THE ABSORPTION SPECTRUM OF THE CHLOROPHYLL IN YELLOW-STRIPED SUGAR-CANE.

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Yellow stripe of sugar-cane is essentially characterized by the mosaic effect produced in the chlorophyll-bearing tissues, and more especially in the leaf blades, by the unequal intensity of the green color in those tissues. This unevenness in intensity is exhibited in areas whose size and pattern vary under different conditions. It ranges from deep green through shades of green, to almost white, and postulates a diminution in the amount of chlorophyll present in the lighter green areas and its almost total absence in the almost white areas.

In only two general ways could the disappearance of the pigment primarily be brought about: (1) By alterations involving the chlorophyll itself; (2) by alterations involving the chlorophyll-bearing bodies, the chloroplasts. The fact that red canes are also decolorized by the disease and, furthermore, that the young, uncolored internodes of certain varieties frequently exhibit faint purplish-red stripes when diseased, suggested an investigation of the possibility of the first alternative—*i. e.*, a general derangement of the chromogenic function in yelow-striped canes. Widbrink and Ledeboer [1] remark in this connection that in some leaves of young plants of the variety G. Z.–247 so much red coloring matter is formed under the influence of the disease that the blades take on a light chocolate tint against which the yellowish stripes and spots stand out. They further state that the cause of the striping in the stems is not due to the same cause in all varieties of sugar-cane and that in the varieties G. Z.–100, 247, 1639, 161, Yellow Batjam and many others the striping is due to the formation of anthocyanin.

Anthocyanin, carotin and xanthophyll, as well as many of the decomposition products of chlorophyll, are known to have characteristic absorption spectra. *Prima facie,* therefore, there appeared to be a strong possibility of any such wholesale modification of the normal chromogenic condition of the cane being detected by comparative spectroscopic examination of the extracted chlorophyll from both healthy and diseased leaves.

[1] Numbers in parenthesis refer to literature cited in bibliography appended.

Only newly attacked leaves were used. They were for a short time and separately extracted with ethyl alcohol after initial heating to boiling. Deep green, fluorescing solutions were obtained of each and their concentration equalized by comparative examination in the colorimeter. The spectroscopic examination of the ethyl chlorophyllide in solution was in all cases made through a ½ millimeter aperture of the jaws of the slit. Electric light was used for the illumination, the temperature of the air surrounding the globe next to the slit being from 30 to 35 degrees centigrade. The dilutions and thicknesses of solution examined will be seen in diagrams given below of the absorption spectrum repeatedly obtained.

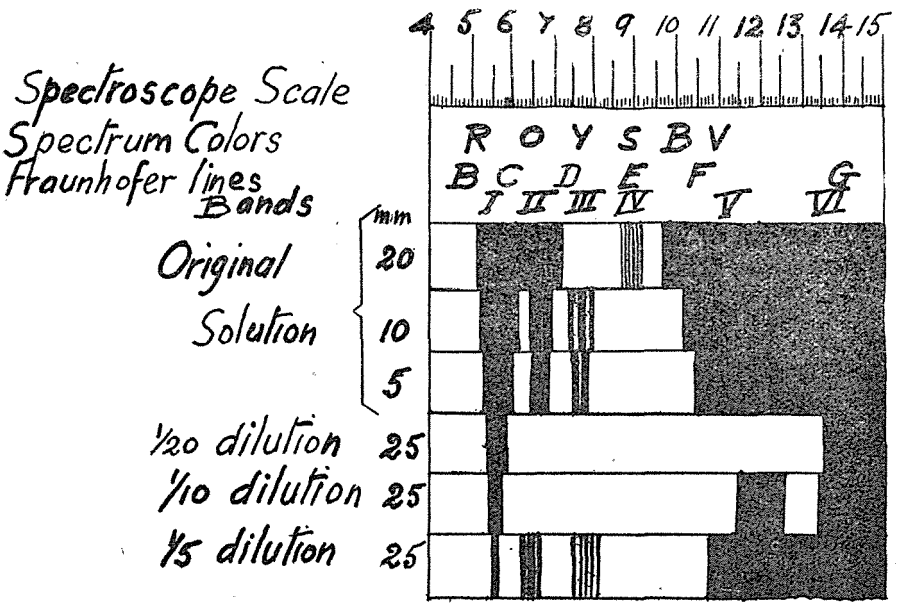

FIG. I.—Absorption spectrum repeatedly obtained by spectroscopic examination of young normal and yellow-striped sugar-cane leaves. The thickness of the layer employed is shown at the left in millimeters; the conventional letters of the Fraunhofer lines are at the top, so also the initials of the spectral colors, the spectroscope scale and the Roman numbers for the absorption bands. Aperture of the slit about one-half mm.; air temperature, 30°–35° C.

It will be apparent after glancing over the diagram that different dilutions and thicknesses of the chlorophyll solutions had to be examined before all the absorption bands could be made out. The absorption area in the more refrangible portion of the spectrum (including bands I, II, III) as well as that in the less refrangible portion (including bands V, VI) were present in all cases, their ex-

tension and distinctness depending on the conditions of examination. Band IV in the middle portion of the spectrum appeared to be the faintest of all, it having been distinctly brought out only in the most concentrated solution and when examined through the second greatest thickness employed (20 millimeters). The resolution of absorption area I, II, III into its three bands was brought about by examination of smaller thicknesses of solution (10 millimeters and 5 millimeters); that of absorption area V, VI by dilution combined with a larger thickness (25 millimeters).

It seems worth noting that band IV has been reported above as a faint band, the faintest of all, not as a dark one as represented in many discussions on the chlorophyll spectrum. In regard to this matter Dr. Edward Schunck states (2): "It should be mentioned that some of the absorption spectra figured in memoirs on chlorophyll really belong to the derivatives of the latter. Whenever in such figures band IV appears rather dark and is followed by another dark band nearer the blue end, we may conclude that the observer has worked with a specimen of chlorophyll that has undergone some change." The dark band nearer the blue end to which he refers has not been reported here as a distinct band, because although the absorption area V, VI may extend to that portion of the spectrum, we have not, nevertheless, been able to make it out as a separate band on further dilution as has been the case with bands V and VI. This has reduced to only six the total number of absorption bands reported here for chlorophyll in ethyl alcohol. Schunck (2), Allen (3), Palladin (4) and Pierce (5) report this number as six. Green (6), Goodale (7), Vines (8), Carracido (9) and Willstätter (10) report seven. Jost (11), reports a total of six, three before F and three beyond F, considering band IV before E as due not to chlorophyll but to a decomposition product of chlorophyll.

The absorption spectrum obtained for chlorophyll can thus be seen to have been fairly typical.

Now, the absorption spectra obtained for the alcoholic (ethyl) solution of chlorophyll from newly yellow-striped young leaves did not in our tests and under the same conditions exhibit any difference from the absorption spectra obtained for the alcoholic (ethyl) solution of healthy young leaves.

The four bands as figured in the diagram in the more refrangible portion of the spectrum are specially characteristic of chlorophyll. They constitute a certain test for this substance; so much so that Schunck (2), referring to the fact, says that chlorophyll "may ac-

cordingly be defined as the substance which in solution shows this particular absorption spectrum."

Should there have occurred any decomposition of the chlorophyll in the diseased leaves, the absorption spectra obtained from the examination of the alcoholic solution of their chlorophyll would not have, in the first place, been identical with the absorption spectra similarly obtained from healthy leaves. New bands or a modification of the old bands would in all probabilities have been noted, since other cane pigments and many decomposition products of chlorophyll are known to have characteristic absorption spectra. The fact that the decomposition of the chlorophyll would, in the case of the sugar-cane, have developed in an acid medium would have defined all the more the changes to be expected since the acid decomposition products of chlorophyll are fairly well known.

Although the tests above described were not as numerous nor performed with as many solvents as might have been desirable, they warrant the belief that the disappearance of the pigment in yellow stripe is not primarily due to a decomposition of the chlorophyll as such.

(To be continued.)

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