HISTOLOGICAL STUDIES OF RICE LEAVES INFECTED WITH HELMINTHOSPORIUM ORYZAE [1]

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INTRODUCTION

The fungus causing helminthosporium leaf spot of rice (*Oryza sativa* L.) was named *Helminthosporium oryzae* by Brede de Haan (*1*)[2] in 1900. According to Nisikado and Miyake (*6, p. 134*): "The first authentic report of the helminthosporiose of rice plant is that of S. Hori [(*4*)], who in 1892 found the disease on the glumes of rice in the suburbs of Tokyo." Of subsequent contributions to the knowledge of this disease and the causal fungus the Japanese have made by far the greatest number.

In 1924 Ocfemia (*7, 8*) reported on the occurrence of the helminthosporium disease of rice in the southern part of the United States and the Philippine Islands and investigated the relation of soil temperature to germination of certain rice varieties and to infection by *Helminthosporium oryzae*. In 1927 Ito and Kuribayashi (*5*) found the ascigerous stage of the fungus in culture and described it as *Ophiobolus miyabeanus*, but they did not find it under natural conditions. In 1928 the writer found on naturally infected rice plants in the vicinity of Crowley, La., an ascomycetous fungus that apparently is morphologically identical with the ascigerous stage of *O. miyabeanus* as described by Ito and Kuribayashi. However, these ascospores, grown on artificial culture media, have not as yet produced *H. oryzae*, nor has *H. oryzae* produced the ascigerous stage in culture for the writer.

Except for minor differences, the manifestations of the disease as it occurs in the United States seem to be the same as elsewhere in the world. The lesions produced by the fungus are usually narrowly elliptical spots with grayish centers and brown margins. Various gradations are found from narrowly elliptical lesions in some varieties to circular spots in others. Typical leaf spots are shown in figure 1.

The fungus has a number of hosts. Suematsu and Okapa (*9*) reported 39 members of the grass family and Nisikado and Miyake (*6*) 25, some of which are the same as those listed by Suematsu and Okapa.

Many ways to control the disease have been suggested, but the most satisfactory, it seems, is the development of resistant varieties. In Japan several varieties have been reported as very resistant to the disease, and the writer has noted a wide range in the resistance of commercial varieties now grown in the lower Mississippi Valley. In the United States commercial varieties have been crossed with resistant varieties from other countries. It is hoped that resistant strains of commercial value may be isolated from these crosses.

[1] Received for publication Oct. 1, 1934; issued April 1935. Investigations conducted in cooperation with the Arkansas, Louisiana, and Texas Agricultural Experiment Stations.

[2] Reference is made by number (italic) to Literature Cited, p. 90.

The purpose of the present study was to determine the nature of resistance and susceptibility to helminthosporium leaf spot in rice varieties.

MATERIAL AND METHODS

Leaves of 12 varieties of rice infected naturally in the field were used in this study. These were the short-grain varieties Kameji, Butte, Bozu, and Aikoku; the medium-grain varieties Blue Rose, Early Prolific, and Shoemed; and the long-grain varieties Storm Proof, Honduras, Fortuna, Lady Wright, and a selection from a Patna rice, J–131. Of these, Kameji, Aikoku, Butte, and Shoemed show considerable resistance and Fortuna some resistance to the helminthosporium leaf spot. The other varieties named are susceptible.

The material, with the exception of J–131, was killed and fixed in the field with strong chromo-acetic fixative.[3] The specimens were allowed to remain in the killing solution from 24 to 36 hours before being washed. As soon as the killing solution was washed out, the specimens were treated for 20 to 30 minutes with concentrated hydrofluoric acid to remove the silicon from the epidermis, and again washed.

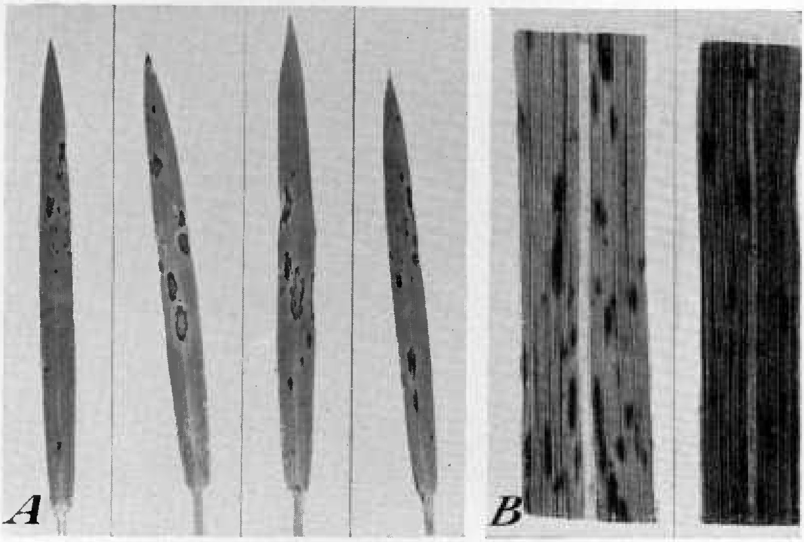

FIGURE 1.—Leaves of Blue Rose rice showing lesions caused by *Helminthosporium oryzae*: *A*, From artificial inoculations in greenhouse. × 0.85. *B*, From natural infections, Beaumont, Tex. × 1¼.

The specimens were then dehydrated in ethyl alcohol up to 70 percent, after which a modification of Zirkle's (*10*) butyl alcohol-ethyl alcohol series was used as follows: From 70-percent ethyl alcohol the specimens were put into a 25-percent solution of *n*-butyl alcohol in 95-percent ethyl alcohol. After 2 hours this was drained off and a solution of 50-percent *n*-butyl alcohol in 95-percent ethyl alcohol was added, and 2 hours later 75-percent *n*-butyl in 95-percent ethyl alcohol. The specimens were allowed to remain in this solution overnight and were then changed to *n*-butyl alcohol. The remainder

[3] Chromic acid 1 g, acetic acid 1 g, water 100 cc.

of the schedule is the same as suggested by Zirkle (*10*). The specimens were then embedded and cut. The cross sections were cut 3μ or 6μ and the longitudinal and tangential [4] sections were cut 10μ. Sectioned material was stained in iron-alum haematoxylin and methylene blue. The former stain also was used for portions of leaves stained in toto.

ANATOMY OF HEALTHY LEAVES

The salient features of the anatomy of a rice leaf are shown diagrammatically in figure 2. The parenchyma consists of the armed-type cells referred to by Haberlandt (*3, pp. 277–278*) in *Bambusa*, *Arundinaria*, *Elymus*, *Calamagrostis*, and *Alistromeria*. This same type of

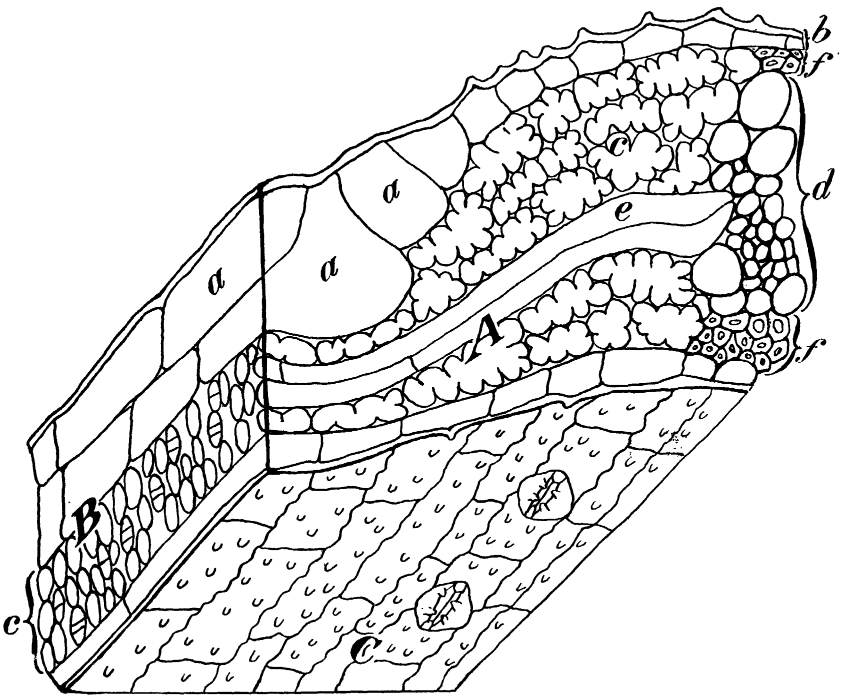

FIGURE 2.—Diagram of a portion of a rice leaf showing (*A*) cross and (*B*) longitudinal section and (*C*) surface view of lower epidermis: *a*, Motor cells; *b*, upper epidermis with papillae; *c*, armed-type parenchyma cells; *d*, fibro-vascular bundle; *e*, transverse bundle; *f*, sclerenchyma cap. × 560.

parenchyma was also found by the writer in *Arundinaria gigantea* (Walt.) Chapm. As shown in figure 2, these parenchyma cells in most cases lie with their long axes crosswise of the leaf.

In cross sections of the leaf the armed-type parenchyma cells are, in general, irregularly rectangular in outline with invaginations at the periphery. In this view their short axes range from 7μ to 15μ and their long axes from 15μ to 40μ (figs. 2, 3, *A*, *B*).

In longitudinal sections of the leaf the armed-type parenchyma cells are rectangular to elliptical in outline, depending on the portion of the cell cut and the location of adjacent cells. In this view their

[4] Longitudinal section, as here used, denotes a section cut through the leaf at right angles to its surface and parallel to its long axis; tangential section, one cut parallel to its surface.

short axes range from 4μ to 10μ and their long axes from 8μ to 20μ. In cases where the cells are cut near the periphery and through the

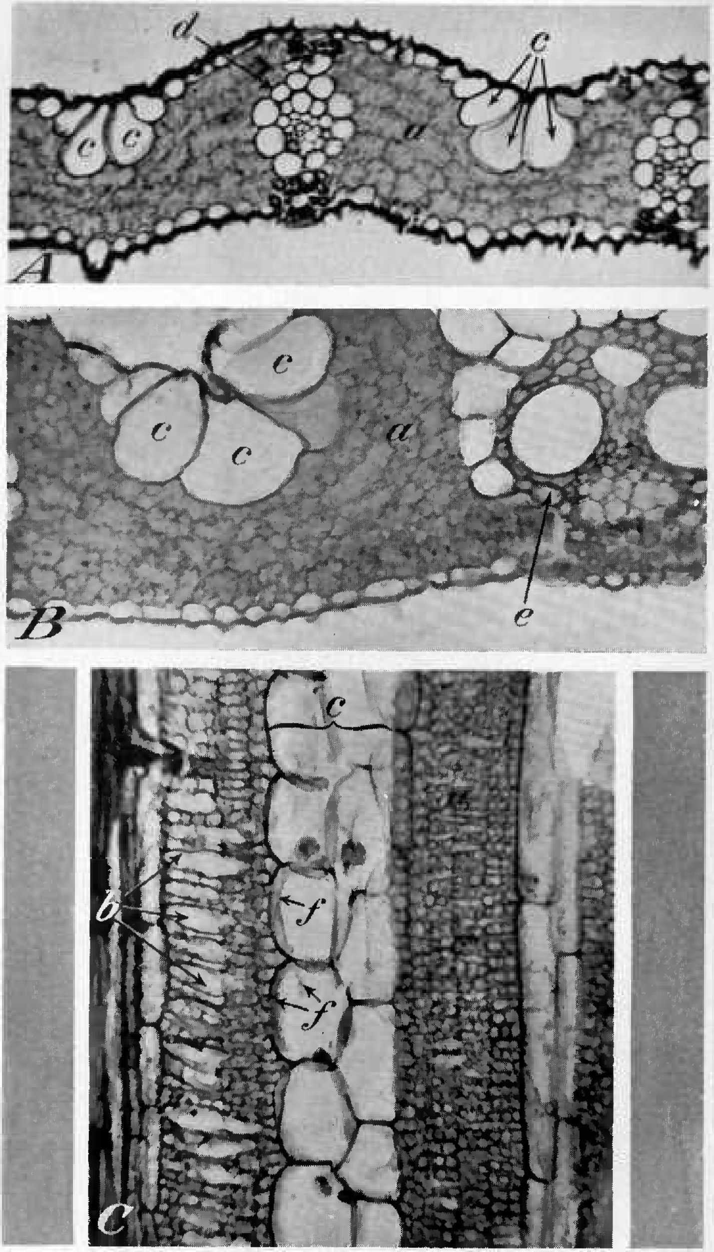

FIGURE 3.—Cross section of portion of leaf of (*A*) Shoemed and (*B*) Blue Rose rice, and (*C*) tangential section of portion of leaf of Blue Rose rice: *a*, Armed-type parenchyma cells; *b*, intercellular spaces; *c*, motor cells; *d*, small bundle; *e*, large bundle; *f*, hyphae of *Helminthosporium oryzae*. All × 288.

invaginations there appear to be numerous small cells. Examination of cross sections, however, shows that what appear to be cross walls of small cells (fig. 3, *C*, *a*) are the invaginations of relatively large cells shown in cross sections in figure 3, *A*, *B*.

In cross sections of the leaves, as shown in figure 3, *A*, *B*, there are no conspicuous intercellular spaces. Only small openings occur

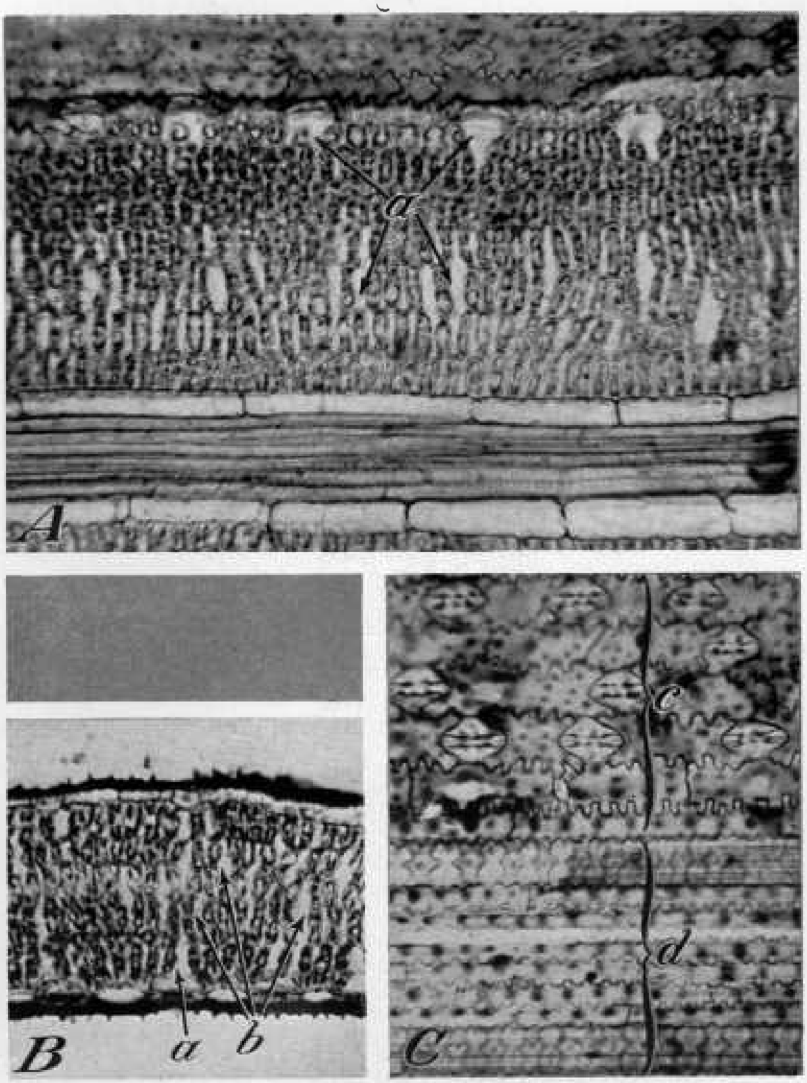

FIGURE 4.—Tangential (*A*) and longitudinal (*B*) sections of portions of leaves of Shoemed and Blue Rose rice, respectively, showing substomatal chambers (*a*) and intercellular spaces (*b*); and surface view (*C*) of portion of leaf of Shoemed rice showing lower epidermis covering parenchyma (*c*) and a large bundle (*d*). All × 320.

opposite the invaginations. There are, however, rather large substomatal chambers which are more evident in longitudinal and tangential sections (fig. 4, *A*).

In longitudinal and tangential sections intercellular spaces are abundant (fig. 3, *C, f*). Frequently they extend from the upper to the lower epidermis (fig. 4, *B*) and occur in bands on each side of the bundles.

Considerable difference was found in the size of the intercellular spaces of various rice varieties when examined in longitudinal sections. In Aikoku the intercellular spaces are small and relatively infrequent. In Kameji, Bozu, and Butte they are somewhat larger and occur more often. In Shoemed, Blue Rose, Early Prolific, Storm Proof, and J–131 they are still larger, and the largest were found in Fortuna and Honduras.

The stomata occur in parallel rows on the upper and lower surfaces of the leaf. The rows of stomata alternate with the bundles and motor cells on the upper surface. The bands of stomata on the lower surface (fig. 4, *C, d*) are opposite those on the upper surface.

The substomatal chambers are relatively large and connect with the intercellular spaces just referred to and, together with the stomata, constitute the system for the exchange of gases in the photosynthetic area of the leaf.

Most of the epidermal cells and the guard cells of the stomata are studded with papillae as shown in figures 2; 3, *A* and *B*; 4, *A* to *C*; 5, *A* and *E*; and 6, *A* to *C*. These papillae differ considerably in size, shape, and distribution as shown in the illustrations. Those on the epidermal cells extend approximately at right angles to the epidermal surface, while those on the guard cells of the stomata are inclined toward the stomatal opening. On the upper and lower epidermis there occur several kinds of hairs.

ANATOMY OF DISEASED LEAVES

The symptoms of helminthosporium leaf spot of rice, as it has been observed by the writer, coincide with the descriptions of previous investigators. Lesions have been found on plants 18 hours after inoculation, as reported by Farneti (*2*) and others. In the lesions that are just appearing, the cells in the vicinity of the invading mycelium are brownish in color and the cell walls have partly collapsed. The invading mycelium from an appressorium may enter the leaf through a stoma or penetrate directly into the cells of the epidermis, as reported by Nisikado and Miyake (*6*) and Ocfemia (*7, 8*). It appears from the specimens examined that invasion is most frequent in the motor cells, and often the invaded motor cells are almost filled with the mycelium of the fungus. Such a condition is shown in figure 5, *A, B,* on a leaf of Kameji and Bozu, respectively. Hyphae in the motor cells are shown in figure 5, *A* to *C*. The section shown in figure 5, *C*, is from a portion of a leaf of Lady Wright inoculated in the greenhouse. This portion of the leaf was stained in toto to show how the hyphae have invaded the motor cells. Toxic substances produced by the fungus soon cause discoloration and death of the neighboring armed-type parenchyma cells.

Lateral branches of the mycelium that penetrate the inner walls of the motor cells are produced, and in this way the mycelium gains entrance into the intercellular spaces of the photosynthetic area of the leaf. Two systems of mycelium are then produced. One develops just beneath the epidermis and spreads through substomatal cavities

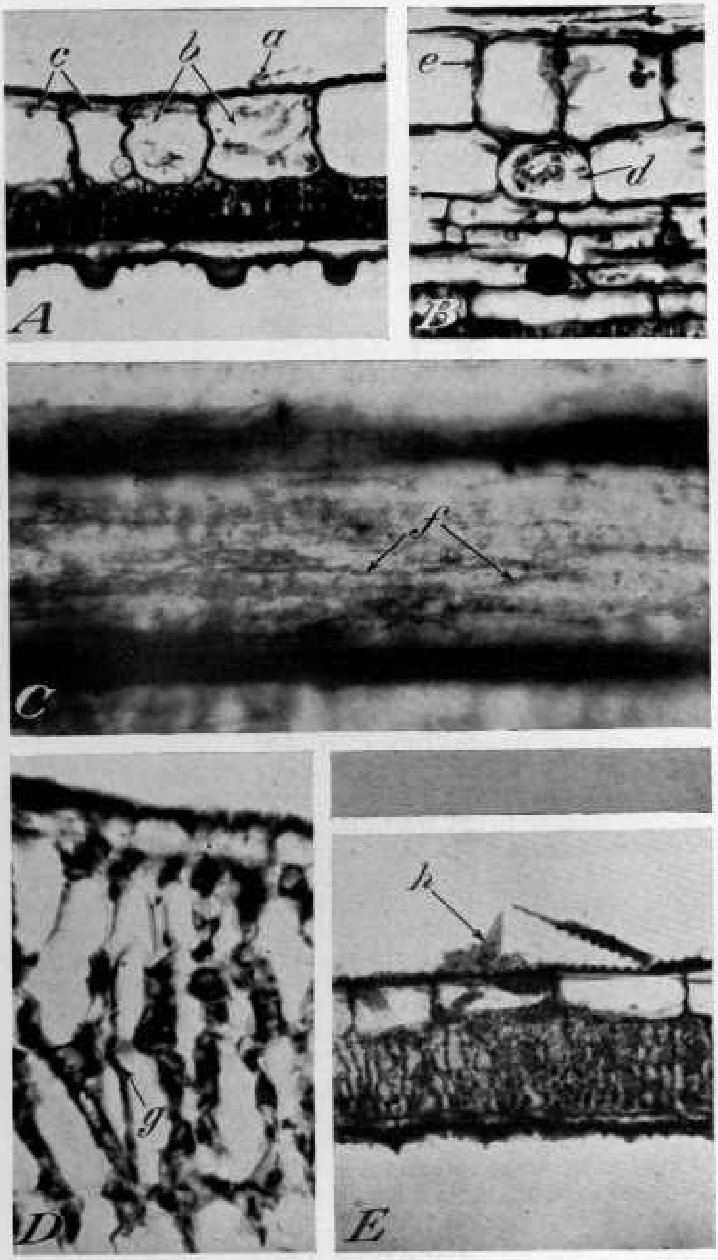

FIGURE 5.—*A*, Longitudinal section of a portion of a leaf of Kameji rice, showing remains of spore on surface of motor cell at *a*, two motor cells nearly filled with mycelium at *b*, and intracellular hypha at *c*. × 288. *B*, Tangential section of a portion of a leaf of Bozu rice, showing a cell almost filled with mycelium of the fungus at *d*, and intracellular hypha at *e*. × 288. *C*, Surface view of a portion of a leaf of Lady Wright rice, showing hyphae of the fungus in the motor cells at *f*. × 228. *D*, Longitudinal section of a portion of a leaf of Storm Proof rice, showing an intercellular hypha at *g* in an intercellular space. × 900. *E*, Longitudinal section of a portion of a leaf of Blue Rose rice, showing conidiophores, at *h*, beginning to form on an infected motor cell. × 288.

from one system of intercellular spaces to the next. The other consists of branches from this system that invade the intercellular spaces between the faces of the armed-type parenchyma cells. A lateral strand of mycelium invading the intercellular space of a leaf of Storm Proof is shown in figure 5, *D, g*.

The most susceptible varieties have large intercellular spaces and large substomatal chambers, invasion progresses rapidly, and the leaf spots frequently extend from the midrib of the leaf to the margin. The spots are at times circular but more often narrowly elliptical. In many cases several lesions coalesce to produce large spots.

In areas of active invasion the host cells first become yellowish in color, the chloroplasts disappear, the protoplasm dies, and the cells partially collapse.

The bundles tend to form barriers against the lateral spread of the fungus. To invade adjacent areas beyond a bundle it is necessary that the fungus penetrate the bundle sheath. Apparently this is accomplished much more easily in some varieties than in others. In very resistant varieties it apparently does not occur.

In resistant varieties invasion of the motor cells occurs, but in most cases the mycelial strands are small and soon become brownish in color and thick-walled with rather sparse contents, whereas those found in susceptible varieties are not brownish in color and are large in diameter and rich in cytoplasm, indicating much greater vigor. The conidiophores are formed from a plexus of the mycelium in the intercellular spaces or in the motor cells. The latter case is shown in figure 5, *E*. Five immature conidiophores can be seen in this group.

In resistant varieties a deposit is formed in the intercellular spaces that aids in restricting the fungus to the area of primary invasion. This ability of the parenchyma cells of an invaded area to protect those of adjoining areas from invasion is apparently the greatest factor in resistance in the leaves. These deposits are found most highly developed in Shoemed, but they also have been found to some extent in susceptible varieties. In the more susceptible varieties they are formed by groups of cells but never in sufficient numbers to isolate a given area completely, as is the case in Shoemed. Changes in the walls of the armed-type parenchyma cells and in the cells of the bundle sheath, in the immediate vicinity of the mycelium are seen in sections stained with methylene blue even before the deposits are formed. The cell walls become thicker, stain more intensely with all stains used, and after a time become somewhat yellowish in color.

The deposits are formed between the armed-type parenchyma cells considerably in advance of any mycelium, discoloration, or disintegration of the host cells. The deposits are solid in the case of the very narrow intercellular spaces and are sometimes hollow in large intercellular spaces or in substomatal chambers (fig. 6.). In resistant varieties these deposits extend from the upper to the lower epidermis, from one bundle to the next, and along the bundles. In this way, openings that may exist from one set of intercellular spaces to the next are filled so completely that in case the fungus has gained entrance into the photosynthetic portion of the leaf no further internal spread is possible. These deposits also protect the bundles so that the fungus is unable to reach additional sources of food or to invade adjacent photosynthetic areas. The protoplasmic content of the motor cells is very limited and as soon as the reserve food obtained from the area of primary invasion is exhausted the mycelium apparently dies for want of nourishment.

The chemical nature of the deposits is not known. In unstained sections they are yellowish brown. They stain intensely with iron-alum haematoxylin, as does the outer wall of the epidermis. When they were tested for cutin, suberin, or pectin, no positive reaction was secured. Because of the high silicon content of the epidermis it is necessary to treat all material with hydrofluoric acid before sectioning. This treatment may so alter the deposits that it is not possible to determine their composition in sectioned material.

Usually the formation of the deposits is first observed between the parenchyma cells near the bundles just under the upper and lower epidermis.

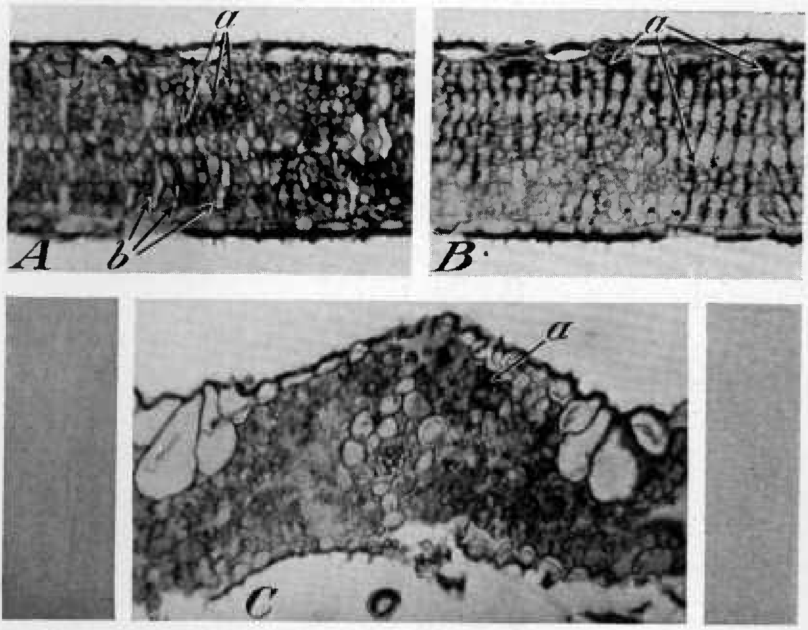

FIGURE 6.—Longitudinal (*A* and *B*) and cross (*C*) sections of portions of infected leaves of Shoemed rice, showing substomatal chambers and intercellular spaces filled with deposit at *a*. In the large intercellular spaces shown at *b* the deposits are hollow. All × 288.

SUMMARY

The anatomy of healthy rice leaves is characterized by papillate epidermis, motor cells, armed-type parenchyma cells, and usually by large intercellular spaces in photosynthetic areas.

In rice leaves affected with leaf spot caused by *Helminthosporium oryzae* the hyphae of the fungus grow intercellularly in the photosynthetic areas and intracellularly in the motor cells and bundle sheath.

The bundle sheaths of resistant varieties are less readily penetrated by the fungus than are those of susceptible varieties. The bundles interfere with the lateral spread of the fungus in the leaf.

In resistant varieties of rice the invading fungus is hemmed in by the formation of deposits, which accumulate in the intercellular spaces about an infection. The chemical nature of these deposits has not been determined.

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