RECENT DEVELOPMENTS IN THE STUDY OF THE NATURE OF MOSAIC DISEASE OF SUGAR CANE AND OTHER PLANTS.

By Julius Matz.

Mosaic diseases of plants, including mosaic of sugar cane, have of recent years come to be generally considered as infectious and obviously caused by microorganisms which were supposed to be ultramicroscopic and invisible even with the aid of the usual staining materials. Most investigators have apparently relied in the past, in the studies of mosaic diseases, on the culture method to detect the existence of organisms; having failed in that and not recognizing any familiar forms of bacteria or fungi in the host tissues the problem was left in the field of inexplicable phenomena. More recently, however, within the last three years, new light was thrown on the subject, and it seems that the causes of mosaic diseases are in a fair way to become clear. The new procedure in the investigation is centered along cytological lines and is based on comparative studies on the contents of cells from diseased plants and contents of cells from similar but healthy plants.

In 1919 the writer started histological studies of sugar-cane mosaic and it was soon found that in advanced stages of the disease small portions in the interior of mosaic cane stalks become slightly bleached, as well as light brown and dark brown. In these discolored or darkly colored tissues the cells are filled with a more or less hardened, or compact, densely but finely granulated, often slightly browned plasma.

Since the publication of the above facts in an article entitled "Infection and Nature of the Yellow Stripe Disease of Cane (Mosaic, Mottling, Etc.)" in the Journal of the Department of Agriculture of Porto Rico, Vol. III, No. 4, Oct. 1919, two investigators, namely, Dr. Kunkel, writing in 1921 on corn mosaic in Hawaii (1), and more recently Dr. Palm, in his Bulletin treating on the cause of tobacco mosaic (2), refered to my findings in sugar-cane mosaic diseased tissues as something similar to what the first author found in corn mosaic and the second author in tobacco mosaic. Kunkel stated that in addition to the larger bodies which he found in the leaves of corn mosaic, he is "able to confirm the observations of Matz as regards the occurrence in diseased cane tissue of cells filled with a hardened, granular slightly brownish substance." He further states that "Cells filled with exactly the same kind of granular material are also present in the stalk tissues of corn suffering from mosaic. * * * What this substance may be or what relation it may have to either the cane or the corn disease the writer is unable to suggest."

He published some very carefully executed drawings (plate 5, figs. A to M.) of what he calls intracellular bodies and which he considers as foreign bodies believed to be a living organism in the diseased cells of mosaic corn plants. These bodies are shown attached in various positions to the nucleus of the host cells.

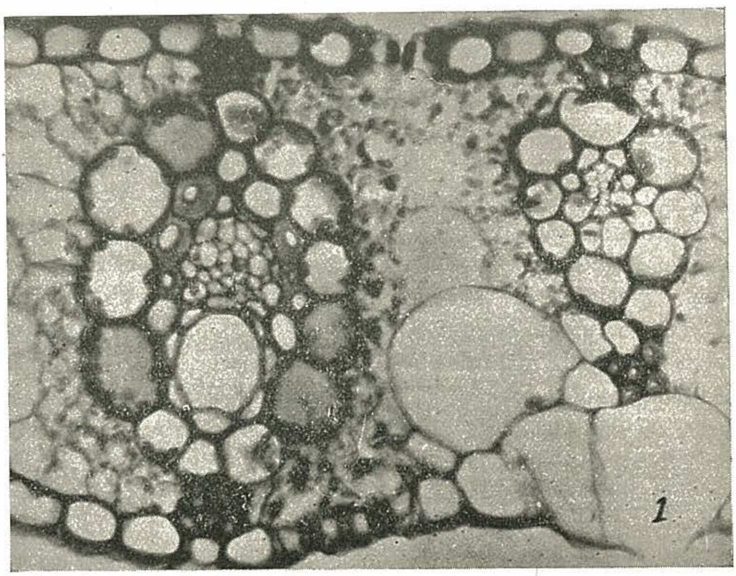

FIG. 1.—Section through a healthy sugar-cane leaf, showing chloroplasts in cells.

Iwanowski in his studies of tobacco mosaic published in 1903 (3) illustrates (in Table II, fig. 8) strikingly similar bodies, to those shown by Kunkel, in identical positions attached to the nuclei of the host cells, but Iwanowski disregards these as a possible cause of the mosaic disease on the grounds that these bodies are altogether too large for the fine pores of filters to pass through. The infectious principle of tobacco mosaic is known to filter through fine-porous filters. This investigator considers these larger bodies as abnormalities in the cytoplasm of the cells due to mosaic infection, but mosaic itself he maintains to be caused by a minute bacterium which he differentiates from the other cell contents by using Loeflers methelene blue and eosin, the chloroplast and protoplasm taking the red stain while the nuclei and the bacteria take the blue stain.

The writer has applied these stains to sugar-cane mosaic leaf tissue and the two colors differentiated the chloroplasts from the nuclei but no blue bacteria were detected. However, the behavior of tobacco mosaic is not like the sugar-cane mosaic, as will be pointed out later, and it is possible that the "bacteria" are not localized in the same elements in the two host plants' tissues.

Palm in his very recent work with tobacco (2) states: "In a large number of cells of the mosaic-diseased tissues it was possible to observe the occurrence of foreign elements, viz., of fairly large, more or less peculiarly shaped corpuscles, or very small granules of varying size." He adds in a note: "Matz (1919) has found such corpuscles in mosaic-diseased sugar cane in West Indie, and Kunkel (1921) in mosaic-diseased maize in Hawaii. * * *" He further states: "In the cells with the above-described larger corpuscles or in others where they apparently did not occur, it was possible, especially in the later stages of the disease as already mentioned, to find a second foreign cell element, consisting of extraordinarily small granules."

"These granules * * * occur in the cells in larger or smaller numbers. They frequently lie in irregularly shaped conglomerates in the cell plasm sometimes the cell lumen being completely filled." This point agrees very well with my observations on sugar-cane mosaic.

In my article above refered to I stated that the granular substance in the cane mosaic is made up of a mass of small hyaline granules, more or less uniform in size, but that their exact size and form could not be ascertained, due to the fact that the whole mass is in the form of a compact plasma, that the granules are smaller in size than ordinary bacteria and appear like nuclear granules in a mass of cytoplasm. This characteristic granular plasma was also found in cells of mosaic leaf sheaths of cane.

While Dr. Kunkel lays more emphasis on the larger bodies in the cells of corn mosaic and is not able to define with certainty the relation of the minute foreign granules in either the corn or cane mosaic, Dr. Palm, however, finds it convenient to compare these minute granules of Iwanowski, Matz and Kunkel to the granules found as a consequence of variola infection and other virus diseases in human beings and animals. From this he draws the conclusions that mosaic disease belongs to the chlamydozoonoses; that the different forms of granules found in mosaic-diseased plants are either different stages of *Strongyloplasma* or by-products of this organism, and he names the organism which causes the mosaic of tobacco *Strongyloplasma iwanowskii,* giving the honor to Iwanowski who twenty years ago maintained that he saw bacteria in tobacco mosaic-diseased plants. Although Dr. Palm states that the organism which is found in tobacco is similar to the substance which

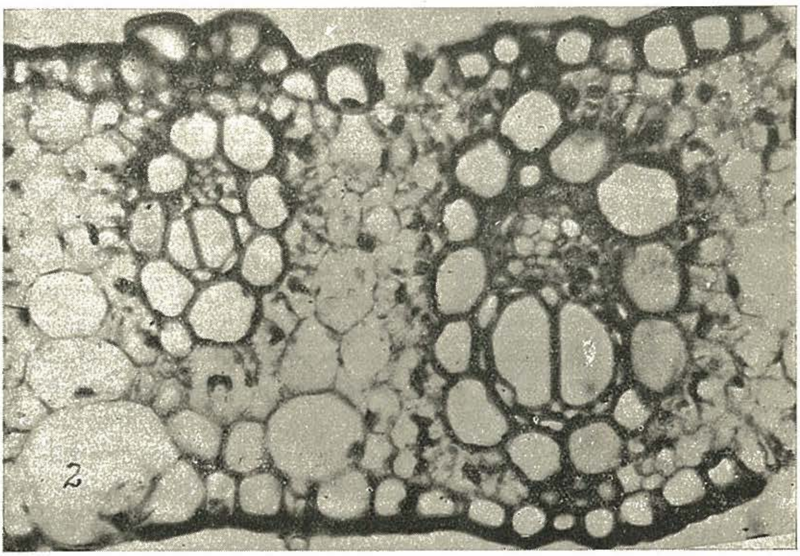

FIG. 2.—Cross section through mosaic leaf of sugar cane, showing destruction of chloroplasts.

I found here in sugar cane, we do not necessarily have to accept *S. iwanowskii* as the name for the plasma found with the sugar-cane mosaic disease in view of the fact that the two diseases do not behave in a similar manner. Tobacco mosaic is transmitted by contact while sugar-cane mosaic is transmitted by special carriers or special methods only. The infection of tobacco mosaic may take place through diseased plant residue in the soil, while such is not the case with the sugar-cane mosaic. Perhaps the most important, distinction between the two diseases is their apparent specific characters. Tobacco mosaic is not known to have been transmitted in the field to cane from the tobacco, nor is it known that cane mosaic is transmitting itself to tobacco. There exist extensive tobacco plantations free from mosaic in the Cayey District where cane is heavily diseased with mosaic. Evidently the organisms which cause mosaics in plants are of a groupe which have common general or generic characters, but there must exist specific differences between these since they exhibit marked differences in their behavior, and the sugar-cane plasma if it is to have a name it will have to have one of its own. Further work was continued at the Insular Experiment Station on the same phase of this problem and new and interesting facts have come to light that may be stated briefly in the following.

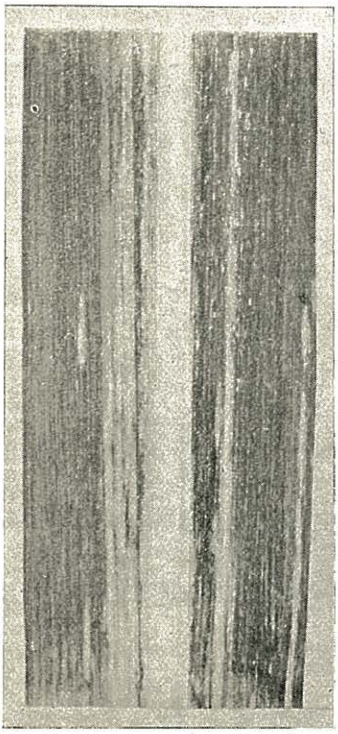

FIG. 3.—Early stages in mosaic diseases of sugar-cane leaf.

Of the numerous methods employed in studying plant tissues, Jeffreys' formula of corrosive sublimate and picric acid, with the addition of 5 c.c. glacial acetic acid, was found convenient to use. Healthy and diseased portions of sugar-cane leaf were killed and fixed in the above mixture for two or three hours, passed through several washings in 70 per cent, 85 per cent and 95 per cent alcohol, then the material was transferred to butyl alcohol and left there until it lost all its green color, then it was placed in melted paraffin and embedded.

Sections of cane-leaf tissue were stained with iron-alum haematoxylin, orange G, and methelene blue and eosin. One feature was very pronounced throughout, namely, the very evident destruction of chloroplasts in the diseased portions of the leaf tissue. Figures 1 and 2 are photographs of a healthy and diseased leaf portions respectively. The two leaves were as nearly alike in size and age as could be estimated. In the healthy leaf the larger cells surrounding the fibro vascular bundles contain many chloroplasts; the parenchyma between the bundles are also filled with numerous chloroplasts, while in the diseased tissue the chloroplasts are few and are evidently misshaped and broken up. In stained sections they look like mere ink spots, one or two in a cell. This destruction of chlorolpasts is a symptom of sugar-cane mosaic and it fixes the seat of the disease more definitely. Apparently the cell walls and other cell contents are not affected, but the chloroplasts are gradually destroyed.

Sections of single discolored stripes of a leaf in an early stage of infection were made (Fig. 3), and it was seen that the breaking up of the chloroplasts begins with a reduction in their size. The chloroplasts in the healthy or green parts of the same leaf were normal in their size and numbers, while in the discolored or pale-green stripes chloroplasts in all stages of reduction were noted.

REFERENCES CITED.

1. L. O. Kunkel.—A Possible Causative Agent for the Mosaic Disease of Corn—Bull E. S. H. S. P. A., Botanical series, Vo. III, Part I, pp. 44, 57. 1921.
3. Prof. D. Iwanowski, Warschau. Uber die Mosaikkrankheit der Tabakspflanze, Deli, Medan Sumatra. 1922.
3. Prof. D. Iwanowski, Warschau. Uber die Mosaikkrankheit der Tabakspflanze, Zeitschrift fur Pflanzen krankheiten, XIII Band, 1 heft, pp. 1–41. April 1903. Three plates.