CONTRIBUTIONS TO THE STUDY OF SPIKE-DISEASE OF SANDAL (SANTALUM ALBUM, LINN.).

Part I. Diastatic Activity of the Leaves.

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It is well known that in the case of many plant diseases the pathological condition is indicated by a marked disturbance in the enzymatic activity of the tissues. From this point of view the oxidising enzymes have been those most investigated and it has been found that the oxidase-activity of the diseased tissue is not infrequently intensified abnormally. Mosaic disease of tobacco, potato "Leaf-roll" disease and "Curly-top" of sugar-beet are examples in which this occurs. Other groups of enzymes have received comparatively little attention. The diastase-content of diseased potatoes has however been found greater than that of the normal tubers. Coleman investigated the diastatic activity of sandal-leaves, both healthy and spiked, in an endeavour to find an explanation for the strikingly abnormal accumulation of starch in the diseased leaves and other tissues of spiked sandal. He came to the conclusion, however, that the diastatic activity of spiked leaves was lower than that of the healthy ones, and explained starch-accumulation on the basis of defective translocation of the photosynthetic carbohydrates due to poor diastatic activity.

During the course of a detailed investigation of the various enzymes of sandal-tissues, both diseased and healthy, our experience has been entirely different and we have consistently found higher diastatic activity in the diseased leaves. Coleman's remark however that the spiked leaves are really comparable in composition with young healthy leaves holds true, not only with respect to their chemical composition but also with regard to enzymic activity. The spiked leaves are as rich in diastase as the growing points of a healthy sandal (see Table IV).

Material and Methods: Collection of Samples.

Samples of leaves from spiked and healthy sandal trees were collected from two different areas, Uttarahalli and Ragihalli, the former lying at a distance of about 6 miles from Bangalore on the

Kankanahalli Road, and the latter near Banneraghatta, about 13 miles south of Bangalore. Both the areas have been under observation for a number of years and the first symptoms of spike were observed in the Uttarahalli area in the year 1914 (Coleman, *Spike Disease of Sandal*, 1917, p. 2). No record is so far available to us with regard to the date of incidence of spike in the Ragihalli area. Samples were taken from a number of selected trees situated at different points on the area. The well-known external symptoms were taken as the criteria for determining the spiked condition of plants. In the present state of our knowledge we are not in a position to state definitely that the apparently disease-free plants from which we have collected our research material are absolutely healthy though we believe this to be the case. As they are growing in a spiked area it is possible they may already have become infected, though they show no symptom from which this could be deduced. Moreover, the healthy trees have, so far as all external appearance goes, remained healthy and, if infected at all, could only have been in the earliest stage of the disease.

Leaves and stems were collected between 8·30 and 9·30 a.m., and were taken from every one of the marked trees so that a representative sample was ensured. The material was placed in glass-stoppered bottles immediately after plucking, in order to minimise any loss of moisture due to transpiration, and carried to the laboratories in cold storage. In the case of the Uttarahalli samples the time taken for transferring the bottle from the site of collection to the ice-chest was about 30 minutes, while roughly two hours elapsed with the samples from the Ragihalli area. The samples collected on any one day were at least comparable between themselves.

Preparation of Material for Analysis.

Expression of saps.—About 100 grams of leaves after separation from the stems were weighed into a nickel-wire-gauze holder which was afterwards immersed in liquid air and allowed to remain there until the liquid ceased to boil. The material was removed, and allowed to attain room-temperature in a closed bottle in order to prevent the condensation of atmospheric moisture. The material was then ground in a porcelain mortar, wrapped in a filter-cloth (previously wetted and pressed out at a pressure of one ton to the square inch) and pressed in a nickel-plated hydraulic press at one ton to the square inch.

To secure uniformity in composition and yield of sap from materials collected on different days the following arbitrary standards were fixed. The charge of leaves was 100 gms., pressure applied one ton; time allowed for expression 15 minutes. The sap expressed was weighed

and also the leaf residue. The losses due to imperfect removal of the liquid from the parts of the press do not usually exceed one or two per cent. The sap was immediately centrifuged for 15 minutes at 3,000 revolutions per minute, toluene was added, and the sap preserved in the ice-chest. The conditions for the expression of saps from the stems were nearly the same, except that a pressure of 2 tons to the square inch was applied.

Preparation of leaf-powders.—The leaves were spread in thin layers and dried over freshly fused calcium chloride in a vacuum-desiccator, 24 hours being usually sufficient for complete drying. The material was then powdered in a porcelain mortar, passed through a 40-mesh sieve, and preserved in glass-stoppered bottles.

Methods of estimating diastatic activity.—The sap (2 c.c.) was pipetted into a 50 c.c. conical flask containing 25 c.c. of 2 per cent. soluble starch and a little toluene, the reaction being allowed to proceed at room-temperature. The necessary controls were run. After 20 hours the mixture was treated with 5 c.c. of dialysed iron and centrifuged. Sugars were estimated in an aliquot portion of the clear centrifugate by Bertrand's method and calculated as maltose.

The diastatic activity of leaf-powders was estimated similarly, 0·25 gm. of the dried powder being allowed to act upon 25 c.c. of 2 per cent. soluble starch solution. The necessary controls were run. The toluene added to the mixture serves not only as an antiseptic but also as an autolysing agent. After 20 hours the mixtures were treated with dialysed iron, centrifuged, and an aliquot portion of the clear fluid used for the estimation of reducing sugars by Bertrand's method. The coloration developed with iodine was noted in each case.

Results.—The diastatic activity of the saps, expressed in milligrams of maltose per cubic centimeter of sap, is shewn in Table I. In every instance without exception the diastatic activity of saps from spiked leaves is definitely greater than that of healthy leaf-saps. In some cases the activity of spiked leaf-sap is found to be four times that of the healthy sample. Table II includes a few results shewing the diastatic activity of diseased and healthy stem-saps. A distinctly higher diastatic activity is again shown by the spiked specimens. Table III comprises the results obtained with the dry leaf-powders, diseased and healthy, expressed in grams of maltose produced per gram of leaf-powder. All the results point to a higher diastatic activity in the case of the spiked leaf. The colour reactions with iodine confirm this result.

TABLE I.

Diastatic Activity of Leaf-sap.

Date in 1927	Locality	Diastatic activity of 1 c. c. of sap			
		Healthy		Spike	
		Maltose in mgms.	Coloration with iodine	Maltose in mgms.	Coloration with iodine
April 27 ...	Uttarahalli	77·5	Red	161·0	None
May 12 ...	,,	66·0	Blue	114·7	Red
,, 19 ...	,,	101·5	Deep Red	130·5	None
June 16 ...	,,	66·0	,,	144·0	,,
,, 23 ...	,,	109·6	,,	168·0	,,
August 5 ...	,,	75·0	,,	152·0	,,
,, 11 ...	Ragihalli	28·0	Blue	126·0	,,
October 27 ...	Uttarahalli	44·2	,,	172·8	,,
November 7 ...	Ragihalli	46·4	,,	86·0	Blue

TABLE II.

Diastatic Activity of Stem-saps.

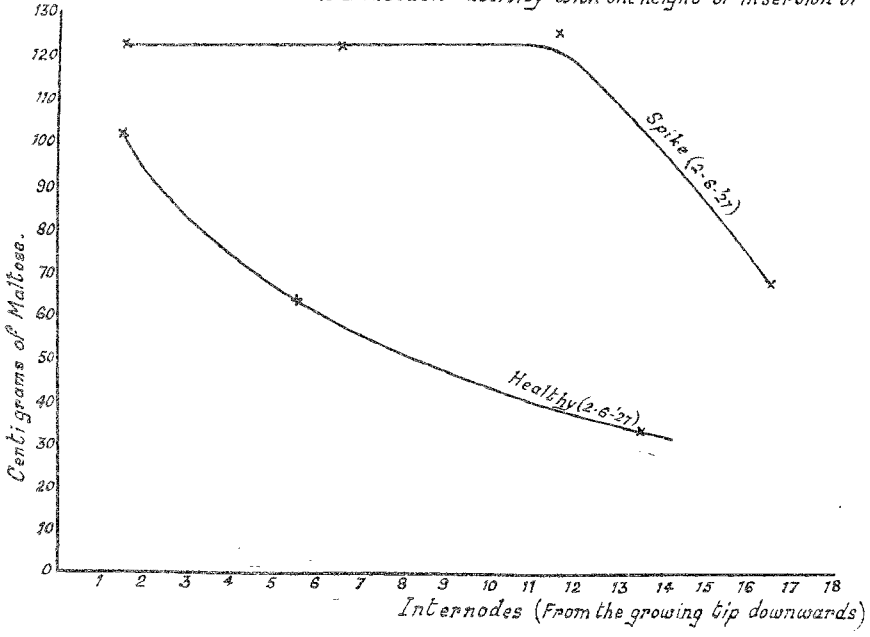
Date in 1927	Locality	Diastatic activity of 1 c. c. of sap			
		Healthy		Spike	
		Maltose in mgms.	Coloration with iodine	Maltose in mgms.	Coloration with iodine
July 20 ...	Uttarahalli	19·2	Blue	37·8	Blue
November 7 ...	Ragihalli	27·0	,,	38·0	,,

TABLE III.

Diastatic Activity of Leaf-powders.

Date in 1927	Locality	Activity of one gram of dry leaf-powder			
		Healthy		Spike	
		Maltose in gms.	Coloration with iodine	Maltose in gms.	Coloration with iodine
April 14 ..	Ragihalli	0·198	Reddish blue	0·980	Red
,, 20 ...	Uttarahalli	0·204	,, ,,	0·356	,,
May 5 ...	Ragihalli	0·610	Bluish Red	1·080	Slightly Red
,, 12 ...	Uttarahalli	0·039	Reddish blue	0·906	Red
,, 19 ...	,,	0·192	Blue	0·294	Bluish Red
June 2 ...	,,	* 0·664	Bluish Red	* 1·100	Colourless
,, 16 ...	,,	0·132	Reddish blue	0·287	Red

* Average of four determinations.


Gradient of the Diastatic activity with the height of insertion of leaves.
Centigrams of Maltose.
130
120
110
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Internodes (From the growing tip downwards)
Spike (2-6-'27)
Healthy (2-6-'27)

Gradient Study.—The variation in diastatic activity of the leaves according to the height of insertion in the stem was followed by a gradient-study. The results collected in Table IV reveal an almost entire absence of any gradient in the case of the spiked leaves, while a steady fall in the diastatic activity from the growing tip downwards is shewn in the healthy shoots. The results have also been represented graphically (Graph I).

TABLE IV.

Gradient of Diastatic Activity (June 2, 1927, Uttarahalli).

Sample	Activity of dry leaf-powders			
	Healthy		Spiked	
	Maltose in mgms.	Coloration with iodine	Maltose in mgms.	Coloration with iodine
Growing tips East...	120·0	Colourless	127·2	Colourless
I ,, ...	114·4	Red	128·0	,,
II ,, ...	89·4	Red	126·4	,,
III ,, ...	—	—	50·0	Red
Growing tips West...	102·0	Colourless	122·4	Colourless
I ,, ...	63·6	Blue	122·0	,,
II ,, ...	—	—	127·2	,,
III ,, ...	33·6	Blue	68·4	Red

TIME-COURSE STUDY.

A study of the time-course of diastatic action was made with the leaf extracts. Leaf-powder by itself could not be employed since the solid material interferes with the subsequent operations of drawing out definite quantities of the reaction mixture for analysis. The enzyme extract was prepared as follows: Desiccated leaf-powder (15 gms.) was digested at room-temperature with 150 c.c. of water containing a little toluene for 20 hours in a 250 c.c. glass-stoppered bottle shaken at frequent intervals. Hide-powder (5 gms.) was added for the abstraction of the tannins which might inhibit the activity of diastase, it having been previously ascertained that hide-powder did not adsorb any diastase. The whole digest was centrifuged, the supernatant liquid passed through a filter and the clear extract used for the experiments. The reaction mixture consisted of 100 c.c. of a 4 per cent. Lintner's soluble starch solution, 100 c.c. of the enzyme extract and 5 c.c. toluene. The hydrolysis was allowed to proceed at 30° in a thermostat. Definite quantities of the mixture were withdrawn from

the experimental and control flasks and examined polarimetrically, the reducing sugars being estimated by Bertrand's method.

An examination of the tables (V and VI) and the curves (graph II) brings out strikingly the higher diastatic activity of the diseased leaf extract. Whether these results indicate differences in the absolute amount of diastase or are explicable by the presence of inhibitors or activators in the respective samples is undoubtedly of great interest. The curves point however to a definitely lower enzyme-concentration.

TABLE V.

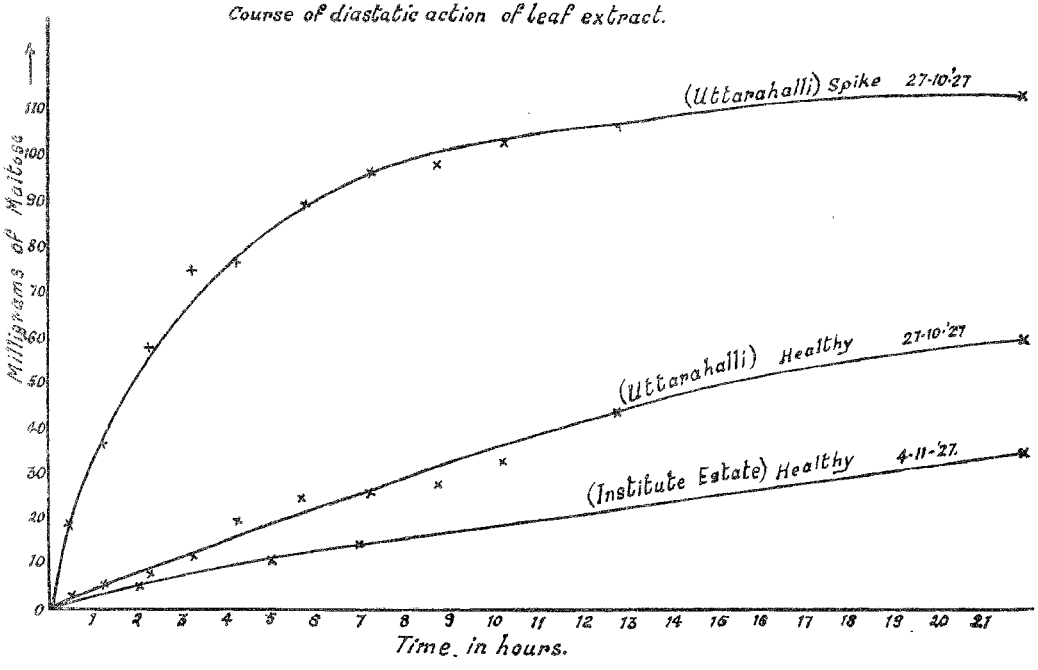
Time-course of Diastatic Action (Healthy Leaf Extract).

Time	Diastatic activity		Remarks
	Maltose in mgms.	Coloration with iodine	
30 minutes	2·4	Blue	Leaves collected at 8-30 A.M. on 4-11-27 from healthy sandal trees on the Institute Estate
60 ,,	4·7	Blue (tinged red)	
135 ,,	10·7	Bluish violet	
180 ,,	12·2	Violet	
300 ,,	18·0	Purple	
420 ,,	23·3	Brick red	
21·75 hours	58·3	Slight red	
47 ,,	78·5	Colourless	

TABLE VI.

Time-course of Diastatic Action (Healthy and Spike Leaf Extracts).

Time		Healthy		Spike	
		Maltose in mgms.	Coloration with iodine	Maltose in mgms.	Colcration with iodine
Hrs.	Mins.				
0	30	2·5	Blue	18·5	Blue
1	15	4·7	,,	37·5	Reddish blue
2	15	8·9	Violet	58·0	Violet brown
3	15	11·5	Reddish violet	75·0	Dull red
4	15	19·2	,,	77·0	,,
5	45	24·5	Brown	89·0	Colourless
7	15	25·0	Red	96·0	,,
8	45	27·0	,,	97·0	,,
10	15	32·4	,,	103·0	,,
12	45	50·4	,,	...	,,
22	30	60·0	Slight Red	114·0	,,


Course of diastatic action of leaf extract.
Milligrams of Maltose
(Uttanahalli) Spike 27-10-'27
(Uttanahalli) Healthy 27-10-'27
(Institute Estate) Healthy 4-11-'27
Time in hours.

in the healthy leaf extract. In addition to a higher enzyme-concentration in the spike-leaf extract however, the lower pH value (4·7), which approximates to the optimum reaction of plant amylases, the lower calcium content, and the higher amino-nitrogen value of the diseased leaf extract, possibly contribute towards the activation of the enzyme derived from the spiked leaf. Further work to examine the influence of these and other factors is in progress.

In conclusion it is our pleasant duty to express our gratitude to Professor Norris for the keen interest he has evinced in the work and for his many helpful suggestions.

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