A Contribution to Pathological Anatomy of Rice Plants Affected by *Gibberella Fujikuroi* (Saw.) Wollenweber. I.

By

Y. Nisikado and K. Kimura.

[Received on July 31, 1940]

The "Bakanae" disease of rice plant has been known for a long time in Japan. The causal fungus was first described by Hori (1908) as *Fusarium heterosporum* Nees. Later, Sawada (1917) discovered its perfect stage and applied the name *Lisea Fujikuroi* Sawada. However, Ito (1930) regarded it in the genus *Gibberella* and suggested to rename it *Gibberella moniliformis*. Wollenweber (1931), from the inoculation experiment of the senior author (Nisikado, 1930, 1931) conducted while in Berlin, adopted the name *Gibberella Fujikuroi* (Saw.) Wollenweber.

The phenomenon of abnormal slender elongation in the diseased rice plant was reported and described by Kurozawa (1926), Hemmi, Seto (1932), Ito and Kimura (1931), Shimada and others. The occurrence of the variations in the pathogenicity of the fungus was demonstrated by Nisikado and his collaborators (1933). Yabuta and Hayasi (1937, 1938) reported that the abnormal elongation of the diseased rice plant is due to a toxin, which they named "Gibberin" and that the abnormal atrophied condition to their "Fusarin Säure".

The diseased plant typically exhibits an abnormal elongation in height but not the other symptoms, such as spotting, wilting etc., at least in the beginning of the disease. There is no published account of observations made on the occurrence and the distribution of the fungus in the diseased rice plant in this early stage of disease. Such a study is important for various reasons, particularly from the point of view of disposal of diseased rice straw as a method of eradicating the disease. The present study dealt particularly with the distribution of the fungus mycelium in the diseased rice culms.

The distribution of the fungus mycelium within plants in the seedling stage was found to be comparatively limited in its extent, and this subject will be discussed in detail later under a separate title. In the present study, the results are taken only from those culms that had approached the period of heading. A preliminary report of this work was read at the annual meeting of the Japanese Phytopathological Society held in April 1939.

I. Features of the Fungus Mycelium in the Affected Rice Culm.

Just prior to the time of heading in September, the rice plants affected with the Bakanae disease are much slender than the healthy uninfected plants and show an etiolated appearance. The photograph of Figure 1 shows a rice field affected with the Bakanae disease. The disease plants appear either in isolated spots or in large groups in a rice field and appear whitish in the illustration. The affected plants at this time are tall, and do not have any discarded dead leaves. On sections of various portions of such diseased culms the distribution of the fungus mycelium was studied under a microscope. The results were as shown in Figures 2–7, the figure number being arranged in the order of the increase in height from which the sections were made. Figure 2 is taken at the base of the culm; and Figure 7, higher up just below a node.

All of these sections show the mycelium and the microconidia in the vascular bundles. Especially they were copious in large pitted vessels and the air space or lacuna of the xylem. On the other hand, the mycelium was not as abundant in the phloem, occurring only in a few severely damaged culms (Figures 2–5). In those culms the mycelium occurred also in the soft parenchyma cells (Figure 3). In general, the macroconidia were found only in those plants advanced in the disease in the inner side of the leaf sheaths near the node.

II. Microscopical Studies on the Distribution of the Fungus Mycelium in the Affected Culm.

Diseased rice plants were collected near the Institute in September 1938. They were washed and separated individually from the point of tiller, and the nodes of each shoot were marked. Sections were cut at both below and above the node; and the occurrence of the fungus mycelium was recorded with + and −, indicating the presence and absence of the mycelium respectively. A − was indicated when mycelium was not observed after making three repeated trials.

The results obtained by such a method of observation is illustrated in Text-Figure 1. The plant No. 1 was 122 cm. tall from the base of the culm to the tip of the spike, and the plant showed no external symptoms except for a condition similar to etiolation. The mycelium occurred below the third node, which is 11 cm. high from the base of the culm, but none was found higher up.

The plant No. 6 was 121 cm. tall, and the mycelium occurred to a height slightly above the 4th node which was 44 cm. from the base of the culm; but none at the 5th node.

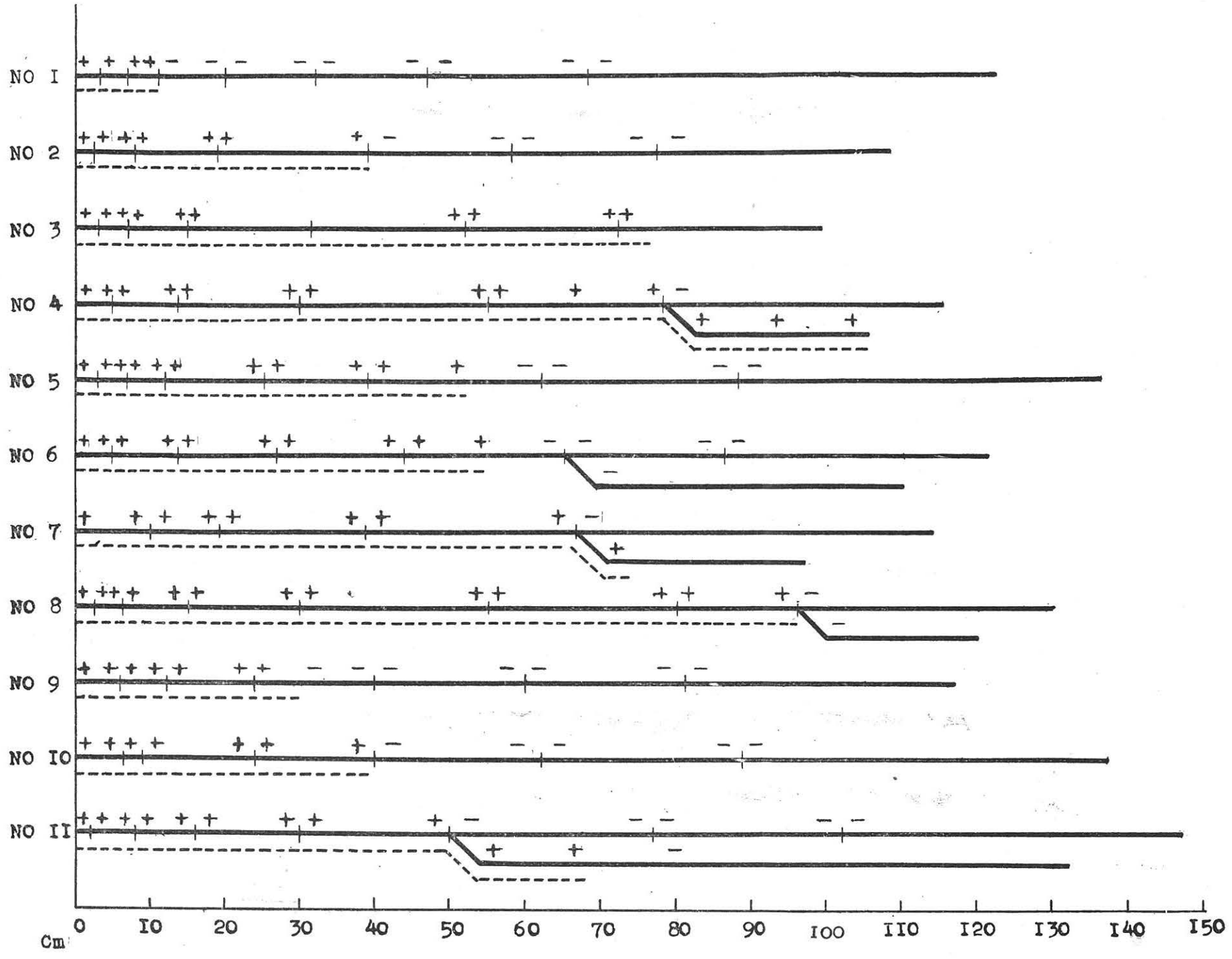
In the plant No. 8, the mycelium was observed to a height of 96 cm. a point immediately below the last node, but none was found in the leaf sheath occurring above the last node.

Text-Figure 1.

Distribution of the mycelium in rice culms affected by *Gibberella Fujikuroi* (Saw.) Wollenweber.

(I) Results of anatomical studies.

Sections were made just below and above every node of diseased rice culms showing an abnormal elongation but no other symptoms. The heavy lines (Nos. 1, 2 etc.) show the rice culms studied, the branch lines, the leaf-sheathes and the short thin transverse lines the place of node. Plus or minus signs means the presence or absence of the Bakanae-fungus in the sections. Figures on the absissa are the length of the rice culms. The part of the culms affected by the Bakanae fungus is expressed by broken lines.



Again, however, in the plant No. 4 the mycelium not only reached to the last node, but also to the leaf sheath arising from the last node. There were many other plants that showed a similar condition to this.

Although the occurrence of the fungus mycelium in the culms depends much on the severity of the disease, the result showed that the mycelium and the conidia of the fungus extended upward to some height in the tissue of the rice plant, spreading especially along the vessels and lacuna in the xylem.

III. Cultural Studies on the Distribution of the Fungus Mycelium in the Affected Culm.

The diseased plants were washed and the nodes were marked as has been done in the previous observation. Small samples of the affected parts of the plant, 3–4 cm. long, were cut away just above and below each node with a sterile scalpel, and the ends of these pieces were immediately sealed with melted paraffin. They were then surface sterilized by dipping into 50% alcohol and 0.1% mercuric chloride solution for one minute, and thoroughly washed with sterile water. Two or three samples of 3–5 mm. in size were cut and each was placed on the rice straw decoction agar medium in the Petri dishes for incubation. The temperature of the incubator was held at 24°C.

The result of the observations is shown in the graph of Text-Figure 2. In plants A and C, the fungus mycelium occurred continuously along the length of the plant; but in plants B and D, there were portions free from the fungus mycelium. In B, the mycelium occurred to a point above the 3rd node, and did not extend into the 4th node; but at the above portion of the 5th node the fungus mycelium reappeared. Again in D, mycelium was present to a point slightly above the 2nd node, and none at 3rd, 4th, 5th or 6th; but reappeared at above the 7th and 8th nodes. (See Text-Figure 2 on next page)

The results obtained from the microscopic and cultural methods of studying the distribution of the fungus mycelium were very similar, though not identical in every respect. One of the main differences between the two is that when studied by cultural method, there was a discontinuous occurrence of the fungus mycelium along the length of the host plant. This difference may have been due to the method of sampling employed in determining the presence of the mycelium culturally; but more likely that such a condition of discontinuity actually exists in the rice plants affected with the Bakanae disease.

IV. Brief Considerations on the Results.

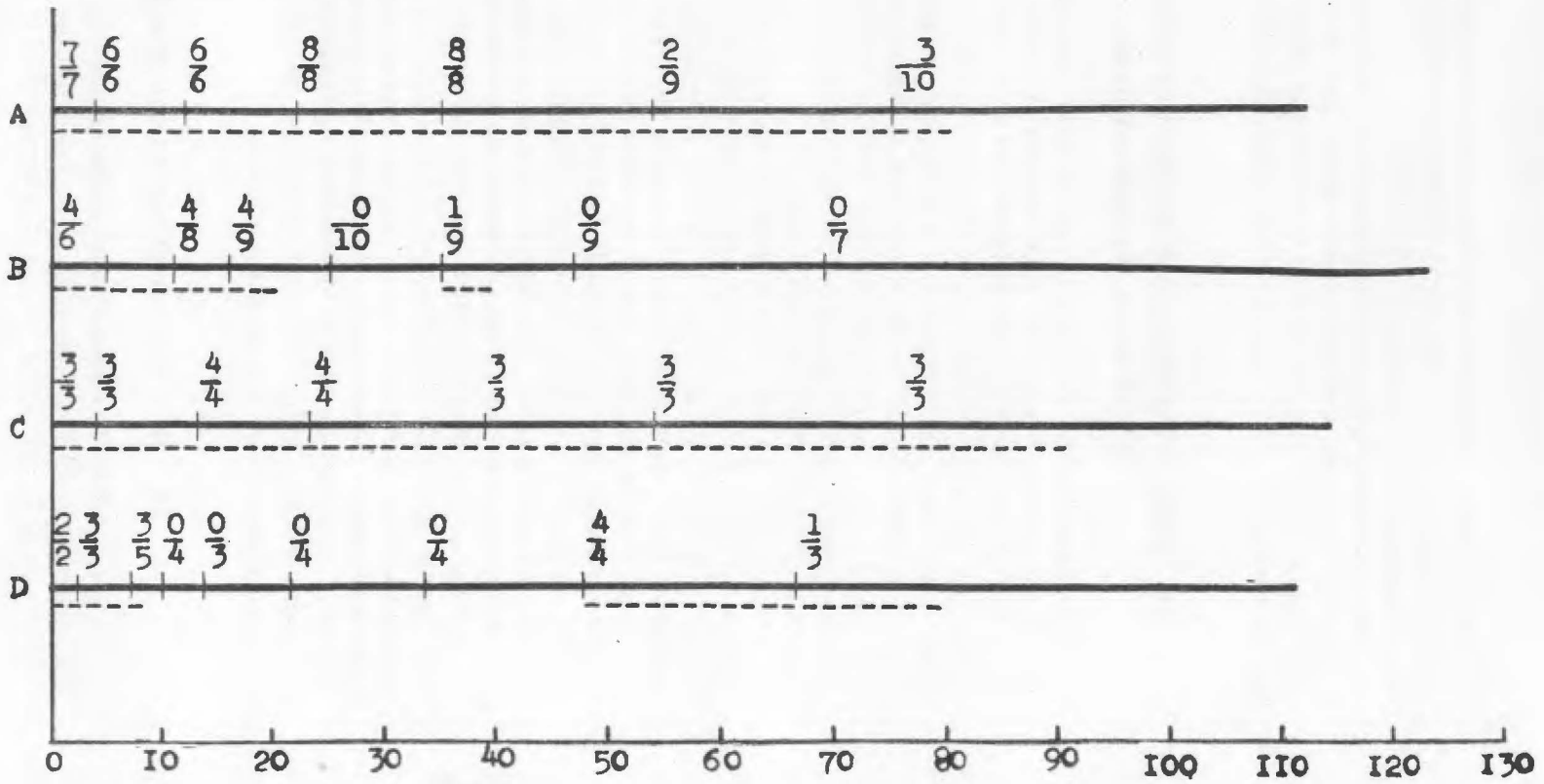
Considering from the results obtained, its firmly established that the Bakanae fungus mycelium and the microconidia occur widely distributed in the vascular bundles even in the region that does not show any symptom of the disease.

Text-Figure 2.

Distribution of the mycelium in rice culms affected by *Gibberella Fujikuroi* (Saw.) Wollenweber.

(II) Results of cultural studies.

The heavy line (A, B etc.) show the rice culms studied and the short thin transverse lines the place of node. The denominator of the fraction given means the number of culture made and the numerator the number of mycelial growth of the Bakanae fungus. Figures on the abscissa show the length of the rice culms. The part of the culms affected by the Bakanae fungus is expressed with broken lines.



The fungus in the conidial stage belongs in the genus *Fusarium*, which contains numerous members of phytopathogenic and nonpathogenic species. Wollenweber (1931) and his collaborator Reinking (1935) separated this genus into 16 sections or "Gruppen". The causal fungus of Bakanae disease, *Gibberella Fujikuroi* (Saw.) Wr. belongs in the 13th section, Gruppe Liseola.

The species in this section does not cause any "Gefässparasitäre Welkkrankheit" or wilt disease; and similarly, the Bakanae fungus conforms to this general rule. But on the other hand, the Bakanae fungus does form abundant mycelium and microconidia in the vascular bundles of rice plant. This fact is of particular importance especially in connection with the disposal of the diseased straw and the surface sterilization of seed as the most effective means of controlling the disease, as has been reported by Ito (1932) and others.

References.

Hori, S. (1898): Ine no Bakanae Siken. Nôzi Siken Seiseki, I-Kan, 12 Hô. (*Japanese*)

——— (1908): Nôsakumotu Byôgaku, p. 114. (*Japanese*)

Ito, S. & J. Kimura (1931): Ine Bakanae-Byô ni kansuru Kenkyû. Hokkaidô Nôzi Sikenzyô Hôkoku, 27 : 1 – 94. (*Japanese*)

Kurosawa, E. (1926): Ine Bakanae-Byô-Kin no Bunpitubutu ni kansuru zikkenteki Kenkyû. Transact. Formasan Natural History, 16 : 87 : 213 – 227. (*Japanese*)

Nisikado, Y. (1931): Vergleichende Untersuchungen über die durch *Lisea Fujikuroi* Saw. und *Gibberella moniliformis* (Sh.) Winel. verursachten Gramineenkrankheiten. Vorläufige Mitteilung. Berichte Ōhara Inst. f. landw. Forschungen, 5 : 1 : 87 – 106.

——— (1932): Über zwei wirtschaftlich wichtige, parasitäre Gramineenpilze: *Lisea Fujikuroi* Sawada und *Gibberella moniliformis* Wineland. Zeitschr. f. Parasitenkunde, 4 : 2 : 285 – 300.

Nisikado, Y., Matumoto, H. & K. Yamauti (1933): *Fusarium*-Zoku Kin no seiriteki Bunka ni kansuru Tiken. (I) Ine Bakanae Byôkin kakusyu Keitô kan no Byôgensei no Sai ni tuite. Nôgaku-Kenkyû, 20 : 320 – 345. (*Japanese*)

Sawada, K. (1917): Taiwan Kinrui-Siryô. XIV. Transact. Formosan Natural History, 31 – 32 : 131 – 133. (*Japanese*)

Seto, F. (1932): Experimentelle Untersuchungen über die hemmende und die beschleunigende Wirkung des Erregers der sogenannten „Bakanae" Krankheit, *Lisea Fujikuroi* Sawada, auf das Wachstum der Reiskeimlinge. Memoirs Coll. Agr., Kyoto Imp. Univ., 18 : 1 – 23.

Wollenweber, H. W. (1931): *Fusarium*-Monographie. Fungi parasitici et saprophyti. Zeitschr. f. Parasitenkunde, 3 : 269 – 516.

Yabuta, T. & H. Hayasi (1937): Ine Bakanae Byôkin no Seikagaku. Agriculture & Horticulture, 11 : 1 : 27 – 33. (*Japanese*)

——— ——— (1938): Ine Bakanae Byôkin no Seikagaku (Zokuhô). Agriculture & Horticulture, 12 : 4 : 1073 – 1083. (*Japanese*)

Explanation of Plates.

Fig. 1. Rice field in the heading stage affected by the Bakanae Disease. In the photograph the diseased culms appear tall and whitish.

Fig. 2–Fig. 7. Transverse sections of rice culms affected by the Bakanae fungus, showing the mycelium and conidia of the fungus in vascular bundles. (×400)

Fig. 2–4. Sections of a rice culm near the base.

Fig. 5–6. Sections of a rice culm, somewhat above that shown in Fig. 4.

Fig. 7. A section of rice culm, above that shown in Fig. 6.

PLATE XXVI.

Fig. 1.

Fig. 2.

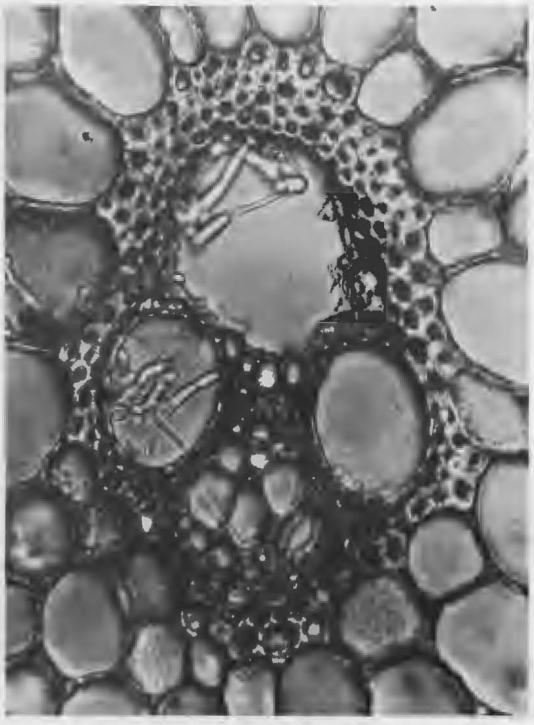


Fig. 3.

PLATE XXVII.

Fig. 4.

Fig. 5.

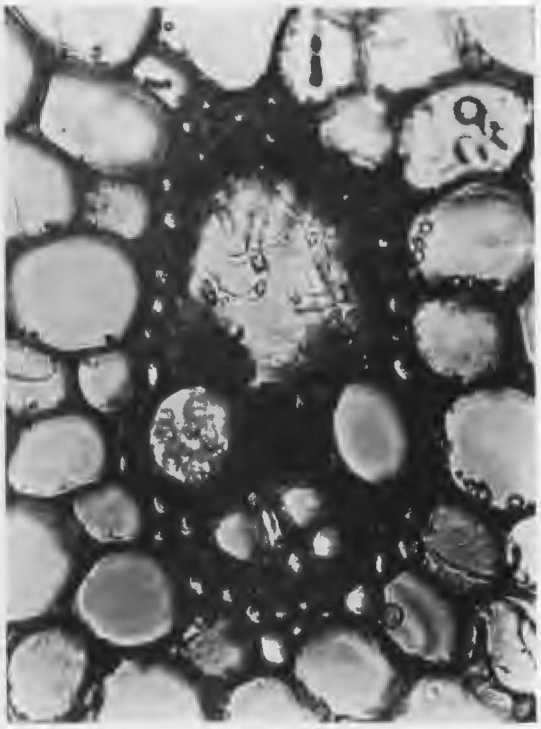


Fig. 6.

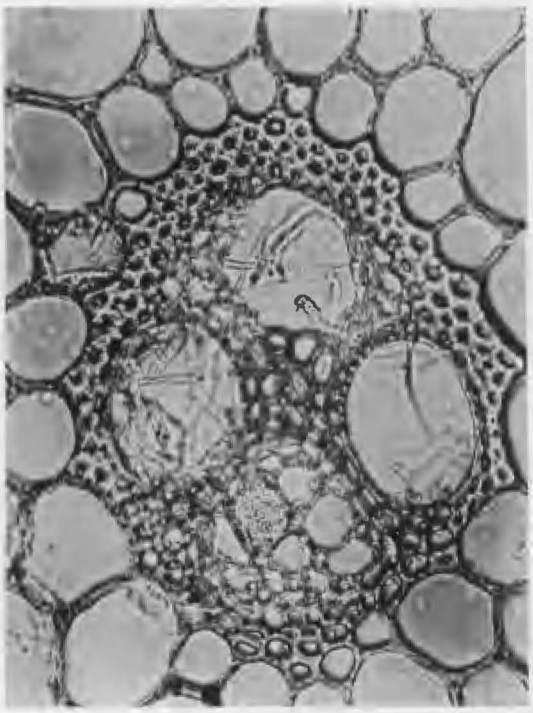


Fig. 7.