HISTOCHEMICAL STUDY ON THE RATOON STUNTING DISEASE OF SUGARCANE

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Introduction

The ratoon stunting disease is now recognized as one of the major diseases of sugarcane in most cane sugar producing areas of the world. The external symptoms of the disease are more evident in ratoon crops than in plant cane. These are stunting and reduction in number and diameter of the stalks in a stool. These symptoms however, are not specific for the disease. The internal symptoms are a pink discoloration of the growing point of young shoots and an orange to reddish discoloration at the base of the node of mature stalks. These symptoms have been used in identification of the disease. However, the fact that no pathogenic organism has been found associated with the disease, together with the fact that the disease is juice transmissible and virus-like particles found to be associated with the disease (Forbes and Ling, 1960) is considered as sufficient evidence to classify the causal agent as a virus. The disease can be controlled by using healthy seed cane or by planting seed pieces treated either with hot-water or hot-air. In order to reduce mechanical transmission in the field, sterilization of cutting knives is recommended.

Since symptoms of ratoon stunting disease are not always reliable, the present study was made in an attempt to find additional diagnostic procedures for determining the presence of the disease in a plant through histochemical methods.

Materials and Methods

Simple Staining Test

In this test, attempts were made to determine whether or not materials which could

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be demonstrated by simple staining techniques were present in diseased tissues. Nine dyes , viz.:

The nitro dye
1. Martius yellow
The azo dye
2. Orange G
The quinone-imide dye
3. Safranin 0
The phenyl methane group of dyes
Diamino-triphenyl methane
4. Light green
Triamino-triphenyl methanes
5. Acid fuchsin
6. Anilin blue
7. Basic fuchsin
8. Methyl violet
The xanthene dye
9. Eosin Y

were used. They were dissolved in distilled water at a concentration of 0.5%. Fresh sections of growing point, internode, node, leaf sheath, leaf blade, and midrib of both healthy and diseased C. P. 36-105, C. P. 44-101, and C. P. 52-68 collected from both field and greenhouse were cut by sliding microtome at a thickness of 50 microns or less. Several sections were used for each staining, as replications. Tests were made from May to August, 1959.

Section Treated with Acid or Alkali

Sections of node and internode of C. P. 44-101 at a thickness of 50 microns were used for determining whether or not any differences between healthy and diseased canes could be induced by treating with acid or alkali. The acid solutions used were glacial acetic acid, 3N hydrochloric acid, and 6N sulphuric acid. In addition to 3N HCl, 1/16, 1/8, 1/4, 1/2, and concentrated HCl were used. The alkalies were 3N ammonium hydroxide, 1% potassium hydroxide, and 3N sodium hydoxide. The tests were made in June 1959.

Histochemical Test

Sections of growing point, internode, node, leaf sheath, leaf blade, and midrib of C. P. 44-101 were used for the histochemical tests. Both healthy and diseased materials were obtained from the field. Only freshly prepared sections were used. Tests were made from June to August, 1959.

There were 51 microchemical techniques as given by Johansen (1940), Chamberlain (1932), Hill and Orton (1938), and Lindner, Kirkpatrick, and Weeks (1950) used for determining the following compounds and minerals:

A. Alkaloids
B. Amino acids:
1. Amino acids
2. Asparagine
3. Leucin
4. Tyrosine
C. Callose
D. Carbohydrates:
1. Amylodextrin
2. Cane sugar
3. Grape sugar
4. Inulin
5. Starch
6. Sugars
E. Cellulose
F. Chitin
G. Cutin, suberin
H. Enzyme: Catalase
I. Fats
J. Formaldehyde
K. Glucosides:
1. Amygdalin
2. Anthocyanin
3. Arbutin
4. Saponin
5. Tannin
L. Hemicellulose:
1. Amyloid
2. Methyl pentoses
M. Lipoids:
1. Lecithin
2. Phytosterol
N. Mineral substances:
1. Ammonium
2. Calcium
3. Iron
4. Manganese
5. Nitrates

6. Phosphate
7. Potassium
8. Silicon
9. Sulphate

O. Organic acid:
1. Citric acid
2. Formic acid
3. Tartaric acid

P. Phenol:
1. Phenol
2. Phenolic compound

Q. Pigment: Carotin

R. Proteins:
1. Aleurone
2. Protein

S. Secretion: Resin

In addition to the above microchemical tests, there were three techniques which gave positive result in studies, as follows:

A. Phloroglucin and hydrochloric acid for lignin: Fresh sections were placed on a slide in a drop of a solution of 0.1 gm phloroglucin in 10 ml of 95% alcohol and a drop of 25% hydrochloric acid and covered with a coverslip. The appearace of a red to red-violet color indicates the presence of lignin (Johansen, 1940, Rawlins, 1933, Sass, 1958). Zinc chloride was prepared by dissolving 32 gm of potassium iodide and 100 gm zinc chloride in 34 ml of water to which metallic iodine was added as much as it would dissolve. This was used as a counterstain (Artschwager, 1960). Since the lignified tissues stained red which is in marked contrast to unstained hyaline tissues, the counterstain was infrequently used. After staining, sections were examined immediately with the microscope.

B. The Maule test (Johansen, 1940): This is a test for lignin oxide. Sections were covered on a slide with 1% neutral aqueous potassium permanganate for 15 to 20 minutes. After thoroughly washing in distilled water, sections were placed in 2% hydrochloric acid, and washed again after the dioxide has been dissolved. A few drops of ammonium hydroxide solution were added to the sections. A deep red color develops in the lignified elements.

C. Feulgen's reaction for nucleoproteins (Pantin, 1948): Fresh sections were placed in normal HCl solution for one minute and then in normal HCl solution at 60°C for 5 minutes, or up to 15 minutes. They were removed to reduced basic fuchsin for 2 hours or longer, and were then washed thoroughly, first in three changes of the HCl-bisulphite washing solution, and later in distilled water for 30 minutes. Finally, they were counterstained with 0.25% light green and examined with the microscope. The reduced basic fuchsin was prepared by dissolving 1 gm basic fuchsin in 200 ml of boiling distilled water. When it cooled to about 50°C, it was filtered and 20 ml of normal HCl were added. Finally, 1 gm anhydrous sodium bisulphite was added. One or two days after preparation, the solution lost the pink color and was used. The washing solution was freshly prepared every time. It was made of 10 ml 10% sodium bisulphite, 10 ml normal HCl, and 200 ml distilled water.

Since the results from the above three staining techniques were very similar, the simpler, more convenient phloroglucin and hydrochloric acid test was used in studies for showing the percentage of vascular bundle with lignified phloem cells. Fresh sections were examined in water under microscope for the presence of discolored vessel and again after being treated with phloroglucin and hydrochloric acid for lignified phloem cells.

Kuijper's (1915) nomenclature was used in this paper. According to his system, the highest leaf with visible dewlap is given the notation +1. The older leaves are consectively number +2, +3, etc., while the younger ones receive the number ±0, −1, −2, etc. Each joint has the same number as the leaf it carries.

Results

Simple Staining Test

Sections of different parts of sugarcane plants were stained with solutions of acid fuchsin, anilin blue, basic fuchsin, eosin Y, light green, Martius yellow,

methyl violet, orange G, and safranin O, respectively. After staining, sections were examined with microscope. Cells and tissues examined were: epidermis, sclerenchyma, parenchyma, bundle sheath, sclerenchyma cap, phloem (sieve tube and companion cells), large pitted vessel (scalariform vessel or metaxylem), smaller vessel (protoxylem, annular or spiral vessel) (Artschwager, 1925, 1940, Edgerton, 1958). Any differences in color intensity, as well as any special substances stained inside the cells of diseased sections were noted. Comparisons of healthy and diseased sections were carefully made. Although there was a total of about 800 sections examined, no significant difference was observed in between healthy and diseased sections as a result of the simple staining test. However, some discolored vessels were often present in sections of nodes and internodes of diseased canes.

Sections Treated with Acid or Alkali

Four acids and three alkalies were used for treating the sections. A total of about 160 sections of both healthy and diseased canes was treated and examined with the microscope. No significant difference between diseased and healthy sections has been found. However, the yellow color of smaller vessels in vascular bundles of +1 to +3 internodes was induced by conc. HCl. In some sections, 1/2 conc. HCl gave the same result but not the further dilutions. The average percentages of such vascular bundles from three repeated tests were 18.4 and 28.1 for healthy and diseased tissues, respectively.

Histochemical Test

Sections of different parts of both healthy and diseased sugarcane plants were tested by microchemical techniques for compounds and minerals, viz., aleurone, alkaloids, amino acids, ammonium, amygdalin, amylodextrin, amyloid, anthocyanin, arbutin, asparagine, calcium, callose, cane sugar, carotin, catalase, cellulose, chitin, citric acid, cutin, fats, formaldehyde, formic acid, grape sugar, inulin, iron, lecithin, leucine, manganese, methyl pentoses, nitrate, phenol, phenolic compound, phosphate, phytosterol, potassium, protein, resin, saponin, silicon, starch, sugar, sulphate, tannin, tartaric acid, and tyrosine. A total of about 2,100 sections was treated and examined. The differences between healthy and diseased tissues were not significant or not repeatable.

Discoloration of Vessel

As mentioned before, in microscopic examination of sections of nodes and internodes of diseased sugarcane plants, almost always the discolored vessels were observed without any staining technique. No such discolored vessels were seen in sections of healthy sugarcane. The color varied from light yellow to orange yellow, under the microscope with natural light. The yellow color was located only on the wall of some vessels but was spread throughout the entire lumen of other vessels, in the cross sections. In the latter case, the vessels

were apparently plugged by the yellow substance. There may be a single large pitted vessel (scalariform vessel or metaxylem), both two large pitted vessels, the smaller vessel or vessels (protoxylem, annular or spiral vessel), a large pitted vessel and smaller vessel or vessels, or entire vessels become discolored and plugged with the substance in a vascular bundle. The discolored vessels were more predominant in sections of nodes of stems but were not found in the vessels of root, leaf sheath, or leaf blade. The discolored vessels were not restricted to the older cane stalks, but were present also in sections at the base of stems of young plants grown from diseased planting material, only one month after planting. These young canes did not show any typical symptoms of ratoon stunting disease. The yellow discolored vessels were considered to be one of the major characteristics of diseased cane, as observed through microscopic examination, as compared with the healthy stalks which were free from other diseases or insect injuries.

The Nature of the Yellow Substance

In cross sections of diseased stems, especially from the nodal region, the vessels were plugged with substance. Steindl (1950), Steindl and Hughes (1953), King and Steindl (1953), Hughes and Steindl (1956), and Artschwager (1960) called it a gummy substance. Sections of C. P. 36–105, C. P. 44–101, and C. P. 53–1, in which the vessels were plugged with such a substance, were immersed in chemical solutions. After two days, they were examined with the microscope again. Several sections were used as replicates for each treatment. The results, as shown in Table 1, indicated that the substance was insoluble in the following solvents: water, alcohol, ether, carbon disulfide, potassium hydroxide,

Table 1. Tests for the reaction to chemicals and stainings of the yellow substance in vessels of ratoon stunting diseased canes.

Chemical or staining	C. P. 36-105		C. P. 44-101		C. P. 53-1
Water	−	−	−	−	−
95% Ethyl alcohol	−	−	−	−	−
Ether	−	−	−	−	−
Carbon disulfide	−	−	−	−	−
3N Nitric acid	−	−	−	−	−
Nitric acid and heated	+	+	+	+	+
3N Potassium hydroxide	−	−	±	−	−
HCl and $KClO_3$	+	+	+	+	+
Phloroglucin and HCl	red	red	red	red	red
Maule test		no		no	no

+ = Color intensity of yellow substance reduced.
− = Not dissolved. no = Not change to deep red color.

sulphuric acid, and cold nitric acid. Hot nitric acid and a mixture of hydrochloric acid and potassium chlorate reduced the intensity of the yellow color, and it was stained red by phloroglucin and hydrochloric acid. The yellow substance did not change to a deep red color which is the indication of the positive reaction of the Maule test for lignin.

Lignified Phloem Cells

In severely diseased canes, the phloem cells in cross sections of node and internode were seen to have a slight orange color without staining. However, this was unusual. When stained, one to several phloem cells, or in some instances, the entire phloem of some vascular bundles of diseased sections from node and internode reacted to the following treatments: 1) phloroglucin and hydrochloric acid; 2) the Maule test; and 3) Feulgen's reaction for nucleoproteins. By the first two techniques, which were known for testing of lignin, the phloem cells stained red while they stained violet in color with Feulgen's reaction. Results from nodal sections of both healthy and diseased canes, collected from field, stained with Feulgen's reaction were given in Table 2. The same results were obtained with the phloroglucin and hydrochloric acid test, and with the Maule test. These positive results indicated that lignin was present in the phloem cells of diseased sections. In other words, the stained phloem cells were lignified. Artschwager (1960) used "lignification of sieve tube" or "lignified phloem" for the description of these stained cells. Phloem consists of sieve tubes and companion cells (Artschwager, 1925, Edgerton, 1958), and both took the stain in studies reported here. Only rarely did the entire phloem of a vascular bundle stain red. "Lignified phloem cells" seems to be a more appropriate description of the material studied here. Seldom was the staining restricted to the cell wall; usually both the cell wall and cell contents were stained. Stained phloem cells were found in sections of diseased nodes

Table 2. Reaction of phloem cells of healthy and ratoon stunting diseased sugarcane to Feulgen's stain.

Variety	Stalk No.	Number of vascular bundles examined		Percentage of vascular bundles reacting to stain	
		Healthy	Diseased	Healthy	Diseased
C. P. 36-105	I	993	710	0	3.66
	II	771	856	0	4.32
	Total	1,764	1,566	0	4.02
C. P. 44-101	I	682	896	0	1.90
	II	1,198	859	0	1.05
	Total	1,880	1,755	0	1.48

and internodes, but not in sections of diseased roots, leaves, or leaf sheaths. The vascular bundles which showed lignified phloem cells were not always associated with yellow discolored vessels. No lignified phloem cells were found in sections of healthy plants free from other diseases or insect injuries so far tested.

Discolored Vessels and Lignified Phloem Cells of Different Parts of Sugarcane

The results of examinations of sections of different parts of C. P. 44-101, grown from diseased seed setts for 6½ months, were shown in Table 3. Neither were discolored vessels nor lignified phloem cells observed in sections of leaf

Table 3. Percentages of vascular bundles showing discolored vessels and lignified phloem cells of different parts of ratoon stunting diseased C. P. 44-101 (6½ months old).

Portion of cane	No. of vascular bundles examined	% of vessels discolored	% of phloem cells lignified
±0 Leaf blade & midrib	943	0	0
+3 Leaf blade & midrib	940	0	0
Leaf sheath	348	0	0
Growing point	8 sec.	0	0
+3 Internode	972	0	0
+3 Node	1,368	0.29	0.88
+4 Internode	1,436	0	0.42
+4 Growth ring	2,169	5.35*	0
+4 Node	2,284	0.26	1 31
+5 Internode	1,594	0	4.02
+5 Node	1,809	0.77	3.98
+6 Internode	1,706	1.70	7.93
+6 Growth ring	2,561	6.21*	5.66
+6 Node	1,821	0.55	13.07
Basal portion of stem	1,810	8.34	7.85
Root	15 sec.	0	0

sec.=Sections.

%=Percentage based on the number of vascular bundle in which showed discoloration or lignification, same meaning in the following tables.

* The wall of vessel discolored only. No plugging substance has been found in the vessels.

blades, leaf midribs, leaf sheaths, or terminal growing points of shoot or in sections of roots of plant. However, discolored vessels were present in sections of stems. They were more predominant in the nodes, especially in the basal part of the shoot than in other parts of the plant. The percentage of vascular bundles showing discolored vessels was higher in sections of growth ring of stem. However, these discolored vessels, only the wall of the vessel was yellow

in color; none of the vessels being plugged with the yellow substance. Lignified phloem cells appeared in vascular bundles of nodes and internodes of plant, there being a tendency for a higher percentage to occur in the lower part of the shoot. Plants grown from seed pieces treated with hot-water at 50°C for 3 hours were used as checks. From treated plants, a total of 11,778 vascular bundles and 23 sections of growing points and roots were examined. None showed discolored vessels or lignified phloem cells.

Discolored Vessels and Lignified Phloem Cells of Different Nodes of Sugarcane

Yellow discolored vessels and lignified phloem cells were more prevalent in nodes than in internodes, sections from nodes were made from diseased stalks of C. P. 36–105, collected from the field, for determining the frequency of vascular bundles which showed discolored vessels or lignified phloem cells. The results were shown in Table 4. It indicated that lignified phloem cells were present in every examined node of the mature diseased stalks, while discolored vessels were found in all nodes of diseased stalks except nodes designated +3 and +4. Percentages of both discolored vessels and lignified phloem cells varied among nodes. However, there was a tendency that higher percentages occurred in the lower nodes. In average, 2.00% and 1.61% were obtained for vascular bundles showing discolored vessels and lignified phloem cells, respectively.

Table 4. Average percentages of vascular bundles showing discolored vessels and lignified phloem cells of different nodes of ratoon stunting diseased C. P. 36–105 from field.

Sequence of node	No. of vascular bundles examined	% of vessels discolored	% of phloem cells lignified
+ 3	805	0	0.75
+ 4	906	0	0.55
+ 5	1,037	0.20	0.59
+ 6	728	0.44	1.28
+ 7	856	0.59	1.31
+ 8	733	0.12	1.23
+ 9	869	1.27	1.63
+10	905	1.43	1.43
+11	884	1.52	1.88
+12	685	2.14	1.71
+13	624	2.36	2.79
+14	541	2.70	1.83
+15	1,020	4.20	1.87
+16	1,019	3.91	2.09
+17	552	6.74	3.81
+18	1,038	5.01	2.40
Total	13,202	2.00	1.61

Table 5. Percentages of vascular bundles showing discolored vessels and lignified phloem cells of the basal portion of shoots of C. P. 44-101 of different ages.

Planting material	Age month	No. of vascular bundles examined	% of vessels discolored	% of phloem cells lignified
Ratoon stunting diseased	1	3,545	0.62	0
	1½	3,726	1.18	0
	2	5,634	1.46	0.04
	2½	3,549	2.65	0.14
	3	5,385	2.79	0.54
	3½	2,362	3.68	1.44
	4	5,450	6.17	0.75
	4½	2,015	15 24	2.78
	5	5,477	7.79	6.77
	Total	37,143	4.17	1.45
Healthy (absence of symptoms)	1	2,103	0	0
	1½	2,126	0.89	0
	2	2,287	0.87	0
	3	2,173	0	0
	4	1,663	0	0
	5	2,324	0	0
	Total	12,676	0.31	0
Hot-water 50°C-2 hours treated	2	1,808	0	0
	2½	1,515	0	0
	3	1,548	0	0
	3½	1,448	0	0
	4	1,346	0	0
	4½	1,521	0	0
	5	1,711	1.11	0.06
	Total	10,897	0.17	0.01
Hot-water 50°C-3 hours treated	2	1,430	0	0
	2½	1,731	0	0
	3	1,499	0	0
	3½	1,696	0	0
	4	1,662	0	0
	4½	1,597	0	0
	5	1,742	0	0
	Total	11,357	0	0

Discolored Vessels and Lignified Phloem Cells of C. P. 44–101 of Different Ages.

Four different sources of planting materials of C. P. 44–101, viz. diseased, healthy (absence of symptom), hot-water 50°C–2 hours treated, and hot-water 50°C–3 hours treated were planted in the greenhouse. They were examined for the vascular bundles showing discolored vessels and lignified phloem cells at a half month to one month intervals. Sections were always made from the basal portion of the shoots. Several sections were usually used for each treatment. A total of 72,073 vascular bundles was examined and the results were shown in Table 5. Plant in which discolored vessels were observed was only one month old. This was the earliest date examined after planting. The youngest plant which showed lignified phloem cells was two months old. Generally speaking, the older the plant, the higher the percentage of vascular bundles that showed discolored vessels and lignified phloem cells. In some instances, discolored vessels and lignified phloem cells were found in shoots arising from healthy seed pieces which showed no symptoms and from seed cuttings treated with hot-water at 50°C for 2 hours, but not in shoots from planting materials treated with hot-water at 50°C for 3 hours.

Discolored Vessels and Lignified Phloem Cells in Different Varieties

Diseased seed pieces of four sugarcance varieties, viz., C. P. 29–116, C. P. 43–47, C. P. 44–101, and C. P. 52–68 grown in the greenhouse were examined for the vascular bundle showing discolored vessels and lignified phloem cells at a half month to one month intervals. Sections were always made from the basal portion of shoots. The results of plants from one to five months old were condensed in Table 6. The youngest plant of all four varieties examined in which discolored vessels were observed was only one month old. This was the earliest date examined after planting. The ages of youngest plants in which lignified phloem cells were found were ½, 2, 3, and 4 months respectively for C. P. 52–68, C. P. 44–101, C. P. 29–116, and C. P. 43–47. There was the tendency that the higher percentages of vascular bundles showing discolored vessels and

Table 6. Average percentages of vascular bundles showing discolored vessels and lignified phloem cells of the basal portion of young shoots (one to five months old) from ratoon stunting diseased planting materials of four sugarcane varieties.

Variety	No. of vascular bundles examined	% of vessels discolored Average (Range)	% of phloem cells lignified Average (Range)
C. P. 26-116	18,334	0.61 (0- 2.43)	1.99 (0-17.38)
C. P. 43-47	18,116	1.44 (0- 8.81)	0.19 (0- 1.14)
C. P. 44-101	37,143	4.17 (0-15.24)	1.45 (0-12.88)
C. P. 52-68	21,455	0.64 (0- 1.45)	0.30 (0- 2.54)

lignified phloem cells were usually found in the older shoots. There were some variations of the number of vascular bundles showed discolored vessels or lignified phloem cells among varieties. Comparatively speaking, the percentages of vascular bundles showing discolored vessels were higher in C. P. 44–101 and C. P. 43–47 and lower in C. P. 52–68 and C. P. 29–116. The percentages of vascular bundles showing lignified phloem cells were found higher in C. P. 44–101 than in other varieties.

Discussion

Nine dyestuffs, four acids, three alkalies, and 54 histochemical techniques for 48 compounds and minerals, were tried in an attempt to differentiate the diseased cane from the healthy through the reactions shown in tissues under the microscope. Except for 1) Feulgen's reaction for nucleproteins; 2) the Maule test for lignin oxide; and 3) phloroglucin and hydrochloric acid for lignin, none gave significant and repeatable results. Steindl (1950) mentioned that diseased stems usually contained a higher proportion of starch than did healthy ones but this factor did not appear to be sufficiently constant to be of much diagnostic value. However, concentrated hydrochloric acid and sometimes 1/2 conc. HCl induced the yellow color of smaller vessels in vascular bundles of +1 to +3 internodes, but the only difference between diseased and healthy materials was a higher percentage of such bundles in the diseased plants.

In cross sections of diseased stems, especially from the nodal region and the basal portion of the young shoot, some vessels were yellow in color and some were plugged with substance. Hughes and Steindl (1956), King and Steindl (1953), Steindl and Hughes (1953), and Artschwager (1960) called it a gummy substance. Khanna, et al. (1958) pointed out that this substance was insoluble in water, acetic acid, alcohol, xylol, and clove oil. Rawlins (1933) defined wound gum as a substance often found in vessels of plants adjacent to wounds and in wood invaded by wood-decaying fungi. It is insoluble in water and stains red with phloroglucin in hydrochloric acid. Schneider (1945) applied wound gum to the substance in the necrotic sieve tubes of buckskin-diseased peach and cherry. He described the substance as giving a positive test with phloroglucin and hydrochloric acid and a negative, or a weakly positive, reaction with the Maule reagents for lignin. It was insoluble in a mixture of potassium chlorate and hydrochloric acid, and in hot 10% nitric acid. Hewitt (1938) regarded wound gum as a water insoluble material which reacted positively to phloroglucin and hydrochloric acid, but did not color with the Maule reaction. According to Küster (1925), wound gum is characterized by the following properties: it does not dissolve or swell in water, it is insoluble in alcohol, ether, carbon

disulphide, potassium hydroxide, sulphuric acid, cold nitric acid, and cold aqua regia. It is soluble in warm nitric acid and in a mixture of hydrochloric acid and potassium chlorate. The results from the present study indicated that the substance in the vessels of ratoon stunting diseased plants was insoluble in water, alcohol, ether, carbon disulfide, potassium hydroxide, sulphuric acid, and cold nitric acid. Hot nitric acid and a mixture of hydrochloric acid and potassium chlorate reduced the intensity of the yellow color of the substance. It stained red by phloroglucin and hydrochloric acid, but it did not change to a deep red color by the Maule test (Table 1). Therefore, the term "wound gum" seems applicable to the yellowish substance found in the vessels of plants having ratoon stunting disease.

Some phloem cells of some vascular bundles of diseased nodes and internodes stained red with phloroglucin and hydrochloric or the Maule test, and they stained violet by Feulgen's reaction for nucleoproteins. The positive results with the Feulgen's reaction indicated the presence of nucleoproteins in the stained phloem cells. Not only were diseased phloem cells stained by this reaction, but also bundle sheaths and vessels of both healthy and diseased sections took the stain. It is doubtful that the Feulgen's reaction is a specific test for nucleoproteins only. The additional reactions of the diseased phloem cells to phloroglucin and hydrochloric acid, and the Maule test, suggested that the staining in phloem cells was because of the presence of lignin in the cells. In other words, the stained phloem cells were lignified. By using phloroglucin and hydrochloric acid, Artschwager (1960) pointed out lignified sieve tubes in sections of diseased plants. Among these three staining techniques, the phloroglucin and hydrochloric acid test was found to be the simplest and most convenient for demonstrating the lignified phloem cells in sections of diseased stems.

Vascular bundles showing yellow discolored vessel and lignified phloem cell have been observed in sections of diseased plants. Both may be considered as diagnostic characteristics for the ratoon stunting disease, as observed through microscopic examination. The lignified phloem cells were clearer in appearance when the sections were stained with phloroglucin and hydrochloric acid. However, both of these characteristics have been seen occasionally in the sections of the basal portion of young shoots arising from supposedly healthy seed pieces and planting material treated with hot-water at 50°C for 2 hours. This reduces the value of using discolored vessels and lignified phloem cells as diagnostic characteristics for the disease. However, as mentioned by Hughes and Steindl (1956), the absence of symptoms does not mean that the plants are healthy,

and the 50°C hot-water treatment of seed pieces for 2 hours is not always entirely effective for ratoon stunting disease control. These facts might be used for explaining why discolored vessels and lignified phloem cells appeared in sections as mentioned above, since there is no way to certify that these materials were completely free from ratoon stunting disease. From results reported here, it is concluded that the discolored vessels and lignified phloem cells could be used as additional diagnostic characteristics for the ratoon stunting disease.

Summary

1. In a total of about 800 sections of sugarcane plant simply stained with nine dyestuffs, no difference between healthy and ratoon stunting diseased materials was observed.

2. No significant difference was found between healthy and diseased sections treated with acids or alkalies.

3. A total of about 2,100 sections of different parts of healthy and diseased canes was treated, using 54 histochemial techniques for 48 compounds and minerals. The only tests which gave significant and repeatable results were staining phloem cells of the diseased sections by 1) Feulgen's reaction for nucleoproteins; 2) the Maule test for lignin oxide; and 3) phloroglucin and hydrochloric acid for lignin. It was demonstrated that the stained phloem cells were lignified. The lignified phloem cells were found in sections of stems, but not in roots, leaves, or leaf sheaths of diseased plants.

4. Yellow discolored vessels, usually plugged with a gummy substance, were usually present in sections of diseased stems. They were prevalent in the nodal region and the basal portion of the young shoot, but not in roots, leaves, or leaf sheaths.

5. The term "wound gum" was proposed for the yellowish substance found in the vessels of diseased sections through its chemical properties.

6. Percentages of vascular bundles showing discolored vessel and lignified phloem cells varied among nodes of mature diseased stalks. However, there was a tendency for higher percentages to occur in the lower nodes.

7. Discolored vessels were observed in sections of young shoots of one month old and older, arising from diseased seed pieces, but lignified phloem cells appeared in older shoots only. There was a tendency for the percentages of vascular bundles showing discolored vessels and lignified phloem cells to increase with the increasing age of the plant.

8. Percentages of vascular bundles showing discolored vessels were higher in young shoots of C. P. 44–101 and C. P. 43–47 arising from diseased seed pieces than in C. P. 52–68 and C. P. 29–116. Lignified phloem cells were found more frequently in C. P. 44–101 than in other varieties.

9. Both discolored vessels and lignified phloem cells could be considerd as additonal diagnostic characteristics for ratoon stunting disease of sugarcane.

甘蔗矮化病組織化學之研究

林 克 治

本研究之目的在於以組織化學方法比較矮化病病蔗與健蔗之差異，應用於矮化病之鑑定。採集病蔗及健蔗，將其各部份做成切片，經藥品處理後，用顯微鏡觀察其異同。供試藥物計有染料九種、酸四種、鹼三種，所用組織化學法則有五十四種。所得結果除下列三種組織化學方法外，其他均不明顯或不穩定。有效三種方法爲：1. Feulgen's reaction for nucleoproteins、2. Maule test for lignin oxide、3. phloroglucin and HCl for lignin。有病甘蔗之莖部，尤其是節部附近，其維管束內之韌皮部細胞，經第一種方法處理後呈紫色，經後兩種方法處理者呈紅色，顯示其木質化。惟病莖內具有此種木質化韌皮部細胞的維管束爲數甚少，而於一個維管束內木質化的韌皮部細胞爲數亦不一致。且此種現象僅見於莖部，病蔗之其他部份，如葉片、中肋、葉鞘、生長點、根部及健蔗之各部份的韌皮部細胞，均無此現象。上記三種方法中，以最後一種最爲簡便。

病蔗之莖部，尤其是節部附近，維管束內的導管，勿須染色，常可在顯微鏡下觀察到其變色者，其顏色由淺黃至桔黃，同時導管內常充滿一種膠狀物質，由此物質之化學性狀，根據 Küster 之描敍，擬稱之爲 "wound gum"。病蔗之其他部份以及健蔗各部份維管束內之導管，均無此現象。

變色之導管及木質化之韌皮部細胞在病蔗莖部各節間之數目不一，以基部者較多，生育期中之蔗莖，以較老者爲多，品種間亦有差異。惟此兩種現象，似均有助於甘蔗矮化病之鑑定。（摘要）

Literature Cited

ARTSCHWAGER, E. Anatomy of the vegetative organs of sugar cane. *Jour. Agr. Res.* 30: 197-221, 1925.

ARTSCHWAGER, E. Morphology of the vegetative organs of sugarcane. *Jour. Agr. Res.* 60: 503-549, 1940.

ARTSCHWAGER, E. Sieve tube lignification in sugarcane and its significance in relation to the ratoon stunting disease. *Proc. 10th Congr. Int. Soc. Sug. Cane Technol.* pp. 1077-1083, 1960.

CHAMBERLAIN, C. J. Methods in plant histology. 5 th rev. ed. The University of Chicago Press, Chicago. 416 pp., 1932.

EDGERTON, C. W. Sugarcane and its diseases. 2nd rev. ed. Louisiana State University Press, La. 301 pp., 1958.

FORBES, I. L. and K. C. Ling. Particles associated with the ratoon stunting diseas of sugarcane. *Sugar Jour.* 23: 15, 1960.

FORBES, I. L. and K. C. LING. Viruslike particles associated with the ratoon stunting disease of sugarcane. (Abstr.) *Phytopathology* 50: 635, 1960.

HEWITT, W. B. Leaf-scar infection in relation to the olive-knot disease. *Hilgardia* 12: 41-71, 1938.

HILL, L. M. and C. R. ORTON. Microchemical studies of potato tubers affected with blue stem disease. *Jour. Agr. Res.* 57: 387-392, 1938.

HUGHES, C. G. and D. R. L. STEINDL. Some further development in the study of ratoon stunting disease in Queensland. *Proc. 9th Congr. Int. Soc. Sug. Cane Technol.* pp. 1012-1022, 1956.

JOHANSEN, D. A. Plant microtechnique. McGraw-Hill Book Co., Inc., New York. 523 pp., 1940.

KHANNA, K. L., S. L. SHARMA, R. C. SRIVASTAVA, and J. N. SINHA. Search for ratoon stunting of sugarcane in Bihar. *Proc. Indian Acad. Sci. Sec. B.* 47: 1-14, 1958.

KING, N. J. and D. R. L. STEINDL. The relationship between varietal yield deterioration and ratoon stunting disease. *Proc. 8th Congr. Int. Soc. Sug. Cane Technol.* pp. 851-861, 1953.

KUIJPER, J. De groei van bladschijf, bladscheede en stengel van het suikerriet. *Archief Suikerind. Ned.-Indië* 23:528-556, 1915.

KÜSTER, E. Pathologische Pflanzenanatomie. 3. Aufl. Gustav Fisher, Jena. 558 pp., 1925.

LINDNER, R. C., H. C. KIRKPATRICK and T. E. WEEKS. A simple staining technique for detecting virus diseases in some woody plants. *Science* 112: 119-120, 1950.

PANTIN, C. F. A. Notes on microscopical technique for zoologists. Cambridge University Press. 77 pp., 1948.

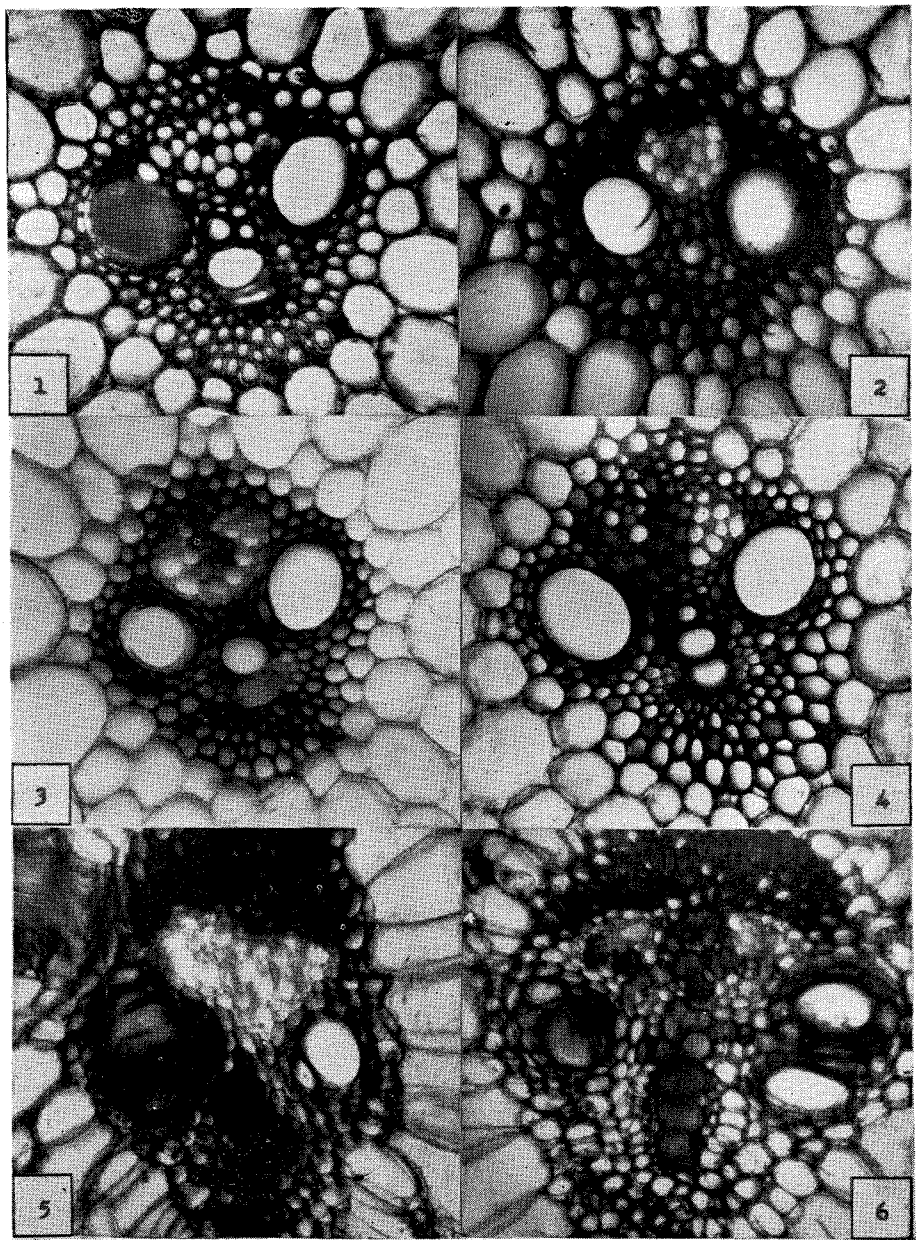
RAWLINS, T. E. Phytopathological and botanical research methods. John Wiley & Sons, New York. 156 pp., 1933.

SASS, J. E. Botanical microtechnique. 3rd ed. Iowa State College Press, Iowa. 228 pp., 1958.

SCHNEIDER, H. Anatomy of buckskin-diseased peach and cherry. *Phytopathology* 35: 610-635, 1945.

STEINDL, D. R. L. Ratoon stunting disease. *Proc. 7th Congr. Int. Soc. Sug. Cane Technol.* pp. 457-465, 1950.

STEINDL, D. R. L. and C. G. HUGHES. Ratoon stunting disease. *Cane Growers' Qurt. Bull.* 16: 79-95, 1953.



Explanation of Plate

Cross sections of internode (Figs. 1-4) and node (Figs. 5-6) of ratoon stunting diseased N:Co 310 collected from field in Tainan. All sections were made by free hand.

Fig. 1. A vascular bundle with one large pitted vessel (metaxylem, scalariform vessel) which was discolored and plugged with the "wound gum".

Figs. 2-4. Sections stained with phloroglucin and hydrochloric acid, showing various number of lignified phloem cells in the vascular bundle.

Fig. 5. A vascular bundle of node, showing one large pitted vessel and smaller vessels (protoxylem, annular or spiral vessels) were discolored and plugged with the "wound gum".

Fig. 6. Section stained with phloroglucin and hydrochloric acid, showing a vascular bundle of node with discolored and plugged with "wound gum" vessels and lignified phloem cells.