Nutritional Status of Soils and the Incidence of the 'Bunchy Top' Disease of Bananas (*Musa* sp.)

PART IV. ANATOMICAL VARIATIONS IN VIRUS INFECTED AND HEALTHY PLANTS AS A FUNCTION OF CALCIUM/MAGNESIUM RATIO IN SOILS*

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Almost all the studies on the 'Bunchy Top' disease up-to-date have been mainly confined to an elucidation of the mechanism by which the disease infection takes place and to prophylactic control measures. Very little work on the anatomical aspects to understand the mechanism of cell degeneration seems to have been carried out, except for a brief report by Magee (1927). He found that in the phloem region there was a suppression of the development of the fibrous sheath in disease-infected plants. The fundamental tissue in the neighbourhood of the phloem had become gorged with chromatophores. In a normal plant, this region was entirely devoid of chlorophyll. it was noticed that the original cells were divided into angular cells by the growth of thin cellulose walls. The newly formed cells contained numerous chromatophores and distinct nuclei, which exhibited wide variation in size and shape. It was suggested that these nuclei were derived from normal nuclei by amitotic or direct division rather than by true mitosis. In subsequent investigations, Magee (1939, 1953) studied further the anatomy of the Bunchy Top infected banana plants. He described the following modifications induced by the presence of the virus: (1) In the phloem the develoment of the fibrous sheath was suppressed, its place being occupied by cells with many chromatophores; (2) The ground tissue contiguous with the phloem became gorged with chromatophores, whereas in the normal plant this region was almost devoid of chlorophyll; (3) The ground parenchyma cells in contact with the phloem were stimulated to division and formed somewhat angular cells by the formation of thin cellulose partition walls in all planes. Each newly formed cell contained numerous chromatophores and a distinct nucleus; (4) Similar appositional growth of walls in all planes took place in the cells of the phloem and gave rise to numerous angular cells along their length. These cells were nucleate. The phloem nuclei in a primary infected plant were particularly noticeable

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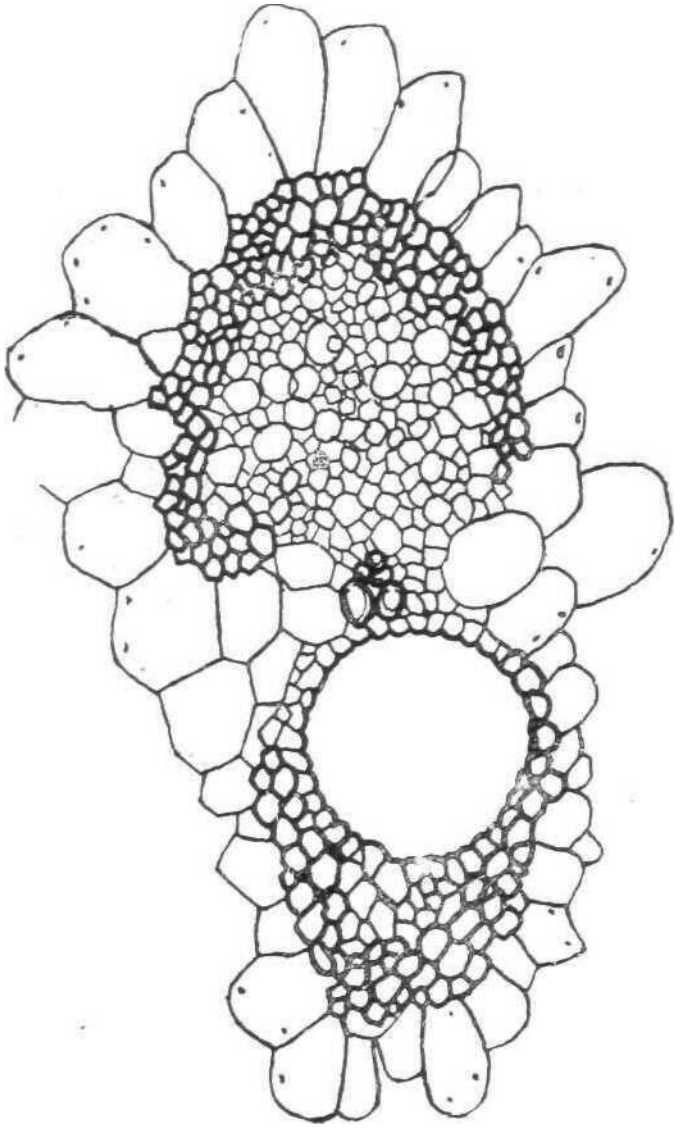

PLATE XL Transverse section of a vascular bundle of the petiole of the young banana plant (Control) (Drawn with the aid of Camera Lucida. x 140)

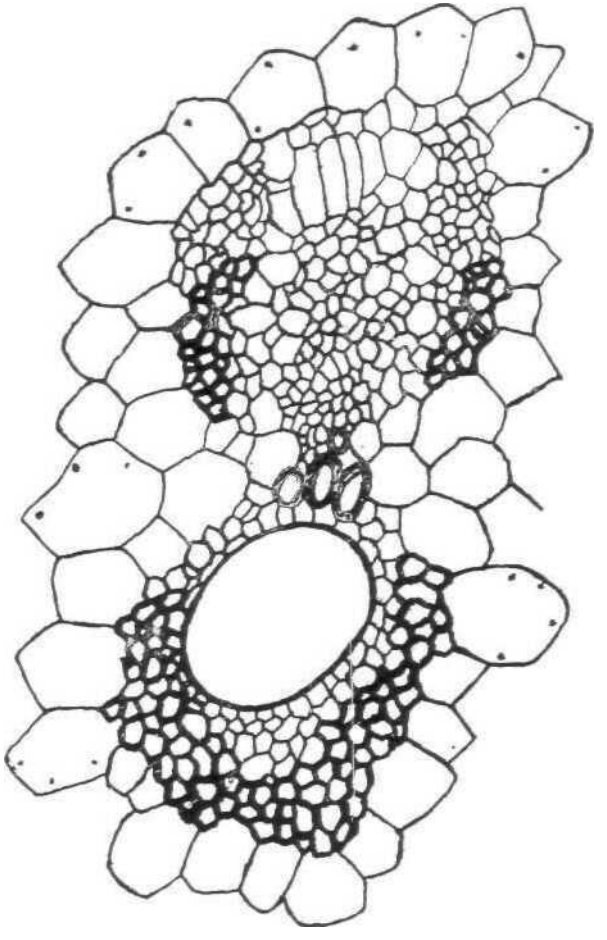

PLATE XII. Transverse section of a vascular bundle of the petiole during the early stage of infection. (Drawn with the aid of Camera Lucida. x 140)

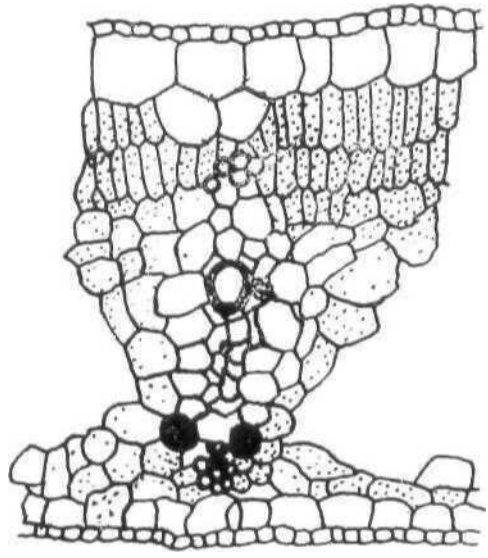

PLATE IX. Transverse section of a vascular bundle of the leaf of a young, healthy banana plant. (Control) (Drawn with the aid of Camera Lucida. X 140)

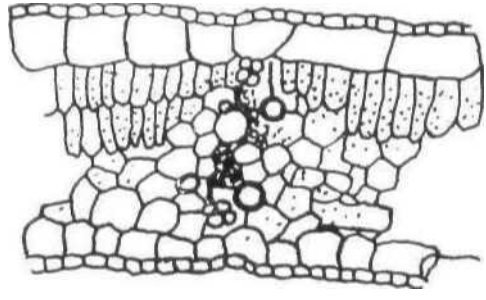

PLATE X. Transverse section of a vascular bundle of the leaf of an infected plant (Treatment **No.** 4) (Drawn with the aid of Camera Lucida. X 140)

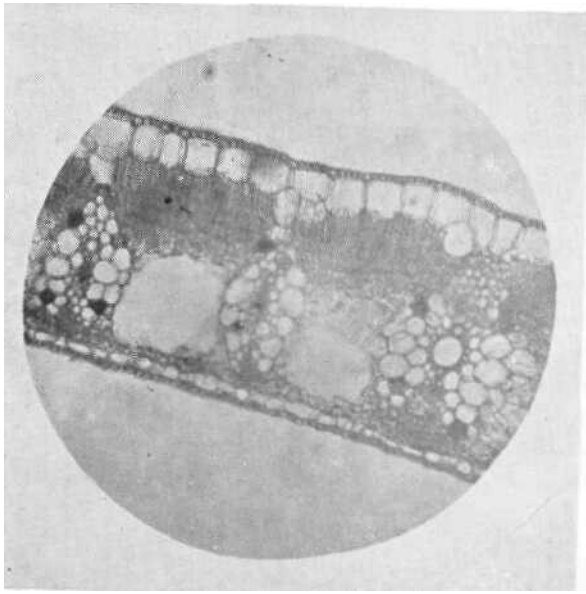

PLATE V. T. S. of leaf of healthy banana

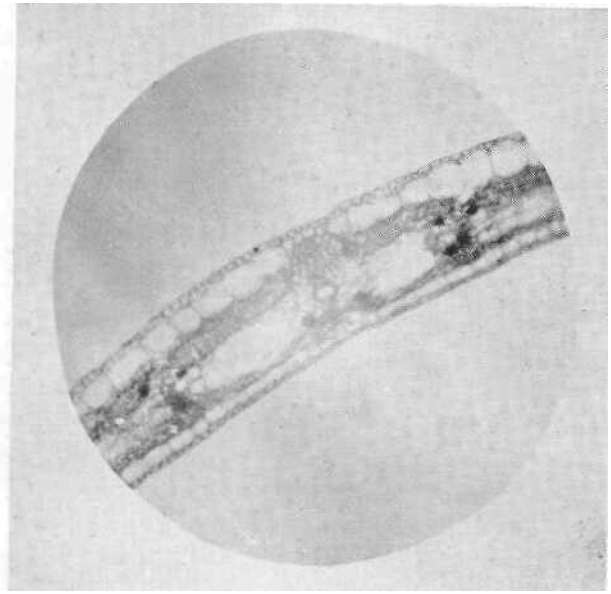

PLATE VI. T. S. of leaf of diseased banana

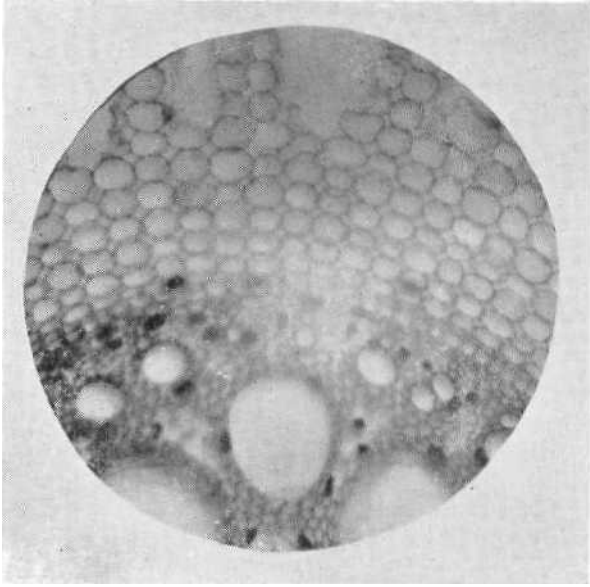

PLATE VII. T. S. of root of healthy banana showing parenchyma, xylem, phloem, endodermis and pericycle

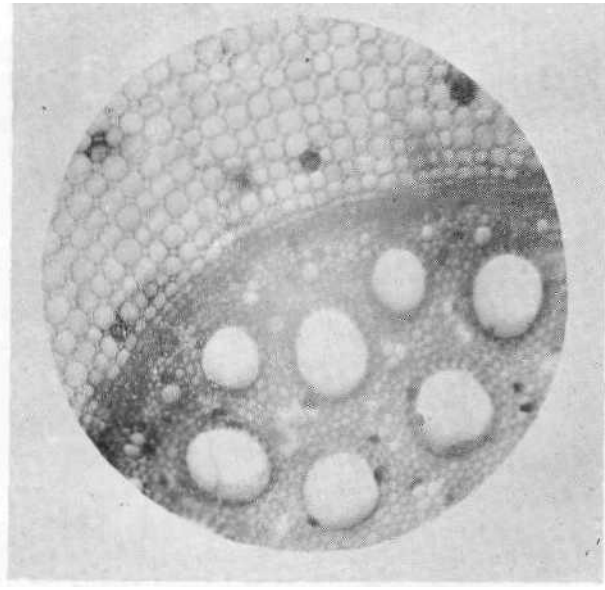

PLATE VIII. T. S. of root of diseased banana

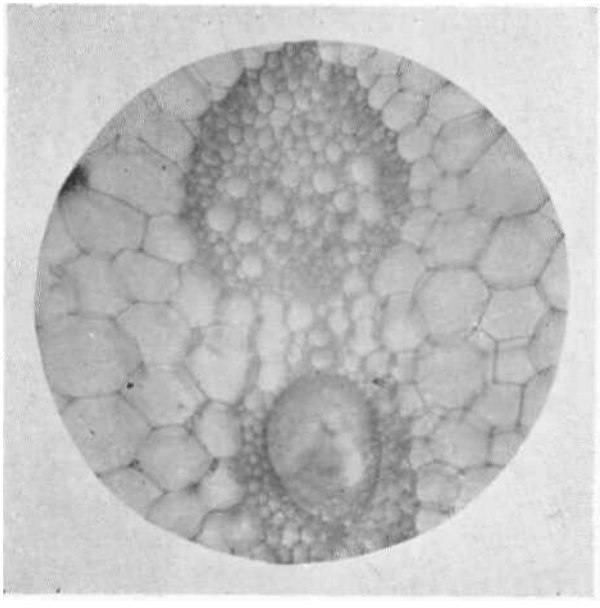

PLATE I. T. S. of Leaf-sheath of healthy banana showing xylem and pholem with adjoining parenchyma

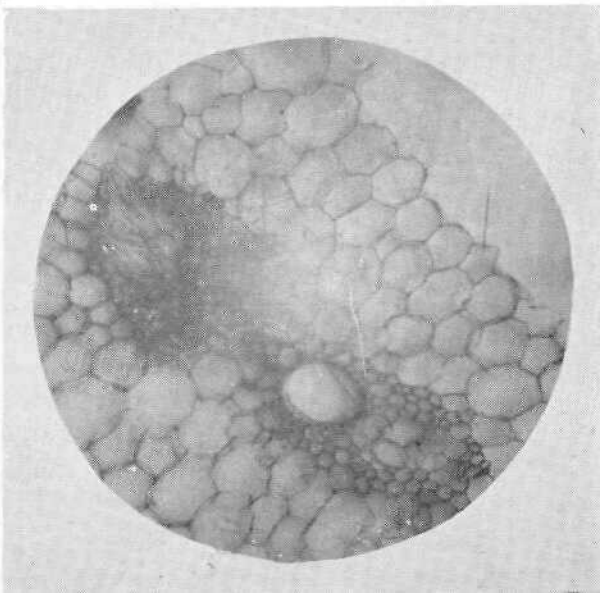

PLATE II. T. S. of Leaf-sheath of infected banana showing xylem and pholem with adjoining parenchyma

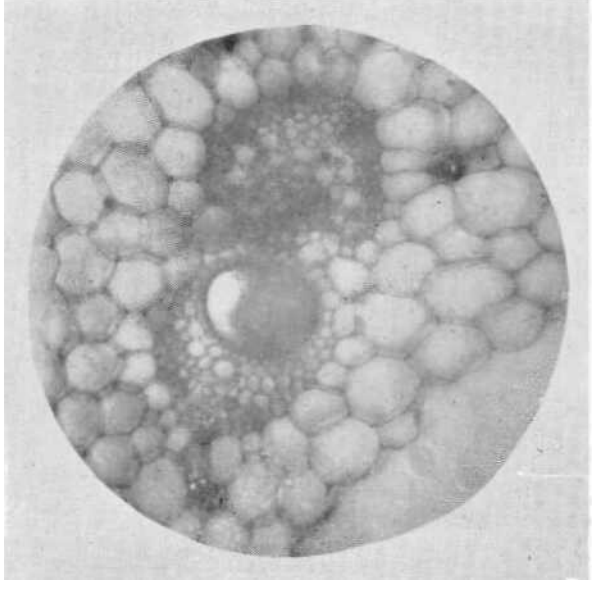

PLATE III. T. S. of petiole of healthy banana showing xylem and phloem with adjoining parenchyma

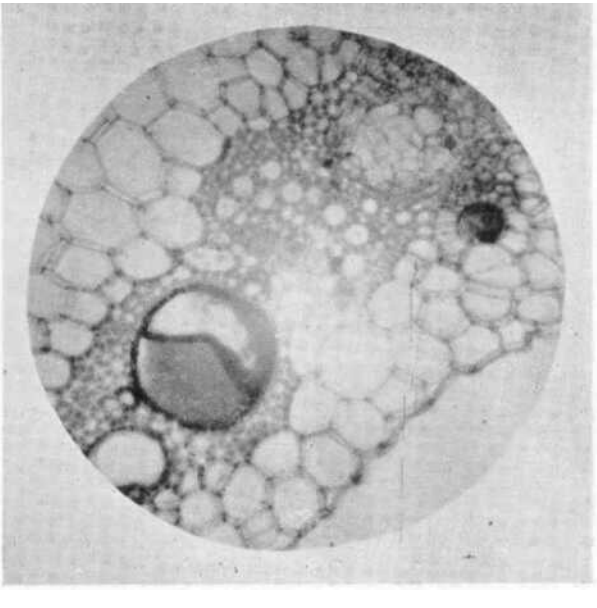

PLATE IV. T. S. of petiole of infected plant showing xylem and phloem with adjoining parenchyma

in any transverse or longitudinal section. They showed wide variation in size und shape and there was evidence that they were formed not by true mitosis but by amitotic or direct division. Changes in and around the phloem similar to those described above also occurred in other regions of infected plants including the pseudostem, the exposed region of the fruit stock and the fruit skin. Wardlaw (1961) in summarising the anatomical work on virus-infected *Musa* sp. by Magee, Allen and some of the Philippine investigators, has stated that a microscopic examination of the pseudostem and bulb tissues revealed no pathological changes of diagnostic value, the yellow or brown streaking (tannin ducts) being characteristic of both healthy and infected plants alike, particularly as they became older. A similar examination of affected leaf tissue, however, revealed a distinctive pathological condition. The action of the Bunchy Top virus resulted in a characteristic disorganisation of the phloem tissue of the vascular strands. The extent to which the vascular bundles were altered depended on the intensity of infection.

Bawden (1950) has stated that internal symptoms of two kinds are common in plants suffering from virus diseases. The first kind is the destruction or modification of normal tissue or cell content and the second is the production of peculiar inclusion bodies which are not found in healthy cells.

Most of the work on intracellular inclusions has been done with Tobacco Mosaic virus. Similar work using Bunchy Top virus does not seem to have taken place to any extent.

Nambiar and Nair (1965) found that the healthy leaves of bananas contained significantly higher quantities of calcium and magnesium than in the case of the Bunchy Top affected plants. They noted that with the incidence of the disease, the translocation of nitrogen, phosphorus and potassium from the leaves is severely affected, possibly due to physiological disturbances occurring as a result of virus infection. In contrast to the accumulation of these elements in the leaf they observed no similar accumulation of calcium and magnesium in the diseased tissues. They concluded that immediately before the incidence of the disease or during infection drastic changes occurred in the absorption and translocation of calcium and magnesium in the plant. It is possible that the physiological disturbances mentioned by Nambiar and Nair (1965) relate to the cellular degeneration that takes place consequent on virus infection. All this would indicate that the anatomical structure of virus infected plant tissues undergo significant transformation. It is of importance in this connection to study the difference in the anatomy of diseased and healthy banana plants under varying nutritional and environmental conditions. It is also necessary to note the anatomical structure of the healthy and diseased tissues as a variant of the time factor, viz., the differences in the period of infection noted under different nutritional treatments. The present study was therefore planned as a complementary part of the investigations on the relationship between the nutritional environment and the incidence of the Bunchy Top disease of bananas.

Meterials and Methods

The plant materials examined in this study were obtained from the banana plants grown under the following treatments in the agronomic investigations reported by Nair and George (1966):

T1—Control

T2—CaO-1.2% and MgO-0.2% in the soil (CaO/MgOratio-6:1)

T3—CaO-0.6% and MgO-0.2% in the soil (CaO/MgO ratio-3:1)

T4—CaO-1.8% and MgO-0.2% in the soil (CaO/MgO ratio 9:1)

In addition to appropriate doses of CaO and $MgCO_3$ to maintain the above ratios in the soil all treatments received liberal applications of farm yard manure (75 kg), ammonium sulphate (500 g), superphosphate (1360g) and muriate ofpotash (1360g), as well as adequate quantities of the trace elements. Planting of banana suckers (var. *Nendran)* was done on 31-8-1965 and the plants exposed to infective aphids twice, first on 27-10-1965 and again one week afterwards. Samples were first collected on 30-9-1965 and thereafter at one month intervals on 30-10-1965, 30-11-1965 and 30-12-1965. These samples are referred to as S1, S2, S3 and S4. The plant parts collected for anatomical studies were the leaf-sheath, petiole, leaf and root. At the time of the first sampling the leaves had not unfurled and hence S1 did not include leaf samples. As the plants under the different treatments had become diseased from the third month onwards it was not considered necessary to continue sampling and analysis of experimental plants beyond the fourth sampling date. However, it was considered useful to compare the results from anatomical studies conducted with a healthy plant of the same age with the data obtained from diseased plants for the fourth sampling. Hence tissues from a healthy plant of the same age were also removed for analysis on the same date on which the fourth sampling was done on experimental plants. The samples of leaf-sheath, petiole, leaf and root from different treatments were kept in separate labelled jars using formalin-aceto-alcohol as the preservative. Hand sections were used for the anatomical studies. The stains used were safranin (0.5% aqueous solution) alone and in combination with fast green (0.5% aqueous solution) and hematoxylin (0.5% aqueous solution). The schedule adopted for staining was as follows: Free hand sections were first treated with 35% alcohol and then washed in water. The sections were then put in 0.5% aqueous safranin for 5 minutes. The stained sections were washed in running water to remove excess stain and mounted in glycerine. For stain combinations with fast green the sections after staining with safranin were stained with 0.5% aqueous fast green for 15 seconds, rinsed in tap water and then mounted. Similarly for combination with hematoxylin the safranin stained sections were treated with 0.5% aqueous hematoxylin for 2 minutes, washed in running water and then mounted in glycerine. For leaf-sheath, petiole and leaf, the parenchyma, xylem and phloem were studied, while for root the endodermal and pericycle cells were also examined. In each case the stained sections were examined under the microscope and 40 cells measured after calibration of the field with a stage micrometer and ocular micrometer. Camera lucida drawings were made and photomicrographs prepared using a Bausch and Lomb microphotographic camera.

Results

Data relating to the influence of different CaO/MgO ratios over time on the tissues of the leaf-sheath, petiole, leaf and root of the banana plants are presented in Tables I–V.

(a) *Leaf-sheath*

From Table I it may be noted that the diameter of the parenchyma cells of the leaf-sheath increased upto the second month after which it decreased. The minimum

TABLE I

Diameter of the parenchyma cells, xylem vessels and phloem cells of leaf-sheath (microns)

Treatment	Sample S_1	S_2	S_3	S_4	Mean
			Parenchyma		
T_1	95.04	111.04	104.32	98.24	102.16
T_2	109.76	118.40	104.96	72.32	101.36
T_3	95.36	117.44	102.28	112.32	107.60
T_4	112.96	118.08	99.84	110.72	110.40
Mean	103.28	116.24	103.60	98.40	
			Xylem		
T_1	63.68	104.64	128.96	109.12	101.60
T_2	51.52	96.00	135.04	90.88	93.36
T_3	62.40	87.04	120.64	118.08	97.04
T_4	54.72	88.96	149.76	78.40	92.96
Mean	58.08	94.16	133.60	99.12	
			Phloem		
T_1	15.22	23.89	27.25	16.19	20.64
T_2	13.18	24.69	27.78	14.24	19.97
T_3	19.20	20.53	28.32	15.22	20.81
T_4	13.09	18.31	23.01	13.45	16.96
Mean	15.17	21.85	26.59	14.77	

	Parenchyma	Xylem	Phloem
C. D. (5% level) for comparison between marginal means	0.116	0.301	0.034
Do for other comparisons	0.232	0.304	0.068

diameters were observed in treatments (Control) and T3 (CaO/MgO = 3:1) compared to other treatments. During the first month the mean diameters were 95.04 and 95.36 microns respectively in treatments Tl and T3 but these values had gone upto 111.04 and 117.44 microns during the second month which was on a par with the means of the other treatments. Hence the decrease in diameter of parenchyma cells was presumably due to infection and consequent cellular diminution. According to visual symptoms the plants had become diseased by the third month.

The diameter of the xylem vessels increased upto the third month after which it decreased. This indicates that the incidence of the disease reduces the diameter of the xylem vessels. Treatment T4 (CaO/MgO = 9:1) showed the least diameter of xylem vessels compared to the other treatment means. The rate of change of the diameter over time corresponding to the four treatments also showed considerable variation and statistical analysis indicated that the variation over time was highly significant.

The variations in the diameter of the phloem cells over time and due to treatments were significant. There was an increase in the diameter of the phloem cells upto three months after which there was a decrease. The reduction in diameter was noticed when the plants were affected by the disease. Treatment T4 recorded the minimum diameter compared to the other treatments, whereas the maximum was for T3 followed by control.

(b) *Petiole*

Data in Table II reveal that the variation in the diameter of the parenchyma cells of the petiole due to treatment are significant. There was an increase in the diameter of the parenchyma cells upto the third month after which there was a decrease. Hence the incidence of disease affects the parenchyma cells of the petiole also. Treatment T2 (CaO/MgO=6:1) showed the least diameter of parenchyma cells compared to the

TABLE II

Diameter of the parenchyma cells, xylem vessels and phloem cells of petiole (microns)

Treatment	Sample S_1	S_2	S_3	S_4	Mean
			Parenchyma		
T_1	74.88	109.44	96.64	91.20	99.04
T	89.92	79.04	85.76	82.88	84.40
T_2	87.68	86.08	99.20	94.08	91.76
T,	97.92	99.20	101.12	85.76	96.00
Mean	87.60	93.44	95.68	88.48	
			Xylem		
T_1	65.28	140.48	141.44	85.44	108.16
T_2	60.16	128.00	126.40	87.04	100.40
T_3	56.32	136.32	134.08	120.00	111.68
T_4	66.88	118.40	15136	72 96	102.40
Mean	62.16	130.80	138.32	91.36	
			Phloem		
T_1	11.50	22.65	23.54	15 31	18.25
T,	13.89	21.94	19 64	15.84	17.83
T_3	15.22	17.61	23 80	14.77	17.85
T_4	15.39	21.41	20.26	13.62	17.67
Mean	14.00	26.90	21.81	14.89	

	Parenchyma	Xyle,m	Phloem
C. D. (5% level) for comparison between marginal means	0.071	0.141	0 026
Do. for other comparisons	0.142	0 282	0.068

other treatments. The rate of change of diameter over time corresponding to the four treatments also showed considerable variation as indicated by the significant interaction. Among the different treatments T2 recorded the minimum diameter and T1 the maximum.

The diameter of the xylem vessels of petiole showed a natural increase for three months till the plants became infected, after which it decreased. Treatment T2 showed the least diameter of the xylem vessels compared to other treatments and treatment T3 showed the maximum. The plants under treatment T3 got the infection only after 37 days, whereas the plants under the other treatments showed symptoms much earlier.

The diameter of the phloem cells also showed considerable increase upto the third month after which it showed a decrease. The variation over time was significant. The maximum dimension was recorded in Tl and the minimum in T4.

(c) *Leaf*

Leaf samples were collected only from the second month onwards and the data relating to the parenchyma cells, xylem vessels and phloem cells are given in Table III.

TABLE III

Diameter of the parenchyma cells, xylem vessels and phloem cells of leaf (microns)

Treatment	Sample			Mean
		Parenchyma		
T_1	80.64	60.80	48.96	63.42
T_2	78.08	55.36	50.24	61.18
T_3	66.24	52.16	47.68	55.32
T_4	75.84	68.48	42.24	62.14
Mean	75.20	59.20	47.28	
		Xylem		
T_1	52.48	50.56	46.08	49.67
T_2	54.40	45.44	43.52	47.75
T_3	56.00	57 92	45.44	53.08
T_4	57.28	54.72	44.48	52.12
Mean	55.04	52.16	44 88	
		Phloem		
T_1	14.04	9.46	12.39	12.30
T_2	13.54	13.09	13.62	• 13.42
T_3	13.80	13.00	13.09	13.30
T_4	13.98	12,83	13.54	13.45
Mean	18.79	16.13	17.55	

		Parenchyma	Xylem	Phloem
C. D. (0·05) for comparison between				
	stage means	0.076	0.056	0.018
Do	treatment means	0.065	0.065	0.016
Do	other comparisons	0.130	0.112	0.031

The variation in the diameter of the parenchyma cells due to treatment and over time was significant. There was a decrease in the diameter from the first sampling to the third in all the four treatments. Control (T1) showed the maximum diameter of the cells. The rate of change of diameter over time also showed considerable variation.

There was significant variation in the diameter of the xylem vessels of the leaf due to treatment and over time. The diameter of xylem vessels decreased from the first sampling to the third sampling. Treatment T2 (CaO/MgO = 6:1) showed the least diameter of the xylem vessels compared to the other treatments, whereas treatment T3 (CaO/MgO = 3:1) showed the maximum diameter.

The diameter of the phloem cells of the leaf showed a decrease during second sampling but increased during the third sampling. The variations due to treatment and over time were significant.

(d) *Root*

The diameters of the different types of cells of the root of the banana plant under the treatments are given in Tables IV & V.

TABLE IV

Diameter of the parenchyma cells, xylem vessels and phloem cells of root (microns)

Treatment	Sample S_1	S_2	S_3	S_4	Mean
			Parenchyma		
T_1	53.44	50.24	62.08	54.08	54.96
T_2	48.00	62.08	59.84	66.88	59.20
T_3	68.19	62.72	64.32	58.88	63.52
T_4	41.60	48.00	71.04	59.84	55.12
Mean	52.80	55.76	64.32	59.92	
			Xylem		
T_1	61.76	44.48	51.52	53.44	52.80
T_2	44.80	52.80	43.52	60.80	50.48
T_3	55.36	53.12	67.52	56.96	58.24
T_4	39 04	47.04	66.56	61.76	53.60
Mean	50.24	49.36	57.28	58.24	
			Phloem		
T,	16.73	14.60	13.81	1292	14.51
T_2	15.75	13.36	15.93	14.34	14.85
T_3	16.02	17.08	18.50	12.74	16.08
T*	14.51	16.73	18.14	13.54	15.73
Mean	15.75	15.44	16.59	13.39	

		Parenchyma	Xylem	Phloem
C. D. (5% level)	for comparison between marginal means	0.065	0.071	0.016
Do.	for other comparisons	0.235	0.142	0.032

TABLE V

Diameter of the endodermal and pericycle cells of root (microns)

Treatment	Sample S_1	S_2	S_3	S_4	Mean
			Endoderm		
T_1	30.53	23.98	22.83	20.70	24.51
T_2	30.26	32.47	19.82	30 62	28.29
T_3	34.78	23.36	35.93	23.89	29.49
T_4	25.75	21.41	30.35	24.07	25.39
Mean	30.33	25.31	27.23	24.82	
			Pericycle		
T_1	31.77	25.90	23.01	16.37	24.49
T_2	24.24	22.47	19.02	31.06	24.20
T_3	27.87	20.79	35.66	21.85	26.55
T_4	22.47	14.95	29.38	21.77	22.14
Mean	26.59	20.26	26.77	22.76	

		Endoderm	Pericycle
C. D. (5% level) for comparison between	marginal means	0.022	0.020
Do.	for other comparisons	0.043	0.040

The variation in the diameter of the parenchyma cells over time was highly significant and that due to treatment was also significant. The mean diameter showed a remarkably high value for T3 as compared to the other treatments. There was an increase in the diameter of the parenchyma cells of the root upto the third month after which it decreased. Treatment Tl (Control) showed the least diameter while T3 recorded the highest value.

The diameter of the xylem vessels showed highly significant variation due to treatment and significant variation over time. There was considerable variation between treatments and between samples. Treatment T2 (CaO/MgO = 6:1) recorded the minimum diameter, whereas the maximum diameter was for T3 (CaO/MgO - 3:1).

The variation in the diameter of the phloem cells over time was significant. After the third month the diameter of the phloem cells decreased, which indicated that the incidence of disease affects the phloem cells of the root. The maximum diameter was seen in the case of T3.

The endodermal cells of the root exhibited significant size variations due to treatment effects and over time. The rate

of change of diameter over time corresponding to the four treatments showed considerable variation as indicated by the significant interaction. The minimum diameter was for treatment T1 (Control) and the maximum for T3 (CaO/MgO = 3:1)

The pericycle cells of the root showed highly significant variation over time. The variations due to treatment were also significant. The minimum diameter was observed for treatment T4 (CaO/MgO=9:1) and the maximum for T3 (CaO/MgO = 3:1) followed by Tl (Control).

Comparison of the tissues of diseased and healthy banana plants

The mean diameters of the different kinds of cells of the diseased banana plants under the various treatments are compared with those of a healthy plant in Table VI

TABLE VI

Comparison of the tissues of diseased and healthy banana plants

Tissue	Diseased			Healthy		
				Mean		
1. *Leaf-sheath*						
Parenchyma cells (dia. μ)	98.24	72.32	11232	92.16	93.76	124.16
Xylem vessels (,,)	109.12	90.88	118.08	78.40	99.12	134 40
Phloem cells (,,)	16.19	14.24	1522	13.45	14.78	30.09
2. *Petiole*						
Parenchyma cells (dia. μ)	91.20	82.88	94.08	85.76	88.48	95.36
Xylem vessels (,, J	85.44	87.04	120.00	72.96	91.36	128.64
Phloem cells (,, j	15.31	15.84	14.77	13.63	14.89	20.08
3. *Leaf*						
Parenchyma cells (dia. μ)	48.96	50.24	47.68	42.24	47.28	83.20
Xylem vessels (,,)	46.08	43.52	45.44	44.48	44.88	62.08
Phloem cells (,,)	11.15	12.30	12.21	9.91	11.39	14.07
4. *Root*						
Parenchyma cells (dia. μ)	54.08	66.88	58.88	59.84	59.92	69.76
Xylem vessels (,,)	53 44	60.80	56.96	59,84	57.76	65.28
Phloem cells (,,)	14.95	1433	12.74	13.54	13.89	15.84
Endodermal cells (,,)	20.70	30.62	23.89	24.07	24.82	32.39
Pericycle cells (,,)	15.75	30.06	21.85	21.77	22.36	27.87

(a) *Leaf-sheath*

There was significant difference in the diameter of the parenchyma cells of the diseased and healthy leaves. The diseased plants under treatment T3 (CaO/MgO = 3:l) showed the maximum diameter of the parenchyma cells compared to the other treatments. The diameter of the parenchyma cells in T3 approached that of the healthy plant. The diameters of the xylem vessels and phloem cells also showed significant variation between the diseased and healthy plants.

(b) *Petiole*

The parenchyma cells, xylem vessels and phloem cells of the petiole exhibited significant difference in diameter between the diseased and healthy plants. In the case of the parenchyma cells the diseased plants under treatments T1 and T3 had more or less the same diameter as for healthy plants. The xylem vessels had the minimum diameter in treatment T4 (CaO/MgO = 9:1) and the maximum in treatment T3 (CaO/MgO = 3:1) which was very close to that of healthy plants.

(c) *Leaf*

The statistical analysis of the data relating to the leaf tissues showed that there was significant difference in the diameter of the parenchyma cells, xylem vessels and phloem cells of the leaf between the diseased and healthy plants.

(d) *Root*

The diameters of the parenchyma cells, xylem vessels and phloem cells of the root of the diseased plants were found to be significantly different from those of the healthy plants. In the case of the diameter of the endodermal cells and pericycle cells also the difference between the diseased and healthy plants was significant.

Discussion

There has been little work done on the anatomy of the Bunchy Top affected banana plants. It is somewhat surprising that even Magee in his classical investi. gations on the many aspects of Bunchy Top infection, has only touched on the fringe of the problem. Wardlaw (1961) has summarised the work on the anatomy of Bunchy Top affected bananas and stated that a microscopic examination of the pseudostem and bulb tissues reveals no pathological changes of diagnostic value. The affected leaf tissue, however, reveals a distinctive pathological condition. The extent to which the vascular bundles are altered as a result of the Bunchy Top disease depends on the intensity of the infection. Few changes are observed first, but as the infection progresses more and more vascular bundles show abnormal development. In severe primary infection every vascular bundle in the leaf is found to have undergone some modification. In transverse section the normal vascular strands of the lamina are seen to have a fibrous sheath on both the phloem and xylem sides. The xylem consists of spiral tracheids and parenchyma, and the phloem of seive tubes and companion cells only.

Magee (1927, 1939, 1953) has described the following modifications induced by the presence of the virus : (i) In the phloem, the development of the fibrous sheath is suppressed, its place being occupied by cells with many chromatophores. (ii) The ground tissue contiguous with the phloem become 'gorged' with tfhromatophores whereas in the normal plant this region is almost devoid of chlorophyll. (iii) The ground parenchyma cells in contact with the phloem are stimulated to division and

form somewhat angular cells by the formation of thin cellulose partition walls in all planes. Each newly formed cell contasns numerous chromatophores and a distinct nucleus. (iv) Similar 'appositional' growth of walls in all planes takes place in the cells of the phloem and gives rise to numerous angular cells along their length. These cells are uninucleate. The phloem nuclei in a primarily infected plant are particularly noticeable in any transverse orlongitudinal section. They show wide variation in shape and size and there is evidence that they may have been formed, not by true mitosis but by amitotic or direct division.

Proliferation of the phloem and adjacent tissues has not been observed in the rhizome or roots. In advanced stages of Bunchy Top, the root system presents a decayed appearance. Soon after infection with the virus, the main and lateral roots show some rotting, but the same condition is often found in apparently healthy plants as well. But as the virus disease progresses, root decay becomes more pronounced. As the main roots rot back towards the rhizome, new ones, at first healthy in appearance and bearing an abnormal number of laterals, are formed. In due course, these also become purple in colour and begin to decay. The Bunchy Top plant, in fact, persists with a scanty root system, which is always in the course of renewal and destruction. The disorganisation of the phloem, together with various correlative developments, may account for the loss of resistance of the roots to common soil fungi.

The foregoing information is practically all what is available in literature on the anatomy of Bunchy Top affected banana plants. The present studies were undertaken to follow the changes, either degenerative or otherwise, that take place in the banana tissue, when plants grown under different nutritional environments were subjected to infection by the virus. The results from these studies have thrown very considerable light on cellular changes that take place in the banana tissue, when plants grown under different nutritional environments are subjected to infection by the virus. The basis of these studies have been the previous observations of Nair and Pillai (1966) that a CaO/MgO ratio of approximately 3. 5 within the plant tissue gave appreciable resistance to infection by the Bunchy Top virus resulting in a delay of 199 days and consequent emergence of the healthy bunch. The samples for analysis taken in the present study were from the experimental plants referred to in the work of Nair and George (1966). Before entering into a detailed discussion of the results of these anatomical studies, it is necessary to remember that a desired CaO/MgO ratio within the plant tissue cannot always be had by changing the soil nutritional environments alone. Nair and George (1966) obtained the following CaO/ MgO ratios in the plant tissue (leaves) corresponding to the treatments selected for anatomical studies :

Treatment	*CaOIMgO ratio in soil*	*CaO/MgO ratio in leaf*
T1 (Control)	0.27/0.08 (3.4)	1.88
T2	1.02/0.2 (6.0)	3.79
T3	0.6 /0.2 (3.0)	2.96
T4	1.8 /0.2 (9.0)	5.61

It may be noted that the lowest CaO/MgO ratios are for the control and T3, indicating that the leaf tissue contains proportionately more of magnesium in these two cases. Such a situation is favoured by a CaO/MgO ratio in the soil of approximately between 3 and 4. With these facts in mind, it would be interesting to

examine the results obtained from the present anatomical studies. All the plants in the experiment became diseased sooner or later, after the release of the aphids, but cellular changes have followed a definite pattern depending upon the CaO/MgO ratio in the soil, which would have vitally affected the plant tissue composition also.

In the case of the parenchyma of leaf sheath, there is a definite decrease in the diameter of the cells after the incidence of the disease, but cell diminution is the lowest in the case of treatments T1 and T3, with a CaO/MgO ratio in the soil of approximately 3. The same trend is seen in the case of the xylem vessels. There is a decrease in diameter after the incidence of the disease, but this decrease is significantly lower in the case of xylem vessels of the sheath of plants grown on soils with a CaO/MgO ratio of 3 and 3.4. Treatment T3 has the maximum diameter of xylem vessels of leaf sheath even during the fourth sampling, that is, three months after the incidence of the disease. In the case of the phloem cells of the leaf sheath, the rate of diminution in size is the lowest for T3 (CaO/MgO=3.0) followed by control (CaO/MgO = 3.4). The minimum diameter of xylem vessels and phloem cells were observed in the case of T4, with a ratio of 9 in the soil with respect to CaO/MgO.

Observations on the parenchyma cells of petiole show that the incidence of the disease has contributed to diminution in cell size. The diminution of parenchyma cells was the lowest in the case of T3 even at the advanced stage of the disease. This was closely followed by T1 indicating that there is a possible correlation between soil ratio of CaO/MgO and the rate of degeneration of tissues, a ratio between 3 and 4 substantially contributing towards delayed cell disintegration. In the case of xylem vessels of petiole also, the maximum diameter was maintained by plants under T3 even at the advanced stage of the disease.

In the case of the root tissue the maximum diameter of parenchyma cells was recorded in the case of T3 even at the advanced stage of the disease corresponding to the fourth sampling date. The same was true in the case of the xylem vessels of the root also. In the case of the phloem cells of the root the maximum diameter was maintained by T3 even at the advanced stage of the disease. The endodermal cells and the pericycle of the root also maintained the same trend.

While a very definite correlation is seen to exist between the rate of cell diminution in the case of tissues of the leaf sheath and petiole & the environmental soil conditions with respect to the CaO/MgO ratio, such diminution being the lowest in the case of plants growing on soils with a CaO/MgO ratio of 3.0 to 3.4, there is no such correlation observed in the case of the parenchyma and phloem cells of the leaf. However, the xylem vessels of the leaf shows, again, a noticeable correlation. Among the diseased plants, the maximum mean diameter of xylem vessels has been observed in the case of treatment T3 (CaO/MgO=3:1). The incidence of the disease has generally resulted in a reduction in the size of the vascular bundles, as also the thickness of the lamina.

In comparative studies between healthy and diseased plants of the same age, it has been noted that there is significant difference in the diameter of parenchyma cells of leaf sheath in both. In the case of plants growing on soils with a CaO/MgO ratio of 3.0 it is seen that the parenchyma cells of leaf-sheath have a diameter approaching that of the healthy plant. This is true also of the xylem vessels. The phloem

vessels too show the same trend. The cells of the petiole in general show the same correlation.

From the results discussed above the following broad conclusions emerge. As a result of virus infection, there is a clear diminution in the size of cells of all tissues. Cell depositions of chromatophores and "X-bodies" as reported by several workers, have not always been found associated with the infection and spread of the Bunchy Top virus in banana. The reduction in the cell size of the parenchyma, phloem and xylem vessels bears a definite correlation to the CaO/MgO ratio in the soil in which the plant grows, a ratio of 3:1 being the most favourable among all the ratios tried, for the lowest rate of cell diminution. Cell degeneration and disintegration are greatly retarded in plants growing on soils where calcium and magnesium are supplied in the above ratio. It is possible that the control plants in the present experiments have turned out to be next best to T3 in respect of resistance to cell changes, because of the fact that the natural ratio of CaO/MgO in the soil used happened to be 3.4. It may be noted that the control plants and T3 both have a plant tissue composition where there is a comparative abundance of magnesium to calcium. Resistance to virus infection, as indicated by resistance to cellular changes such as diminution, degeneration, disintegration etc. may well be a function of tissue composition, which again is largely dependent on the nutritional environment in which the plant grows. Although virus can pass through vascular tissue without reproducing in all its cells, many cells become infected and undergo necrotic changes (Luria, 1956). Phloem necrosis is reported to be wide-spread in the case of many virus diseases and probably accounts for most of the circulatory disturbances in infected plants. It is clear from the results of the present studies that changes in the parenchyma and phloem cells as well as xylem vessels in the plant tissues are remarkably slowed down, depending on the supply of calcium and magnesium to the plant. It is possible that a definite ratio in the plant tissue, rather than in the nutrient medium, may contribute towards resistance to cellular changes consequent on virus infection. Detailed further studies on the anatomy of plant tissues, under cellular compositions differing with respect to calcium and magnesium, seem desirable to throw further light on the possible role of these elements in imparting resistance to infection by Bunchy Top virus in banana.

Summary and Conclusions

There are noteworthy anatomical changes that take place in the banana tissue consequent on the incidence of the Bunchy Top disease.

1. The parenchyma cells of the leaf-sheath undergo diminution in size after the incidence of the disease, such diminution being the lowest in the case of diseased plants growing on soils with a CaO/MgO ratio of 3.0. The same is true of xylem vessels and phloem cells of the leaf-shealh.
2. The parenchyma cells of the petiole undergo significant diminution in size after the incidence of the disease. The reduction in cell size is the lowest in the case of plants growing on soils with a CaO/MgO ratio of 3.0. The same effect was noticed in the case of vascular tissues. The degeneration and disorganisation of vascular bundles were the slowest in plants growing on a soil with a CaO/MgO ratio of 3.0.
3. In the case of the root tissue, maximum diameter of parenchyma cells, xylem

vessels and phloem cells was maintained by plants growing on soil with a CaO/MgO ratio of 3.0, even at an advanced stage of the disease. The endodermal cells and the pericycle of the root also maintained the same trend.

4. The root tissue is comparatively less affected by the secondary infection by Bunchy Top disease.

5. No correlation is observed between size of parenchyma and phloem cells of the leaf tissue and the CaO/MgO ratio of the soil. However, the xylem vessels of the leaf are the largest in the case of diseased plants growing on soil with a CaO/MgO ratio of 3.0. The disorganisation of vascular bundles is the slowest in such plants.

6. In the case of diseased plants growing on soil with a CaO/MgO ratio of 3.0, the parenchyma and phloem cells of leaf sheath and xylem vessels have a diameter approaching that of healthy plants.

7. The cells of the petiole also show the same general correlation (as stated in 6 above).

8. Cell degeneration and disintegration are greatly retarded in plants growing on soil with a CaO/MgO ratio of 3.0.

9. Resistance to virus infection, as indicated by resistance to cellular changes such as diminution, degeneration, disintegration etc. may be a function of tissue composition, which again is largely dependent on the nutritional environment in which the plant grows.

10. It is possible that a definite CaO/MgO ratio in the plant tissue, rather than in the nutrient medium, may contribute towards resistance to cellular changes consequent on virus infection.

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