HISTOLOGY AND CYTOLOGY OF SUGAR CANE MOSAIC [1]

MELVILLE T. COOK

The various mosaic diseases of plants have been extremely puzzling from the time they were first recognized as diseases. Although they have been the subjects of study by some of the leading scientists of the world, the causes are as yet not definitely known. Many theories have been advanced as to the causes of which the following are some of the most important:—(1) bacterial, (2) virus, (3) physiological, (4) enzymatic, (5) protozoan.

All students of the mosaic diseases are familiar with these theories and therefore, it is not necessary to discuss them further than to say that the "virus" and "protozoan" theories are the most prominent at this time. Probably the most important facts that we have learned about these diseases are (1) that they can be transmitted from generation to generation through vegetative parts, (2) that some of them may be transmitted by means of insects, (3) that some of them will attack plants other than the economic crops in which we are interested, (4) that they may be present but not visible in some plants.

The studies recorded in this paper were undertaken (1) for the purpose of studying histological and cytological differences between diseased and healthy plants and (2) for the purpose of gaining some light if possible as to the cause of this particular form of mosaic. Before taking up the results of these studies it is desirable to review the literature bearing directly on this phase of the subject, but since this paper deals only with the histological and cytological characters of mosaic sugar cane as compared with apparently healthy cane we will not refer to any literature other than that which has a direct bearing on these phases of the subject. It will be readily seen that the most extensive studies on these phases of the mosaic disease have been made on tobacco which is a dicotyledonous plant and therefore, not strictly comparable to the sugar cane which is a monocotyledonous plant.

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HISTORICAL

The first histological studies of mosaic were made by Köning **(7)** on tobacco but very little difference was noted between healthy and diseased tobacco other than that in the latter the chloroplasts were disorganized and the cell walls disintegrating.

A brief reference to this phase of the subject was made by Woods **(19)** in his studies on tobacco mosaic, who noted the abnormal condition of the light-colored areas and said that—

"This difference consists in the fact that in the badly diseased plants the palisade parenchyma of the light-colored areas is not developed at all. All the tissue between the upper and lower epidermis consists of a spongy or respiratory parenchyma rather more closely packed than normal. In moderately diseased plants the palisade parenchyma of the light area is greatly modified. Normally the palisade parenchyma cells of a healthy plant are from four to six times as long as broad. In a moderately diseased plant, however, the cells are nearly as broad as they are long, or at most, not more than twice as long as broad. As a rule, the modified cells of the leaf passes abruptly into the normal cells of the green area."

Heintzel **(5)** does not refer to any variation in palisade cells but does note difference in the intercellular spaces in both palisade and parenchyma. He also notes that the chloroplasts were congregated irregularly in small groups.

Bouygues **(2)** reports an absence of the epidermis and a disappearance of the cell contents in the old spots.

The first extensive studies on this phase of the disease was made by Iwanowski **(6)** who also worked on tobacco. Although the cane and tobacco mosaics are probably due to different causes his studies are of special importance at this time. His results bearing on these studies may be summarized as follows:

(1) The infectious principle is not dissolved in the juice.

(2) Calcium oxalate crystals is characteristic of diseased plants.

(3) Transition zone between light and dark green areas consists of only 2 or 3 more rows of cells.

(4) Cross section of green part of a leaf shows the tissues well differentiated.

(5) Pale part is thin; pallisade modified; crystals prominent: no necrosis.

(6) Contents show lack of chloroplasts which are not regularly distributed. (In live cells the chloroplasts are swollen.)

(7) Films seen in the cells.

(8) Sometimes cells have granulations resembling bacteria.

(9) Nuclei normal or magnified and granular. Plasma bodies near them.

(10) Finds bacteria.

(11) Argues that this must be bacteria and not amoeba because amoeba can not go through a filter.

Westerdijk (18) reports that there was no microscopic difference worthy of mention other than that the chloroplasts were smaller and yellowish and that there was but little starch.

The next studies on this phase of the subject were made on the tomato mosaic by Melchers (14) who found that the light green or yellow areas were thinner than the dark green areas and that this was due to a shortening of the pallisade cells. The chloroplasts in the yellow areas were somewhat fewer in number and a pale yellowish green color.

Chapman (3) in his studies on mosaic of tobacco very generally agrees with Woods, but he found that occasionally the parenchyma of the dark green areas was more compact than in the normal plant. He also noted that the dark green areas contained some of the infecting agent. He found no histological differences in the roots and leaves but says that—

"The causal agent * * * is without question present in *all parts of the plant.*"

Artschwager (1) in his studies on the leaf roll of potato found certain changes in the vascular tissues but did not always find "a definite relation between external symptoms and internal changes." However, he reports an accumulation of starch in the diseased leaves. Histological studies on the leaf roll of the potato have been made by a number of European workers but since these studies do not appear to parallel the studies on the sugar cane mosaic, we will omit a discussion of them.

The first studies on the histology of mosaic sugar cane was made by Matz (11) who noted the following: (1) "distinct, single, spherical, darkly colored and densely protoplasmic bodies" in the parenchyma and collenchyma cells of the cankered stem tissues. They resembled "spore bodies" but were soluble in alcohol and he afterwards found them in non-yellow striped cane. (2) As a result of comparative studies on healthy and diseased stems (upper nodes) and leaves he says:

"It seems that a foreign plasmodium-like substance is apparently present in the cells of the yellow-striped cane leaf and stem tissue."

"Microscopic sections of the discolored areas in yellow-striped cane stalks

show that some parenchyma cells are full of a more or less hardened or compact but finely granulated and slightly browned plasma.''

(3) He also says:

"From a study of the internal structure of cankered cane it is clear that actual deterioration and breaking down of cells in the interior of cane in an advanced stage of the yellow-stripe disease takes place. This effect is due to no other causes than to the destructive action of the infective substance of yellow-stripe disease, as there is apparently no connection between these interior sick cells and outer outside mechanical or organizd agencies. Furthermore, this substance resembling a *Plasmodium,* in some of the interior cells was found to be constantly associated with yellow-striped cane in an advanced stage of disease.''

A little later Kunkel (8) made some most interesting studies on the mosaic disease of corn in which he describes intracellular bodies which he finds in the diseased but not in the healthy plants. These bodies are variable in shape, irregular and amoeboid but never spherical. They are closely associated with the host nucleus which becomes enlarged. This work is of special interest to us because the mosaic of the corn is supposed by some workers to be the same as the mosaic of cane. If not identical they are probably closely related.

In commenting on Matz paper he says:

"The writer is able to confirm the observations of Matz as regards the occurrence in diseased cane tissue of cells filled with a hardened, granular, slightly brown substance. Such cells are constantly associated with the disease in mature tissues. This substance, however, does not resemble any of the plasmodia with which the writer is acquainted. It does not stain like protoplasm, does not show a protoplasmic structure, is not vacuolate, does not contain any structure that could be taken for a nucleus, and is not plastic. When crushed, this substance breaks up into irregular-shaped angular masses which keep their form indefinitely and do not dissolve in water.''

He also says that—

"Cells filled with exactly the same kind of granular material are also present in the stalk tissues of corn suffering from mosaic.''

In his discussion Kunkel says:

"While it is not possible at the present time to prove that the bodies are living parasites, there is considerable evidence in favor of this view. They grow, show a structure like that of protoplasm, stain like protoplasm, and tend to be amoeboid in shape. It seems hardly probable that waste products accumulating in diseased cells or the products of protoplasmic degeneration would show these characteristics and would always be so closely associated with the host cell nucleus.''

In a latter paper Kunkel (9) reported the finding of amoeboid bodies in mosaic diseased plants of *Hippeastrum equestre*.

In a more recent work on tobacco Palm (15) described "amoebiform corpuscles" which he apparently considers similar to the bodies described by Kunkel in corn mosaic. Kunkel believes that these "amoebiform corpuscles" of Palm and the "Plasmaanhanfungen" of Iwanowski to be the same as the "intracellular bodies" which he found in corn, tobacco and other plants. Palm also reports the finding of numerous extremely small granules which he believes to be alive and the same as the bodies which Iwanowski described as bacteria. He has given these bodies the name of *Strongyloplasma iwanowskii*.

In a second paper on mosaic of sugar cane, Matz (12) reviews the works of Iwanowski, Kunkel and Palm in the light of his own studies and further states that—

"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 chloroplasts 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."

He furthermore says—

"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."

The next paper bearing directly on the subject was also by Kunkel (10) who found intracellular bodies in Chinese cabbage, *Brassica pekinensis* (Lour) Gagn., sugar cane, *Saccharum officinarum* L., and in tobacco, *Nicotiana tabacum* L. In discussing these bodies he says:

"While no attempt will here be made to fully describe the bodies found in the mosaic tissues of Chinese cabbage, sugar cane, and tobacco, a brief statement regarding them may be of interest. In each of these plants the amoeboid bodies are quite similar to, but somewhat different from the bodies associated with the mosaic in corn and Hippeastrum. In the leaves of the Chinese cabbage they reach about the same size that they do in the leaves of Hippeastrum. They are usually not in contact with the host nucleus and may occupy any part of the cell. They frequently have a number of chloroplasts clustered around them. In structure and in staining reaction they are similar to the bodies of corn mosaic. The bodies associated with the sugar-cane mosaic tend to stain more deeply than do those in any of the other plants studied, and are more irregular in shape. * * * Because of the fact that the older tissues of the sugar-cane plant be-

come very hard they are not suitable for cytological studies. Satisfactory sections showing the bodies of cane mosaic have been made from stalk tissues only. The best sections were obtained from tissues a short distance back of the growing point."

"After having found intracellular bodies associated with mosaic in corn, Hippeastrum, Chinese cabbage, and sugar cane, a study was made of stained sections of healthy and mosaic tobacco leaves. In some of the sections of mosaic leaves, bodies similar to those described in corn and Hippeastrum may plainly be seen. In morphology of the host cell, they are much like the bodies found in the cells of the other plants."

At about this time extensive histological studies were made by Dickson (4) on a number of species of plants infected with mosaic.

In the case of tobacco he found diseased leaves about two-thirds as thick as the normal leaves; the palisade parenchyma cells of diseased leaves shorter than those of normal leaves and in the case of severely infected leaves these cells were cuboidal or isodiametric. In some cases there were two layers of short palisade cells in severely diseased leaves. The cells in the spongy mesophyll of diseased areas were smaller than those in the dark green areas or in normal leaves and the total area of intercellular spaces in healthy parts 50 per cent greater than in diseased parts. The trichomes were more abundant in the light than in the dark-green areas. The number of chloroplasts in the light-green areas was much less than in the dark-green areas in healthy leaves and retrogressive changes were frequent. He also "observed occasional instances in severely diseased specimens where the nuclei were irregular in shape, being subspherical to angular, or considerably hypertrophied."

He also found a very similar condition in the tomato, petunia, potato, black henbane, pepper, sweet pea, kidney bean and broad bean.

A little later McKinney, Eckerson and Webb (13) published the results of what may be a similar disease of wheat. They found intracellular bodies in rosette and mottled wheat but did not find similar bodies in healthy plants. In commenting on the findings in this paper it should be borne in mind that these diseases of wheat may be carried in the soil which is not true of the mosaics. The authors of this paper find that these bodies are usually single; that they are variable in size and that they increase in size with the age of the host cell; that the relation to the nucleus is variable, occasionally or completely surrounding it; that they appear to be surrounded by a membrane; that the infected cells are not different from the healthy cells and that the host nuclei show little or no abnormalities.

Smith (17) made a study of the potato mosaic and noted amoeba-

like bodies in the yellow chlorotic areas. These bodies "tended towards pear-shaped or round." "These corpuscles seemed to be possessed of a definite wall and had in addition one or more very clearly defined vacuoles." "In many cases they were closely associated with the cell nucleus." He failed to find nuclei in these bodies and the chloroplasts were much reduced in number. The cell walls were frequently ruptured and there was a general disintegration of tissue in the light green or yellow areas and the "nuclei themselves are in a state of complete degeneration." He believes the intracellular bodies to be "degenerate products of the host cells."

The last paper on the subject is by Rawlins and Johnson (16) who made cytological studies of tobacco and who found two types of bodies in mosaic tissues, one type being very generally associated with the nuclei.

<div align="center">INVESTIGATIONAL</div>

The studies on which this paper is based have been directed primarily along lines suggested by the researches of Iwanowski (6), Palm (15) and Kunkel (8, 9, 10). The killing fluid most generally used was weak Fleming but Carnoy's fluid and several mixtures of picric and acetic acids both with and without corrosive sublimate were also tried. Weak Flemming fluid proved to be most satisfactory. The most satisfactory stain was found to be Haidenhain's Iron-alum-Haematoxylon, care being taken not to destain too much. The bodies which are associated with the mosaic disease showed to the best advantage when the nuclei of the cane were stained too deep for satisfactory study. A number of matrix stains such as Orange G and Eosin were used but were not necessary to good results. Safranin and Gentian Violet were found to be much less satisfactory than the Iron-alum-Haematoxylon. Carmin stains were tried but proved unsatisfactory.

These studies have to do with both leaves and stems. A few studies were made on the roots, but since no difference between roots from healthy and diseased plants were noted, they will be omitted. In making the studies of diseased leaves, sections were cut from both dark and light green or yellowish spots; and from cane of the same variety and about the same age, supposed to be healthy. In the study of the diseased stems, buds and roots, preparations were also made from corresponding parts of supposedly healthy canes of the same variety and age.

In order to aid in the discussion, we will present a somewhat digramatic drawing (Fig. 1) of a cross section of a normal cane

leaf to be used as a map in our further discussion. In this drawing it will be noted that the leaf is made up of a mesophyll consisting of large and small cells, the former being near the upper surface and usually without chlorophyll; there is no pallisade as in most plants; the mesophyll is supported by fibro-vascular bundles of various sizes, and the larger ones extending almost from epidermis to epidermis; the smaller ones lying near the lower surface; the upper epidermal cells are slightly larger than the lower and the upper cuticle slightly thicker; the stomata are found on both surfaces. The large fibro-vascular bundles consist of xylem cells surrounded by sclerenchyma cells which are in turn surrounded by a single layer of sheath cells. In the smaller bundles the sclerenchyma cells are reduced in number and may be absent. The sheath cells are more or less tubular; in cross section they appear more or less circular and in the longitudinal section rectangular. The nuclei are readily seen in the irregular parenchyma unless concealed by chloroplasts. They are usually more prominent in the large parenchyma and epidermal cells. They are more prominent in longitudinal than in cross sections of the sheath cells, because the diameter of the cells is so narrow that practically every section through a cell shows the nucleus while the length of the cells is great enough that many sections of a cell do not pass through the nucleus. The chloroplasts are larger in the sheath than in the other mesophyll cells and the markings more regular and distinct. There is also much less chlorophyll in the large than in the small cells of the mesophyll.

It is well known that the leaf symptoms of mosaic are most conspicuous on actively growing cane and on the new leaves. In a mosaic cane bearing fifteen or twenty leaves the symptoms on the oldest leaves have almost entirely disappeared. This has been observed repeatedly on canes that had been under observation from time of germination. A study of the cells of these leaves shows that the chloroplasts are always more numerous in the dark-green than in the light areas. Therefore, it appears that in the case of the light-green areas the formation of chloroplasts is inhibited but that with the exposure to sunlight this inhibition is gradually overcome.

Measurements of a number of specimens showed that the light-colored areas were always slightly thinner than the dark-green areas of the same leaf. This difference is so slight in most cases as not to be perceptible except as the result of careful measurements. Similar differences in thickness have been noted by Iwanowski and Woods on tobacco and by Melchers on tomatoes. There was no perceptible

difference in form, size, compactness of cells such as have been mentioned by Woods, Heintzel, Bouygues, Iwanowski and Chapman in tobacco and by Melchers in tomato. However, it should be remembered that tobacco and tomato are both dicotyledonous while cane is monocotyledonous and that the normal leaf structures are quite different in most species of these two great groups of plants. Therefore, the comparisons of structure of the one with the other are not altogether pertinent in this discussion.

There was no great variation in size and character of cells in mosaic cane as compared with healthy canes such as have been noted by Woods Heintzel, Bouygues, Iwanowski and Chapman in tobacco, by Melchers in tomato, and by Artschwager in potato. However, there was a very slight reduction in size of cells in mosaic canes, especially in the sheath cells of the fibro-vascular bundles.

The intracellular bodies which have been described by Kunkel could be found by careful search (Figs. 2–22, 35, 36) but they were not abundant or conspicuous. They were found in the white areas of the leaves and near the growing points in the stems of the canes and sometimes in other places, but never in healthy canes and never in the roots of diseased canes. They were more readily found in young rapidly growing plants than in old plants. They were not always found in severely diseased plants. It is doubtful if they would have attracted the attention of the writer if he had not been looking for them.

These bodies correspond quite well to those described by Kunkel. They appear to be protoplasmic and usually have a more or less definite outline. They were closely associated with the nuclei and are irregular in size and character. In most cases the bodies were in direct contact with the nuclei, but in a few cases they were slightly removed (Fig. 35). Although irregular in shape, they were seldom amoeboid (Figs. 15, 16). In some cases the plasmodial body was dense and in others very delicate and staining very slightly. In some cases these bodies appeared to be surrounded by delicate membranes while in others no such membranes could be detected. This condition has been noted by Kunkel. In some cases peculiar bodies (Fig. 24) were found in cells in which their nucleus could not be seen, but it was impossible to determine if they were or were not the same as those associated with the nuclei in other cells. No nuclei could be detected in any of the bodies under observation. In no case was the veil-like structure described by Kunkel observed.

The variations in appearance of the host nuclei (Figs. 25–39,

55–58) were quite noticeable. In some cases there was no visible change in the character of the nuclei while in others the nuclei were very much enlarged as has been previously noted by Kunkel. In some cases there were two or more nuclei in a single cell but this was also true in tissues from plants that were apparently healthy. Two or more nuclei were much more frequent in the cells of the stems than in the cells of the leaves. In some few cases a tendency to disintegration (Figs. 23, 37) was noted but the writer is very doubtful if this had any relation to the disease. However, the greatest abnormalities in the nuclei were in the white or yellow areas of the leaves. The nuclei in these areas were usually very large and very irregular in shape as compared with nuclei in the dark green areas and in normal leaves of the corresponding age and development. In these green areas and in normal plants the nuclei were comparatively small and spherical in form. A very puzzling feature of this study is that the writer found very few of the intracellular bodies in those cells which showed the greatest abnormalities (Figs. 35, 36). Many of the abnormal nuclei were very much elongated and resembled flagellata (Figs. 28–34) but there was no difficulty in determining their true character. These elongated nuclei must not be confused with the elongated nuclei of the vascular tissues; they are in the parenchyma cells and the size and form has all the appearances of being the results of stimulation. In several cases there was evidence that the nucleus had been destroyed and that the plasmodial bodies (Fig. 24) alone remained in the cells but it was difficult to say definitely whether these bodies were or were not distinct from the normal protoplasm of the cells. The enlargement of the nuclei of host cells in the case of diseases caused by fungi and bacteria has been recorded by a considerable number of workers. It is also very generally conceded that host cells which are not in direct contact with the parasite may undergo modifications. The modifications may be due indirectly to the parasite, through the direct action of its enzymes on the host cell. Therefore it is possible that the modifications of the host nuclei in the mosaic sugar-cane cells are due to enzymes. This may explain the fact that intracellular bodies were not found more frequently associated with the abnormally developed cells. It is well known that cells in host plants which are not in contact with the parasitic organisms, such as nematodes and *Plasmodiophora brassicae* develope abnormal characters. In this connection it is interesting to recall that the enzyme theory was advanced by Heintzel in 1900 and was further elaborated by Woods in 1902.

Another interesting phase of this study was brought out by a careful study of nuclei in the white areas of all the leaves of a diseased plant (Fig. 39 *a–j*). In these cases the nuclei in the outer leaves are very nearly normal while those in the inner leaves are abnormal. It appears that the nuclei tend to become normal with age and exposure to light.

The mitosis of nuclei was observed several times and was normal in every case. No intracellular bodies were seen in cells in which the nuclei were in process of division.

The variation in character of the chloroplasts (Figs. 40–50, 55–58), in mosaic and healthy tissues was fully as marked as the variations in character of the nuclei. In considering this phase of the subject the reader must remember that there are two very distinct types of chloroplasts; those found in the sheath cells and those found in the parenchyma tissues which have already been referred to (see page 12.) The chloroplasts in the white or yellow areas were fewer in number and smaller than in the green areas. In making these comparisons it is necessary to compare with the corresponding tissues, since the chloroplasts in the sheath cells are normally larger and have more definite markings than those in the mesophyll cells. Similar differences in size and number of chloroplasts in cells from white and green areas have been noted by Koning, Iwanowski, Melchers, Matz and others in their studies on mosaic of tobacco, tomato and cane.

The chloroplasts in normal sheath cells are spherical or nearly so and are usually near the cell wall, leaving an open space in the center of the cell (Fig. 40). In the case of diseased cells they are fewer in number, very much smaller and are grouped near the wall in one part of the cell (Fig 41, 43). A close examination of these chloroplasts shows that those that are normal are covered with an anastomosing net work of ridges (Fig. 49 *a*). Those from diseased cells have these ridges very much reduced (Fig 49 *b*). These points can be readily seen in lightly stained chloroplasts.

The chloroplasts in the normal mesophyll cells (Figs. 44, 46 *a*, 47 *a*) next to the sheath cells are somewhat smaller than the chloroplasts in the sheath cells. They are spherical or nearly so and have no regular distribution. In the corresponding cells of a diseased plant they are very much smaller and usually fewer in number (Figs. 45, 46 *b*, 47 *b*). The markings on these chloroplasts are much less distinct than on those in the sheath cells.

The small chloroplasts of the mosaic cells are usually spoken of

as having undergone a process of degeneration. The writer has studied the chloroplasts in the very young unrolled leaves and is satisfied that these chloroplasts are undeveloped rather than disintegrated. They have never reached normal development. Furthermore a study in a series of leaves from those just unrolling to the outermost on a plant shows that the chloroplasts of both normal and mosaic cells (figs. 50 a–i) increase in size after being exposed to light. These changes which are apparently due to age or light are so pronounced that there is very little difference in size of chloroplasts in the old leaves of a diseased plant and the chloroplasts in a leaf of the same age from a healthy plant. The writer is very doubtful if disintegration ever occurs in the case of primary infection (i. e., infection from the seed).

Matz (11) called attention to the formation of internal cavities as a result of the collapse of parenchyma cells. These lesions have been referred to later by Kunkel (8) as symptoms of the mosaic disease. These lesions are without doubt common to mosaic sugar cane, but are also found in cane with other diseases. Our studies indicate that these lesions may start very early in the growth of the cane, especially if the plant is severely infected. The writer found the early stages of these lesions (Fig. 51–54) starting by a gradual disorganizing of the walls of the xylem cells of the fibro-vascular bundles and of the parenchyma cells in contact with the bundles in both stems and leaves. This disorganization of the cell walls was not preceded by a thickening of the walls as described by Kunkel. The disorganization of cell walls by the action of enzymes produced by fungi and bacteria is well known in the case of many plant diseases, and the disorganization of the cell wall in this case is certainly analogous if not homologous to the disorganization of cell walls in a number of other well-known plant diseases. The behavior of these cells before the disintegration of the cell walls was by no means uniform. In most cases they were filled with a dense mass of deep-staining protoplasm (Fig. 51 a), but in other cases the protoplasm was not dense (Fig. 51 b). In many cases there were small deep staining bodies scattered through the protoplasm mass (Fig. 51 b). With the disintegration of the cell walls (Figs. 52, 53), these masses of protoplasm became very prominent, like numerous islands floating in a clear liquid. Numerous deep staining bodies were observed scattered among these islands. They stained exactly the same as nucleoli set free by the disintegrating protoplasm. However similar bodies were observed in section of normal tissues. The fate of this

disintegrating protoplasm was not determined. None of the intra-cellular bodies were found in it. This disintegration of cell walls was observed in the fibro-vascular bundles in both stem and leaf. The fact that it was always associated with the fibro-vascular bundles may be of some significance.

Matz has called attention to the presence of a "granular plasma material" in the parenchyma cells. Material which is probably the same as that noted by Matz has been observed by the writer. This material is frequently found in canes in advanced stages of the disease, especially old canes, but it is also found in canes with other diseases and in old canes that are apparently normal. Therefore, it is doubtful if it can be looked upon as important. However, the writer frequently noted a peculiar finely granular or protoplasmic material in cells, but it was impossible to determine its significance.

The photosynthetic action of diseased plants was very much dis-turbed. Studies were made from leaves collected before daylight and from coresponding leaves collected about 2 P. M. In the case of healthy leaves the behavior was normal; there was an abundance of starch at 2 P. M. and very little at 5 P. M. In the case of diseased leaves there was much less starch formation than in healthy leaves. A more extensive report on this phase of the subject will be given in a later paper.

SUMMARY

1. The symptoms of mosaic are more conspicuous on actively growing than on slow growing plants; and on the new than on the old leaves of a diseased plant.

2. The light areas of a diseased leaf are slightly thinner than the green areas.

3. The green areas correspond in every way to the leaves from healthy plants of the same age and variety.

4. The cells in the light area of a diseased leaf are slightly less in size than those in the green areas.

5. Intracellular bodies were difficult to find but corresponded quite well with those reported by Kunkel.

6. The host nuclei were usually very much enlarged in the dis-eased tissues, especially in the tissues of the leaves. They were frequently very irregular in form and it was very difficult to find intracellular bodies associated with them.

7. The chloroplasts were smaller and fewer in number in the mosaic than in the healthy tissues.

8. The internal cavities in both stems and leaves start with a disintegration of the cell walls.

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EXPLANATIONS OF PLATES

FIG. 1.—Cross section of normal cane leaf. Semi-diagrammatic. (*a*) Upper epidermis. (*b*) Lower epidermis. (*c*) Cuticle. (*d*) Stomata. (*e*) Large parenchyma cells, usually without chlorophyll. (*f*) Small parenchyma cell with an abundance of chlorophyll. (*g*) Sheath cells of the fibro-vascular bundles. (*h*) Schlerencyma cells of the fibro-vascular bundles. (*i*) Xylem cells of the fibro-vascular bundles.

FIGS. 2–16.—Intranuclear bodies found in the leaves of mosaic sugar cane, showing variations in character.

FIGS. 17–22.—Intracellular bodies from the stems of mosaic canes.

FIG. 23.—A degenerating nucleus and a protoplasmic mass which appears be an intracellular body.

FIG. 24.—Two bodies from cells that do not show nuclei.

FIG. 25–34.—Enlarged and abnormal nuclei from white areas of mosaic leaves.

FIG. 35, 36.—Cells from a white area of a mosaic leaf showing enlarged nuclei with intracellular bodies in close contact.

FIG. 37.—Two cells from the stem of a mosaic cane. One showed an enlarged and the other a degenerating nucleus.

FIG. 38.—Normal nucleus from healthy cane.

FIG. 39 (*a–j*).—A series of outline drawings of nuclei from mosaic tissues beginning with the outside leaf and working inward. Each letter represents a leaf. The nuclei in the outer leaves are apparently normal while those in the inner leaves are abnormal in form and size.

FIG. 40.—Cross section of normal sheath cell, showing normal nucleus and chloroplasts.

FIG. 41.—Cross section of mosaic sheath cell, showing abnormal nucleus and chloroplasts.

FIG. 42.—Longitudinal section of normal sheath cell. Nucleus 6.6 mm. Chloroplasts 4.4 mm.

FIG. 43.—Longitudinal section of mosaic sheath cell. Nucleus 6.6 mm. Chloroplasts 2.2 mm.

FIG. 44.—Normal parenchyma cells, next to sheath cells.

FIG. 45.—Mosaic parenchyma cells, next to sheath cells.

FIG. 46 *a*.—Parenchyma cell from an outside normal leaf.

FIG. 46 *b*.—Parenchyma cell from an outside mosaic leaf.

FIG. 47 *a*.—Parenchyma cell from inside mosaic leaf. Nucleus 4.4 mm. Chloroplasts 3.3. mm.

FIG. 47 *b*.—Parenchyma cell from inside mosaic leaf. Nucleus 7 mm. Chloroplasts 1.5 mm.

FIG. 48.—Parenchyma cell from mosaic leaf showing abnormal nucleus and reduced chloroplasts.

FIG. 49.—Chloroplasts from sheath cells of leaf. (*a*) Normal tissues. (*b*) Abnormal tissues. Note variations in size and markings.

FIG. 50.—Series of outline drawings of chloroplasts from leaves of

mosaic plant. The letters start with the outside leaf; the small drawing for each letter represents the relative size of the chloroplasts from the mosaic area as compared with the size of the chloroplast from the cells of the normal area. The drawings on the left in each case are from normal, while those on the right in each case are from mosaic cells.

Fig. 51. (*a* and *b*).—Two cells from fibro-vascular bundle of mosaic plant just before the breaking of the cell wall.

Fig. 52.—First stage in the disappearance of the cell walls.

Fig. 53.—Later stage in the disappearance of the cell walls.

Fig. 54.—A protoplasmic mass soon after the disentegration of the cell walls.

Fig. 55.—A section through a normal leaf.

Fig. 56.—A section through white tissue in the early stage of the disease. Parenchyma cells. Note the enlarged nuclei and reduced chloroplasts.

Fig. 57.—A section through the white area of a leaf in the more advanced stage of the disease. Parenchyma cells. Note the enlarged nuclei and reduced chloroplasts.

Fig. 58.—A section through the white area of a leaf in the more advanced stage of the disease. Sheath cells. Note the enlarged nuclei and reduced chloroplasts.

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