SEARCH FOR RATOON STUNTING DISEASE OF SUGARCANE IN BIHAR

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Received April 13, 1957

I. Introduction

Ratoon stunting disease, a mysterious retarding of growth in sugarcane ratoon, was first noted in Q 28 in Mackey District, Queensland (Australia) in 1944-45, and has since been recorded in 19 countries scattered all over the world,[1] India also being one of them. It was reported to have been spotted at Gola gokaran nath (Distt. Kheri) in Uttar Pradesh by Prof. S. P. J. Chilton during his visit to this country in 1956.[5] In Bihar where ratoons of sugarcane are poor, it was suspected that they suffered from this disease. Investigations were, therefore, taken up to see whether or not the poor growth was due to the disease and are reported in this paper.

II. Nature of the Disease

The latest findings on the disease in other countries are summarised below:—

(*a*) *Symptoms.*—The symptoms of the disease are nebulous because the effect of the disease can be measured to some extent only in large populations and are so ill-defined that " as yet there is no way by which the presence or absence of the ratoon stunting disease can be positively ascertained in individual stalks of sugarcane in field conditions in Louisiana".[10] The crop as a whole shows retarded growth with deficient root system and has poor stand due to the deterioration of lateral buds. As a result the number of stalks per stool is reduced in some varieties. The decline in yield is clearly visible even in the very first season after infection and the yield goes down by as much as 10·7 tons in crop and 20·3 tons in ratoon.[7]

Even these indications of the disease are masked to a great extent if the variety happens to be tolerant as was the case with Q 50[6] and Co. 301, Co. 281, Co. 331 and N: Co. 310 though infected, were not found to show any appreciable reduction in yield in Natal.[4] There is no difference between the juices of healthy and diseased crops so far as their sugar content is concerned.[6]

Individual stalks in an infected crop would be slender and somewhat shortened without any apparent reduction in the length[10] and girth[6] of internodes. Under unfavourable conditions of growth, the leaves may show very slight chlorosis. They may be abnormally stiff and erect.[11] Internal tissues also do not give any indication of the disease except that vascular bundles, especially the larger leaf-traces, show orange-red to brown-orange discoloration at the level of entrance into the nodal region. It is brought about by the formation of gummy substances which block xylem vessels and disorganize the phloem.[6] Reddening of vascular bundles, though found to be a fairly reliable indication of the disease in Queensland,[9] was often faint or variable and was masked by red rot infection or borer damage in Louisiana. But in Southern Florida where sugarcane is grown on muck soil, it was intense and easily recognizable.[10] Further, these discoloured vascular bundles are found only in the lower portions of relatively mature stalks. Young diseased shoots of plant crop, though not consistently of a ratoon, when split longitudinally, would show an unmistakable general salmon-pink colour in nodes up to half an inch of the growing point. Some varieties particularly Q 28 and Q 44 develop this symptom within about two months of planting, when stalks hardly 2 inches long, have not even emerged. Finally the disease may not manifest itself in this way in secondary shoots at all.[6] Obviously a symptom which is dependent on so many factors to appear, as the discoloration of vascular bundles seems to be, is not likely to be a reliable diagnostic feature to go by.

Besides this disease, other unfavourable conditions of growth, such as submergence, high alkali contents of soil and draught also bring about the formation of gummy substances within vascular bundles and their discoloration.

(*b*) *Causal agent*.—The disease has been found to be due to infection by a virus which does not show itself, in various preparations of diseased sap under ultraviolet absorption, spectrophotometer and electron microscope and is not easy to separate by the more common methods of virus purification. It passes through a No. 41 Whitman filter-paper when in high concentration but is retained by a " Chamberland I.F. " filter candle.[6] It has not been possible yet to demonstrate the virus serologically.[8]

The virus is present in all parts of infected sugarcane plant, *i.e.*, stem, leaves and roots in more or less equal concentration. It is easily transmitted by inoculating diseased sap into setts, young growing stems and roots of young plants[5] and produced distinct symptoms of the disease after five months.[12] Since the sap is infective even at such a high dilution as 1 : 10,000,

the disease is transmitted in nature by harvesting knives which have been used before for cutting infected stalks.[6,11] Even rats were found to transmit the virus.[12]

III. Material and Methods

In view of the fact that the disease was most likely to be pronounced in the stunted ratoons, survey of such crop was undertaken at ten places dispersed over the white sugar belt of the State and discoloration of vascular bundles at nodes in all canes found in at least five clumps of as many varieties as available was noted.

Since it was not possible to test the presence of virus by transmission study, nor to make quantitative observations on the occurrence of discolored vascular bundles in all the varieties found at these places, detailed work was confined to only those available at Hassanpur (District Dharbhanga). Small stunted canes of six varieties from that place were treated as given in Section IV and planted under optimum conditions of growth for two years. Also representative samples were taken for anatomical work from different treatments. Hand sections were taken in very close proximity to the nodes and after staining with Safranin and dehydration, were mounted in Canada balsam. The percentage of discoloured vascular bundles was found in different treatments. For comparison their normal material collected at Pusa was also given similar treatments.

IV. Observations and Results

A. Survey of the crop.—Observations on the standing crop were made from 12th February to 4th March 1955, on 26 varieties in reserved areas of eight factories and at Pusa and Patna. Five to ten clumps showing stunted growth but free of diseases and insect-pests were selected for each of the more common varieties available at these places. All the canes in them were split longitudinally and nodes were examined carefully for typical discoloration of vascular bundles. In very large number of cases the nodes were found to show no discoloration whatsoever (Table I). Only at Majhaulia, Motihari (both in Champaran District) and at Motipur in Muzaffarpur District, orange-coloured vascular bundles were met with in some varieties, the percentage of nodes with such bundles being below 5%. In B.O. 17 and B.O. 30 ratoons (not given in Table I) at Majhaulia however, 6·8% and 12·6% of nodes respectively were found to show discoloured vascular bundles. Apart from the absence as a whole, of discoloured vascular bundles in the nodal region, stalks were reduced not only in length but also in girth whereas reduction was brought about in the former only by the ratoon stunting disease which does not affect the girth of cane.

TABLE I

Survey of sugarcane crop made for ratoon stunting disease
(Number of nodes examined)

Varieties*	Plant or Ratoon	Pusa†	Patna	Hari-nagar	Narka-tiaganj	Maj-haulia	Lauriya	Motihari	Motipur	Guraru	Dalmia-nagar
B.O. 10	Plant	391	254	660	..	..	521	..	..	..	..
	Ratoon	..	..	..	..	204	..	..	..	..	..
B.O. 11	Plant	1481	325	..	525	..	..	..	..	370	227
						304		183			
	Ratoon	..	..	436	..	——	..	——	..	..	..
						6‡		2			
B.O. 14	Plant	402	426	..	..	..	768	..	..	..	..
	Ratoon	..	..	853	..	..	..	..	..	..	..
B.O. 15	Plant	537	..	..	..	..	734	..	..	..	520
	Ratoon	..	..	..	..	..	866	..	..	..	484
B.O. 17	Plant	320	..	711	..	..	698	..	..	..	261
						237		194			
	Ratoon	..	..	577	..	——	..	——	..	..	473
						16		1			
B.O. 21	Plant	500	554	..	..	..	652	..	..	..	262
						422		164			
	Ratoon	805	..	..	..	——	..	——	..	..	..
						1		1			
B.O. 22	Plant	526	..	820	1356	..	..	..	..	..	266
								207			
	Ratoon	..	..	589	..	..	..	——	..	..	465
								3			
B.O. 24	Plant	..	341	..	496	..	..	..	..	..	229
									383		
	Ratoon	..	..	..	..	..	..	..	——	..	483
									12		
B.O. 28	Plant	..	303	..	529	..	487	..	..	..	..
	Ratoon	511	..	620	..	..	..	..	..	..	..
B.O. 29	Plant	894	..	1264	455	..	..	..	..	..	231
						436					
	Ratoon	..	..	680	..	——	..	..	..	..	494
						1					
Co. 453	Plant	298	611	735	576	..	..	..	..	290	205
	Ratoon	..	..	605	555	..	..	..	..	329	..
Co. 513	Plant	906	392	..	..	..	475	..	..	..	..
	Ratoon	..	..	..	..	336	..	..	..	..	..

* Varieties on which observations were made at less than three places have been omitted.

† At Pusa, upto 20 clumps were taken in some varieties.

‡ Lower figure in this column and others indicates the number of nodes with discoloured vascular bundles.

B. Experimental work.—An experiment was laid out to test the presence of the virus in stunted canes (i) by treating the setts with hot water at 52° C. for 2 hours before planting[10] and (ii) by dipping for 5 minutes each cut-end of setts from normal material in the juice of stunted canes of the same variety. Material from stunted and normal canes was planted on

29–30–4–1955 as controls for (i) and (ii). Normal material was also given hot-water treatment to compare its effect with that on stunted material.

In 1956–57, all the five treatments were ratooned on 12–4–1956, and material from the first four treatments was also planted the same day without any treatment. As regards the fifth treatment its material was planted both without any treatment and also after hot-water treatment. Thus there were in all 16 treatments as given in Table II.

Stunted material of six varieties namely Co. 331, Co. 453, Co. 513, B.O.11, B.O. 21 and B.O. 22 was collected from Hassanpur factory reserved area in Darbhanga District and planted after measuring the length and diameter of the canes. Normal material of all the six varieties except Co. 331 (not available) was taken from Pusa Farm. Both the kinds of material were given similar and optimum conditions of growth for two seasons. Each year, 20 mother shoots in plant crop and best developed tillers in ratoon were tagged for growth measurements, which were taken fortnightly till the beginning of winter. Averages of the final observations for length and diameter are presented in Table II.

Stalks formed in the stunted material whether planted as such (Treatment I) or after hot-water treatment (Treatment II) were longer and thicker than the original material collected at Hassanpur except in B.O. 21 in Treatment I in which the mother shoot of the only clump died after 5–10–1955.

On being ratooned in 1956–57 season, there was further appreciation both in the average length and diameter of stalks of all the varieties except B.O. 11 in Treatment II which happened to grow on the edge of the experimental plot. The plant crop raised from seed taken from both the treatments was much superior to the original material and definitely better than the crop raised from the latter in 1955–56 (Table II, Text-Fig. 1).

A comparison of the stalks formed in Treatments I and II in 1955–56 and their plant and ratoon crop in 1956–57, shows that increase in size of the stalks was more or less of the same order in corresponding treatments.

It would thus appear that the original material when given optimum conditions of growth made up the reduction in size brought about by lack of proper environment. Greater increase effected by hot-water treatment in Co. 331 and B.O. 11 was due to its stimulatory rather than curative action. Stalks of Co. 513 and B.O. 22 in this treatment were smaller than those of Treatment I while in Co. 453 they were equal in size in both the treatments.

The behaviour of the normal material was more or less similar to that of the stunted material so far as this treatment was concerned. Stalks of

TABLE II. *Average length and diameter in cm. of stalks in a ratoon stunting disease experiment*

(a) Diseased (?) material from Hassanpur

Variety	Character	Original material	No treatment			Hot-water treatment		
			1955–56	1956–57		1955–56	1956–57	
				Ratoon	Plant		Ratoon	Plant
Treatments ..			I	VI	VII	II	VIII	IX
Co. 331 ..	Length	128·0	156·1	257·8	..*	196·1	277·1	197·7
	Diameter	1·29	1·54	2·15	..	2·02	2·09	2·10
Co. 453 ..	Length	139·8	201·9	245·6	240·8	201·5	250·6	206·2
	Diameter	1·52	2·00	2·36	2·35	2·10	2·89	2·16
Co. 513 ..	Length	92·0	153·5	184·1	179·4	129·3	142·9	178·2
	Diameter	1·18	1·73	1·93	1·88	1·64	1·76	1·89
B.O. 11 ..	Length	135·3	145·0	184·6	156·3	174·8	155·9	168·2
	Diameter	1·58	1·83	2·70	2·23	2·13	2·13	1·93
B.O. 21 ..	Length	110·8	..†	229·0	165·8	125·0	270·9	166·3
	Diameter	1·12	..	2·31	2·00	1·83	2·51	1·88
B.O. 22‡ ..	Length	168·8	188·3	268·2	170·5	173·3	256·9	158·7
	Diameter	1·82	1·85	2·38	1·69	2·22	2·19	1·72

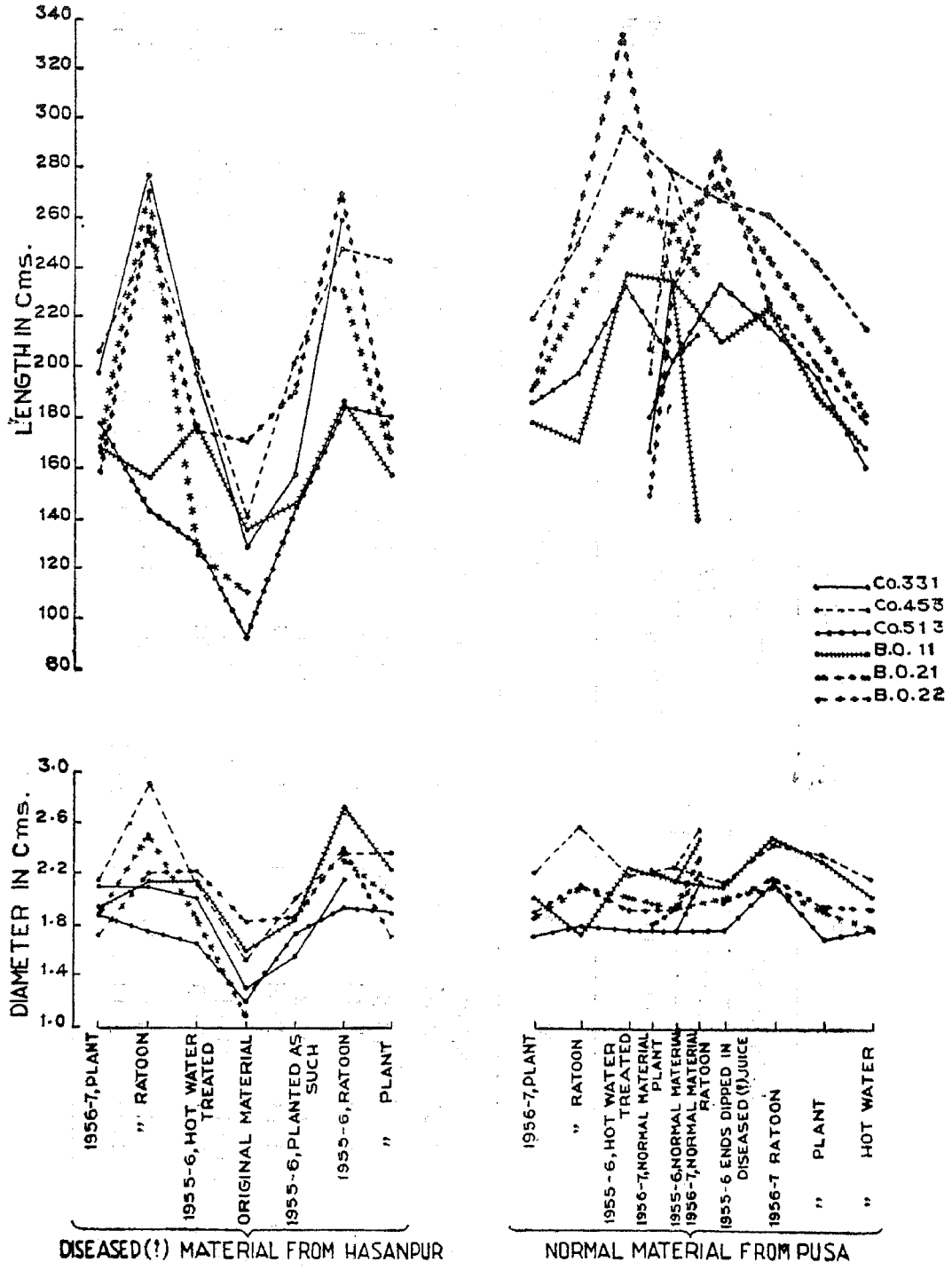
* Material not available as stalks had dried up by harvest.

† The mother shoot of the only clump dried after 5th October 1955.

‡ The original material was identified as B.O. 22 but later on it proved to be Co. 285.

(*b*) *Normal material from Pusa*

Variety	Character	No treatment			Hot-water treatment			Ends dipped in diseased (?) juice			
		1955–56	1956–57		1955–56	1956–57		1955–56	1956–57		
			Ratoon	Plant		Ratoon	Plant		Ratoon	Plant	Hot-water treatment
	Treatment	III	X	XI	IV	XII	XIII	V	XIV	XV	XVI
Co. 453	Length	276·6	243·9	196·3	267·1	247·1	217·8	293·4	256·5	236·3	211·1
	Diameter	2·23	2·51	2·19	2·12	2·54	2·21	2·17	2·40	2·33	2·16
Co. 513	Length	200·2	210·5	178·0	229·4	196·0	184·4	229·4	213·6	193·5	158·4
	Diameter	1·76	2·13	·75	1·76	1·78	1·71	1·74	2·21	1·67	1·91
B.O. 11	Length	232·7	164·2	137·9	207·4	168·0	176·6	234·4	220·5	185·3	166·2
	Diameter	2·13	2·44	2·21	2·09	1·72	2·09	2·23	2·46	2·27	2·00
B.O. 21	Length	254·8	235·4	206·7	270·1	228·6	188·4	261·5	221·3	211·9	175·1
	Diameter	1·92	2·30	·94	1·97	2·09	1·83	1·99	2·08	1·87	1·76
B.O. 22	Length	230·8	246·1	147·0	283·6	258·0	185·2	331·2	248·9	197·9	178·8
	Diameter	1·92	2·18	1·79	1·94	2·11	1·89	1·91	2·16	1·92	1·90



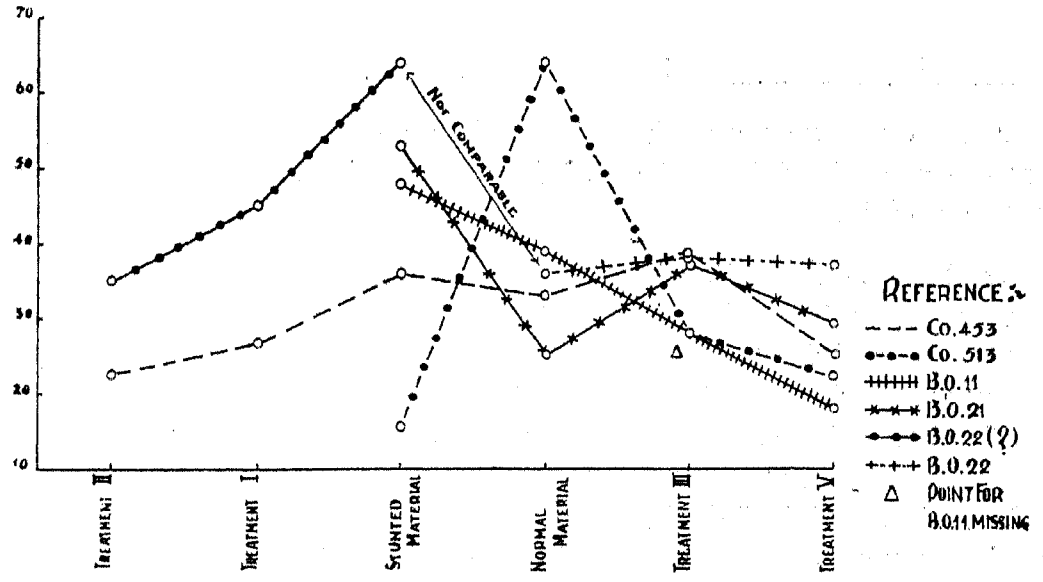
TEXT-FIG. 1. Length and Diameter of Stalks in Ratoon Stunting Disease (?) Experiment.

Co. 453, Co. 513 and B.O. 22 in Treatment IV were distinctly longer than those formed in Treatment III while those of B.O. 11 and B.O. 21 were more or less equal in both the treatments.

In Treatment V in which stalks should have shown a reduction in size if the juice were infective, they were longer than those of Treatment III in three varieties, *viz.*, Co. 513, B.O. 21 and B.O. 22 but shorter in B.O. 11 and Co. 453.

In 1956–57, the ratoon as well as plant crop raised from seed taken from Treatments III, IV and V showed a general decline in the stalk-size except in Co. 513 and B.O. 22 in Treatment XI and in B.O. 11 in Treatment XIV. Stalks formed in Treatment XVII were also smaller than those found in Treatment XVI (Table II, Text-Fig. 1). The general decline in the size of stalks in all treatments in 1956–57 plant crop was due to its being raised on a piece of land which had cane crop in 1955–56.

C. Anatomical study.—In transverse sections taken through the transitional region between node and internode and through the latter just below that region (Pl. I, Figs. 1 and 2), xylem vessels and phloem, more often together (Pl. I, Fig. 3), were found choked to varying degrees with a substance which was insoluble in water, acetic acid, alcohol, xylol and clove oil, similar substance being present in isolated parenchymatous cells (Pl. I, Fig. 4). A study of the average number of vascular bundles and of the percentage of those blocked per microscope field (5·31 sq. mm.) shows that the latter was found to be higher in the stunted than in the normal material in B.O. 11 and B.O. 21, lower in Co. 513 and almost equal in Co. 453, it being not comparable in the case of B.O. 22 (Table III, Text-Fig. 2).



Text-Fig. 2, Discoloration of Vascular Bundles.

TABLE III

Percentage of blocked vascular bundles in different treatments

Variety	Collected in April 1955			Collected in April 1956			Remarks
	Nature of material	Average No. of vascular bundles in 5·31 sq. mm.	% of blocked vascular bundles	Treatments in 1955–56	Average No. of vascular bundles in 5·32 sq. mm.	% of blocked vascular bundles	
Co. 453 ..	Stunted	9·4	36·2	I	15·5	22·6	
				II	6·6	27·2	
	Normal	5·3	33·3	III	8·1	38·5	
				V	8·4	25·4	
Co. 513 ..	Stunted	12·8	15·6	..	..	..	
				..	..	..	
	Normal	7·0	64·3	III	8·6	27·9	
				V	8·6	22·0	
B.O. 11 ..	Stunted	11·0	47·7	..	..	..	
	Normal	7·1	39·0	V	7·6	17·4	
B.O. 21 ..	Stunted	9·5	52·6	..	..	..	
				III	7·3	37·2	
	Normal	7·2	25·6	V	8·9	29·6	
B.O. 22 ..	Stunted	11·5	64·1	I	10·5	45·2	Not comparable because stunted material belonged to Co. 285 and normal B.O. 22
				II	17·0	35·3	
	Normal	6·3	36·0	III	7·4	37·8	
				V	8·8	37·1	

After one year's growth under optimum conditions, their percentage went down in Co. 453 and B.O. 22 (as collected from Hassanpur) in Treatments I and II.

In the material collected from Treatment III, it was more or less equal to that in normal material of Co. 453 and B.O. 22, but was higher and lower respectively in B.O. 21 and Co. 513. In Treatment V, where this should have gone up, due to infection with the virus, it was lower in Co. 453 and B.O. 21 than in Treatment III and equal in the case of B.O. 22 and Co. 513 in both the treatments. As compared with the normal material, it was less in Co. 453, Co. 513, B.O. 11, equal in B.O. 22 and greater in B.O. 21. It, therefore, appears that Treatment V did not bring about any increase in the percentage of blocked vascular bundles.

V. Discussion

From Table I it would appear that the survey of crop covered fairly wide field both with respect to varieties and its area. The percentage of nodes with discoloured vascular bundles was almost negligible and these too were confined to varieties released very recently, while varieties like Co. 453, Co. 513, B.O. 10, B.O. 11, given out for general cultivation respectively in 1947, 1940, 1947 and 1948, were free of them. In view of the poor standard of farming in general, the cultivator being ignorant of and without any means to practise a measure like sterilizing, harvesting implements to prevent infection of seed material, it is hardly likely that these varieties would escape infection during the course of 8–12 years. Further, the synergistic factors like draught,[10] flooding and water-logging which lower the resistance of the host and reflect themselves in poor growth and yield as well, are operating year after year. The environment coupled with climate over the whole of sugarcane-growing tract is so conducive for the spread of a virus disease like ratoon stunting disease, that once it enters the area, there is no natural factor to prevent it from assuming alarming proportions.

These observations coupled with the general reduction in the size of stalks, *i.e.*, both in the length as well as in the girth, as distinct from that in length only, brought about by the ratoon stunting disease,[6] do not appear to indicate the presence of the disease in the areas surveyed specifically for this purpose. Also, regular routine observations for almost two decades on crop grown under various edaphic and climatic conditions and different standards of cultivation of State-owned farms on one hand and that of cultivators' plots on the other, have not suggested the existence of such a disease in this State.

The poor growth of ratoons generally met with in this State appears to be due to lack of proper conditions of growth rather than to the infection with the virus because if the stunted material were suffering from this disease, there should not have been any appreciable increase even when it was given optimum conditions of growth. On the contrary, stalks gained in size to various extents in the stunted material without any treatment (Treatment I), the increase being of the order of 48·4% and 66·6% in length respectively in Co. 453 and Co. 513 and nearly 30% in girth in both the varieties in the very first year. Its ratoon in the ensuing year showed still further increase in size. Moreover, there was nothing to suggest that hot-water treatment had any curative effect because its effect was not consistently beneficial as was found to be the case elsewhere also.[3] Stalks of Co. 513 and B.O. 22 were longer in Treatment I than in Treatment II, those of Co. 453 being equal in both the

treatments. The general pattern of behaviour of stunted material was similar in these two treatments (Text-Fig. 1) thus showing thereby the absence of the disease.

Finally, the failure of juice of stunted stalks to transmit the virus (if at all present in them) to normal material (Treatment V) gave conclusive evidence of its absence especially when virus was found to retain its infectivity at as high a dilution as 1 : 10,000. Instead of showing any reduction in size, the stalks of Co. 513, B.O. 21, and B.O. 22 showed appreciable increase in length. Also, material from this treatment was not found in the succeeding year to be in any way, worse than that in Treatments III and IV (Text-Fig. 1).

The anatomical study too did not give any indication of the presence of the disease in the stunted material. No doubt, the percentage of choked vascular bundles in Co. 453, B.O. 11 and B.O. 21 was greater in stunted than in normal material though reverse was the case in Co. 513. But its fall in Treatment I gave additional evidence of stunting being due to adverse conditions of growth. Similarly, the behaviour of normal material also did not suggest that the presence of discoloured vascular bundles was an indication of this disease alone, because their percentage was, on the whole, respectively greater and less in Treatments III and V than in the normal material (Text-Fig. 2).

On the basis of these investigations, namely survey of crop, experimental work in field and anatomical study in laboratory, it may be, for the present, stated that there is little likelihood of the disease being present in this State. Still a careful watch is being maintained to locate the disease within the State for which regular observations on crop are made throughout during these years.

VI. SUMMARY

1. The latest findings about the disease as regards its symptoms and causal agent are summarised.

2. Observations made on 26 varieties in 8 factory reserved areas and Research Farms at Pusa and Patna in February–March 1955 showed that its two main symptoms, namely the discoloration of vascular bundles at nodes and the reduction in length of stalks without a corresponding decrease in their girth, were generally absent.

3. Experimental work with the stunted material of six varieties showed that (*a*) it gained both in length and girth of stalks when it was planted without any treatment but given optimum conditions of growth and (*b*) that the juice of these stunted canes failed to transmit the virus to healthy material.

4. (*a*) The percentage of blocked vascular bundles also fell when the stunted material was grown under favourable conditions and (*b*) there was no rise when healthy material was brought in close contact with the diseased (?) juice.

5. It may, therefore, be stated for the present that there is little likelihood of the disease being present in the State. Careful watch, however, is beeing maintained.

VII. Acknowledgements

These investigations were carried out at Sugarcane Research Institute jointly financed by the Bihar Government and the Indian Central Sugarcane Committee to whom grateful thanks of the authors are due. Assistance rendered by the staff of the Mycology Section in the survey and by Shree J. N. Chand who was associated with experimental work in early stages of these investigations is duly acknowledged.

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