. The epidermal cells immediately react by turning brownish in the cellulose cell walls and the cytoplasm.

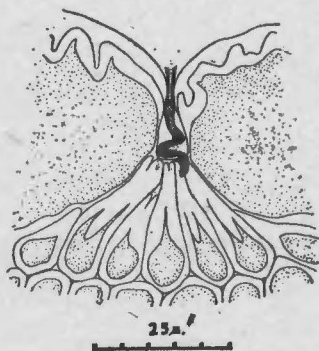


Fig. 3. Cross section of a hull showing the invasion of the laterally arranged epidermal cells by the hyphae of *Helminthosporium oryzae* from the base of the small hair.

As the fungus progresses in the epidermis the area of the discoloration increases and soon extends not only into the neighboring epidermal cells as shown in Fig. 4 but also into the sclerenchymatic and parenchymatic cells directly underneath. The cell contents are definitely altered to colloidal state and give the intense staining reaction with many dyes. The lateral movement into other epidermal cells takes place by piercing through the thinner portion of the walls or through the protoplasmic connections between them.

The invasion of the sclerenchyma seems to occur mainly through the protoplasmic connections from the epidermis. The sclerenchyma walls were not only colored brown, but also under certain cases the primary cell wall separated from the secondary cell walls apparently by the secreted toxic substance of the fungus.

The affected cytoplasm also gave a deep staining similar to the case observed in the epidermal cells. As the cell walls are very thick, the advance of the fungus mycelium in sclerenchyma seemed to be through the numerous protoplasmic connections rather than by the direct penetration of the cell wall.

When the fungus had reached the sclerenchyma tissue there remains but thin walled parenchyma tissue before reaching the kernel itself. The fungus readily permeated the parenchyma tissue which is 2 or 3 cells in thickness as they are crushed to some extent by the pressure of the kernel exerted on the glumes.

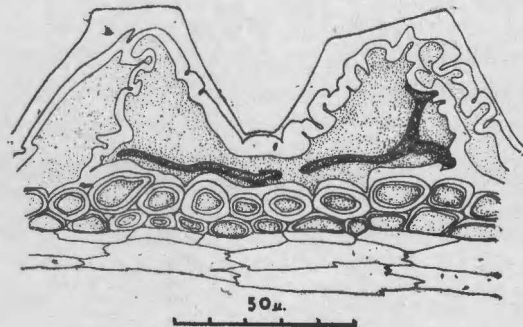
When the disease spot is less than 1 mm. in diameter, the fungus mycelium is invariably confined to only one or two of the epidermal cells. This fact was disclosed after sectioning that the reaction of the host cells is highly sensitive and thus the appearance of the discolored area extends to some distance beyond the point where the fungus mycelium actually rests. The disease spot appears after about two days after inoculation, but at this stage the mycelium is largely limited to the basal portion of the small hair and extending but little into the neighboring epidermis. At the end of four days, most of the mycelium is in the epidermis but it is beginning to affect the other epidermal cells and sclerenchyma tissue. It appeared that after some seven days that the mycelium is able to extend beyond the parenchyma into the contacting grain proper.

IV. Observations on the Kernel.

The fungus advances into the kernel proper after penetrating the various tissues composing the hull. The kernel has in its outermost a layer of crushed or compressed cells which are seed coat and pericarp. These tissues are highly suberized or cutinized as made evident by the reddish staining of Sudan III. Inside of this, there is the aleurone layer of usually one cell in thickness. Its cell walls are rather thick and contains besides the cellular content of starch grains, granular protein and fat bodies. The starch cells are large and thin walled, colorless, and contain masses of minute starch granules.

Sections were made from the kernels that showed discoloration. These kernels all showed severe lesions and were from the same lot as the grains used in

Fig. 4. Cross section of a hull showing the epidermal cells containing the hyphae of *Helminthosporium oryzae*.



the previous observations. The sections of the whole grain were made possible by the use of the celloidin method.

The results of observations of several such kernels disclosed the presence of the fungus in the outermost layer of the grain which consisted of seed coat and pericarp as shown by the discoloration of the cell walls. However, in the immediately neighboring aleurone layer, there was no sign of abnormality; consequently, the starch cells or endosperm were unaffected. This is shown in Fig. 5.

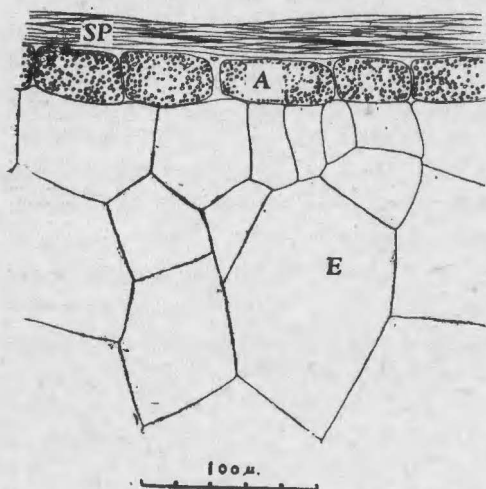


Fig. 5. Portion of a cross section of rice kernel affected by *Helminthosporium oryzae* showing the presence of the mycelium in the seed coat and pericarp SP, but not in the aleurone layer A and the endosperm E.

SUZUKI (1928) reported that fungi, without mentioning specifically, occur in the interior of the rice grain; but from the results of the present observations, it seems that the penetration of the fungus *Helminthosporium oryzae* into the rice kernel proper is confined, in the main, under the normal condition of relatively low moisture content and very hard nature of the kernel when they attain the certain stage of development, to the outermost layer. Should the fungus have reached the kernel that was still very young or if the very wet weather condition continued for days, the kernels would be in the condition of being almost water soaked, and under these special conditions the fungus could be assumed to have invaded the interior of the kernel.

To further ascertain the writers' results of observations, following isolation tests were made with the diseased kernels. 23 kernels that showed similar degree of discoloration as the ones used in making the sections were selected, and each kernel was cut into halves. With the one halves, the discolored seed coat and pericarp layers were scraped off with a sharp dissecting knife; while with the other halves no treatment was made and retained as the control for each kernel. Both halves were then surface sterilized in each of the 45% alcohol and 1:1000 mercuric chloride solution for 30 seconds. The two groups of halved kernels were carefully washed in sterile water separately and were placed on the malt extract agar medium poured in Petri dishes. Observations were made on the appearance of the fungus for seven days. From this experiment, of the 23 kernels used, 11 in

the control halves showed the fungus growth, while none appeared from the halves that had the seed coat and pericarp removed.

This test further supports the fact that the fungus, if ever present in the rice kernel, it invariably confined to the surface seed coat and pericarp and rarely does it extends into the endosperm.

V. Summary.

1. *Helminthosporium oryzae* first infects the base of the small hair which is surrounded by four epidermal cells. The invaded hyphae remain in the lumen of the hair in dormant condition in many instances, thus giving no apparent indication of the occurrence of the disease.

2. The hyphae spread from the hair by penetrating the wall into the surrounding epidermal cells; and the lateral invasion into other epidermal cells occurs by the penetration of the walls or passing through the protoplasmic connections. Into the sclerenchyma cells directly beneath the epidermis and further into the innermost parenchyma tissue of the hull, protoplasmic connections afford a ready means in the fungus' progress.

3. The cells of the hulls are highly sensitive to the fungus and thus, the discolored lesions are observed to extend quite a distance beyond the advancing hyphae. The fungus was apparently able to invade the kernel proper only after about one week.

4. From the results of observations of the sectioned kernels and from the results of actual isolation of the fungus, the mycelium in the kernel is invariably limited to the thin layer composed of seed coat and pericarp.

V. References.

- (1) NISIKADO, Y. and T. NAKAYAMA, The disinfection of rice seeds affected by *Helminthosporium oryzae*, with special reference to the hot water treatments. Ber. Ohara Inst. landw. Forsch., 9: 214 - 228, 1943.
- (2) RAULINS, T. E., Phytopathological and botanical research methods. 156 pp., New York and London, 1933.
- (3) SANTOS, J. K., Morphology of the flower and mature grain of Philippine rice. Philip. Jour. Sci., 52: 475 - 503, 1933.
- (4) STOUGHTON, R. H., Thionin and orange G for the differential staining of bacteria and fungi in plant tissues. Ann. Appl. Biol., 17: 162 - 164, 1930.
- (5) SUZUKI, H., Experimental studies on the possibility of primary infestation of *Piricularia oryzae* and *Ophiobolus Miyabeanus* internal of rice seeds. (In Japanese). Ann. Phytopath. Soc. Japan, 2: 245 - 275, 1930.
- (6) VAUGHAN, R. E., A method for the differential staining of fungous and host cells. Ann. Mo. Bot. Gard., 1: 241 - 242, 1914.
- (7) YOSHII, H., On the mode of infection of *Helminthosporium oryzae* (*Ophiobolus Miyabeanus* ITO et KURIB.) to rice plant. (In Japanese). Ann. Phtopath. Soc. Japan, 9: 170 - 172, 1939.