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

PART X. Seasonal studies on healthy and partially spiked trees.

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It was shown in a previous communication (*J. Indian Inst. Sci.*, 1928, **11** A, 97) that the leaf tissues and tissue fluids of spiked sandal trees were abnormal in composition. In view of these findings, it was considered necessary, for a proper understanding of the disease, to study the metabolism of the leaf during the progressive stages of spike. Simultaneous observations on healthy sandal were also made and were expected to throw considerable light on such metabolic processes as vegetative growth, flowering and fruiting; and to show by contrast the effect of the disease on spiked trees.

The leaves used in the present investigation were collected from one experimental area (Uttarahalli), and possible errors due to soil and climatic variations were thus obviated.

Four fairly large trees, two healthy and two diseased, located near each other and of nearly the same age and height, were chosen for collection of specimens. The diseased trees had a few small branches still unaffected and actually flowering. In the affected branches the internodes were shortened; the leaves were narrower and smaller than those on the unaffected ones and no flowers were seen. In the later stages, the disease was observed to have spread to one of the healthy twigs. The healthy trees were bearing fruit in the beginning of the investigation and after June, buds developed, thereby indicating that these trees remained healthy throughout the period of investigation. To exclude differences arising from age, only mature leaves from large branches were collected for examination; other details relating to the collection and handling of specimens are set forth in an earlier part (*loc. cit.*, 103).

The season covered by the investigation was from early April to late September in 1928 and represented a hot and dry spring, a cool and rainy summer and a mild and humid early autumn. Table I presents the weekly averages for the more important meteorological observations in the period.

TABLE I.

1928	Rainfall, inches	Sunshine, hours	Max. temp. F.	Min. temp. F.	Relative humidity per cent.
April 10	0·01	9·1	93·8	68·9	52·0
,, 20	0·06	9·0	97 0	70·5	44·0
,, 30	0 00	9·5	97 5	72·0	42·3
May 8	0·01	8·8	97·7	72·0	49·1
,, 22	0·29	9·3	96·2	71·5	49·4
June 8	0·01	6·2	87·9	68·4	61 3
,, 22	0·25	5·7	76·4	66·9	60·0
July 9	4·31	5·1	85·1	67·0	70·0
,, 23	0·40	3·1	80·9	65·9	70·0
August 8	1·73	2·8	81·7	66·3	77·3
,, 22	3·07	2·5	80·5	65·6	79·1
Sept. 7	0·02	6·6	84·6	64 8	64·1
,, 21	0·70	5·1	83·4	65·8	66·7

It may be noted that while April and May were very hot, had over nine hours of bright sunshine per day and under 50 per cent. of relative humidity, the late part of July and the whole of August were cool, had under three hours of bright sunshine per day and over 70 per cent. of relative humidity; June and September were intermediate between the two extremes. Such marked variations in season were expected to influence the soil conditions and physiological activities of the trees, and indicate the extent to which the conditions of the latter were affected in both health and disease.

Analytical Methods.

Extraction of sap was made according to the method detailed in an earlier communication (*J. Indian Inst. Sci.*, 1928, **11A**, 23) with the difference that a longer time was allowed for the material to thaw and the cells to plasmolyse, and time of extraction reduced to 10 minutes.

Freezing-point determinations were made by the micro-Beckmann method, with corrections for super-cooling. True depression of the freezing-point was calculated according to the formula $\Delta = \Delta' - 0{\cdot}0125\mu\,\Delta'$ where Δ' represented the observed freezing-point minus that of distilled water and μ the degrees of cooling below the observed freezing-point. Osmotic pressure P was calculated using the formula, $P = 12{\cdot}06\,\Delta - 0{\cdot}021\,\Delta^2$. Specific electrical conductivity, hydrogen-ion concentration and titration values were determined by methods already described (Varadaraja Iyengar, *loc. cit.*, 97).

The sap-contents were estimated by the refractometer method using Schönrock's and Geerlig's tables (*Bureau of Standards*, 1918, 44, 133) respectively, for water and solids. The values thus obtained were checked from time to time by the evaporation method.

Extraction and clarification of the leaf-sugars were conducted by the method previously adopted. Estimations of the individual sugars were made by the method of Tollenaar Dirk (Omzettingen van Koolhydraten in het Blad van *Nicotiana Tabacum*, L., 1925, 21). Reducing sugars were estimated by the Bertrand method, 25 c.c. of centrifuged aqueous extract being used for this purpose; sucrose by hydrolysing 25 c.c. portions of extract with 1·8 c.c. of 37 per cent. hydrochloric acid at 70° for 5 minutes, neutralising, diluting to 50 c.c. and determining reducing power as in the previous case; maltose by a method similar to that adopted for sucrose, but differing in the time of hydrolysis which was extended to 24 hours.

After removing sugars with 96 per cent. alcohol, dextrins in the dried residue were extracted with 10 per cent. alcohol at 50° for 30 minutes. The alcoholic extract was concentrated to a small volume and extracted with water. The aqueous extract was clarified, filtered, treated with one-tenth its volume of hydrochloric acid (*d.* 1·125) and hydrolysed at the temperature of boiling water for 2 hours. The hydrolysate was neutralised, made up to volume and the reducing power determined as glucose. The residue from the above was used for determination of starch by the taka-diastase method.

The residue after starch determination was refluxed with 2·5 per cent. sulphuric acid for 1 hour, filtered, neutralised, clarified, made up to volume and the reducing power estimated. This was denoted as hemicelluloses calculated as dextrose.

The other chemical constituents were determined by the methods detailed before (*loc. cit.*, 98). In addition to the above, the leaves and twigs of the healthy portion of the partially spiked trees were examined histochemically for starch, and chemically for nitrogen and calcium.

The effect of season on the tissue fluids and the several chemical constituents of the tissues are presented in the following tables.

TABLE II.

Sap extraction, healthy and diseased leaves: total weight, 100 gms.

Date	Healthy			Spiked		
	Wt. of sap gms.	Wt. of residue gms.	Loss during expression of sap	Wt. of sap gms.	Wt. of residue gms.	Loss during expression of sap
April 10	59·25	35·60	5·15	57·20	39·40	3·40
,, 20	55·45	39·30	5·25	59·80	35·00	5·20
,, 30	60·65	34·00	5·35	57·50	39·15	3·35
May 8	62·35	34·65	3·00	61·55	34·90	3·55
,, 22	62·50	35·05	2·45	65·40	31·80	2·80
June 8	57·00	40·25	2·75	62·65	34·55	2·80
,, 22	49·80	46·35	3·35	55·95	40·55	3·50
July 9	57·10	40·55	2·35	...	...	...
,, 23	56·90	41·25	1·85	63·65	34·25	2·10
Aug. 8	60·45	37·55	2·00	64·45	...	...
,, 22	65·20	33·10	1·70	60·35	36·20	3·45
Sept. 7	66·20	31·30	2·50	...	...	...
,, 21	55·80	40·80	3·40	...	...	...

TABLE III.

Composition of saps (per cent.).

From refractometer readings.

Date	Healthy		Spiked	
	Water	Solids	Water	Solids
April 30	82·58	17·65	84·35	15·82
May 8	83·34	16·80	84·30	15·85
,, 22	82·35	17·84	86·13	13·95
June 8	85·09	15·10	86·79	13·35
,, 22	82·30	17·87	84·26	15·80
July 9	81 72	18·48	...	...
,, 23	82·43	17·78	87·63	12·49
Aug. 8	86·03	14·19	...	...
,, 22	84·52	15·65	87·07	13·02
Sept. 7	83·83	16·32	...	...
,, 21	81·65	18·56	...	...

TABLE III A.

Composition of Saps (per cent.).

By evaporation method.

Date	Water		Solids	
	Healthy	Spiked	Healthy	Spiked
22-5-28	82·68	84·84	17·32	15·16
22-6-28	84·71	85·33	15·29	14·67
23-7-28	83·18	87·91	16·82	12·09

TABLE IV.

Freezing-point Depression (Δ), *Osmotic Pressure* (P) *and Electrical Conductivity* (K) *in Healthy and Diseased Leaf-saps.*

Date	Healthy				Spiked			
	Δ	P	$K \times 10^5$	$\frac{K \times 10^3}{\Delta}$	Δ	P	$K \times 10^5$	$\frac{K \times 10^3}{\Delta}$
il 10 ...	2·47	29 7	...	...	2·31	27·8	...	...
20 ..	2·30	27·6	...	...	2·18	28·2	...	...
30 ...	2·75	33·0	2625	9·55	2·49	29·9	3190	12·81
y 8 ...	2 27	27·2	2190	9·65	2·11	25·4	3295	15·60
22 ...	2·43	29·2	2001	8·22	2·23	26·8	2785	12·51
e 8 ...	2·50	30·0	2521	10·08	2·20	2·64	3517	16·01
22 ...	2·43	29·2	2241	9·16	2·34	28·2	2777	11·84
y 9 ...	2·28	27·4	2292	10·03	...	...	...	...
23 ..	2·53	29·8	2633	10·42	2·07	20·9	2829	13·69
g. 8 ..	2·05	24·7	2204	10·74	...	...	...	...
22 ...	2·06	24·8	2338	11·35	1·94	23·3	2439	12·59
t. 7 ...	2·01	24·1	2489	12·41	...	..	...	...
21 ...	2·11	25·3	2426	11·52	...	...	...	...

TABLE V.

P_H and Titration Values of Leaf-saps, Healthy and Spiked.

Litmus = P_H 6·81 ; Phenolphthalein = P_H 8·37 : 4 c.c. of sap was titrated in each case against 0·966 N/10 alkali.

Date	Healthy			Spiked		
	P_H	Litmus, c.c. alkali	Phenol-phthalein, c.c. alkali	P_H	Litmus, c.c. alkali	Phenol-phthalein, c.c. alkali
April 10 ...	5·64	1·83	4 69	5·73	1·02	3·17
,, 20 ...	5·75	1 58	4·27	5·70	1·71	4·05
,, 30 ..	5·60	1·47	3·56	5·67	1·28	3·18
May 8 ...	5·65	1·90	4·50	5·85	1·12	3·03
,, 22 ...	5·75	1·57	4·00	5·83	1·33	3·40
June 8 ...	5·60	2·06	4·92	5·73	1·11	2·91
,, 22 ...	5·68	1·60	4·20	5·58	1·37	3·37
July 9 ...	5·71	1·52	3·90	...	...	...
,, 23 ...	5·54	2·18	4·64	5·51	1·23	3·02
Aug. 8 ...	5·40	2·10	4·49	...	...	...
,, 22 ...	5·35	2·17	4·41	5·19	1·80	3·83
Sept. 7 ...	5·47	1·68	3·71	...	...	...
,, 21 ...	5·40	1·83	4·21	...	...	...

TABLE VI.

Moisture-content of Healthy and Partially Spiked Leaves (per cent.).

Date	10·IV	20·IV	30·IV	8·V	22·V	8·VI	22·VI
Healthy	67·1	65·8	69·0	68·0	69·5	67·6	65·0
Diseased	70·1	69·3	69 2	69·7	75·1	72·0	69·0

Date	9·VII	23·VII	8·VIII	22·VIII	7·IX	21·IX
Healthy	66·1	67·1	65·7	69·4	67·5	65·8
Diseased	...	72·2	...	71 9	...	...

TABLE VII.

Ash, Ash-constituents and Total Nitrogen; Healthy (H) and Diseased (D) Leaves.

Percentage on dry weight.

Date	Ash		SiO_2		P_2O_5		CaO		K_2O		Total Nitrogen	
	H	D	H	D	H	D	H	D	H	D	H	D
April 10	12·91	16·25	0·09	4·1	0·20	0·97	3·76	0·88	1·97	2·94	2·35	4·21
,, 20	12·40	19·25	0·29	5·96	0·44	0·59	3·50	1·22	2·57	3·74	2·99	3·71
,, 30	13·61	20·98	0·53	7·11	0·21	0·74	3·53	0·99	2·97	4·08	2·74	3·67
May 8	10·72	22·96	0·32	8·49	0·23	0·55	2·96	1·04	2·29	4·11	2·82	3·63
,, 22	12·71	26·56	0·69	10·91	0·29	0·92	3·35	1·16	3·66	3·92	2·81	3·38
June 8	14·04	25·04	0·62	8·43	0·45	0·65	3·21	1·07	3·02	3·84	2·91	3·27
,, 22	13·14	21·83	0·13	7·90	0·43	0·76	2·77	1·08	2·58	3·85	2·98	3·26
July 9	12·40	...	0·33	...	0·35	...	3·37	...	2·83	...	2·53	...
,, 23	12·26	21·07	0·28	7·45	0·44	0·64	2·90	0·89	3·53	3·94	2·86	3·42
August 8	12·63	...	0·40	...	0·50	...	3·84	...	3·22	...	2·70	...
,, 22	13·12	19·31	0·51	7·73	0·41	0·76	2·84	0·99	2·94	4·57	3·03	3·77
September 7	13·61	...	0·61	...	0·49	...	3·60	...	2·88	...	2·90	...
,, 21	13·91	...	0·72	...	0·45	...	3·42	...	2·97	...	2·83	...

The diseased tissues contained larger amounts of the total ash, silica, potash and nitrogen while their lime contents were significantly lower than those of the healthy ones. The higher ash-content is to a large extent accounted for by increased uptake of silica. Chemical and histochemical examinations of the healthy-looking leaves of the partially spiked trees showed that the former were identical with those from the healthy trees.

TABLE VIII.

Sugars in Healthy and Diseased Leaf-tissues.

Percentage on dry weight.

Date	Reducing sugars other than maltose		Cane sugar		Maltose	
	H	D	H	D	H	D
April 10 ...	0·057	0·083	0·239	0·577	0·506	0·020
20 ...	0·197	0·022	0·352	0·277	0·091	0·118
30 ..	Nil	Nil	0·277	0·461	0·366	0·248
May 8 ...	,,	,,	0·062	0·120	0·284	0·086
22 ...	0·013	,,	0·052	0·061	0·078	0·068
June 8 ...	0·076	,,	0·284	0·223	0·170	0 263
22 ...	Nil	0·066	0·136	0·203	0·301	0·055
July 9 ...	,,	..	0·252	...	0·260	...
23 ...	,,	0·149	0·141	0·158	0·266	0·012
Aug. 8 ...	,,	...	0·177	...	0·342	...
22 ...	,,	Nil	0·234	0·390	0·120	0·096
Sept. 7 ...	,,	...	0·054	...	0·080	...
21 ...	,,	...	0·055	...	0·224	...

The starch and the hemicellulose contents of the affected leaves were invariably higher than those of the healthy ones. They also appeared to increase with time.

The sap extraction figures shown in Table II do not exhibit any significant difference between the healthy and diseased specimens. The total dissolved matter, either from refractometer readings or by evaporation method (Tables III and IIIa) was less in the affected

TABLE IX.

Dextrins, Starch and Hemicellulose in Healthy and Diseased Leaf-tissues.

Percentage on dry weight.

Date.	Dextrins as Dextrose		Starch		Hemicellulose as Dextrose	
	H	D	H	D	H	D
April 10 ...	1·01	2 11	0·41	0·64	5·53	6·58
,, 20 ...	...	...	0 35	0·60	4 53	6·26
,, 30 ..	1·82	2·19	0·57	0·87	5·13	6·68
May 8 ...	0·95	1·03	0.68	1·04	6·47	7 78
,, 22 ...	1·60	1·70	0·58	0·87	6·45	7·39
June 8 ...	0·33	0·86	0·14	0·69	5·52	8·08
,, 22 ..	0·15	0·22	0·25	1·60	7·98	9·49
July 9 ...	0·36	...	0·24	...	6·52	..
,, 23 ...	0·83	0·78	0·13	1·35	8·35	9·12
Aug. 8 ...	0·69	...	0·09	...	6·62	...
,, 22 ...	0·60	0·40	0·09	3·34	5·04	9·80
Sept 7 ...	0·71	...	0·05	..	5 82	...
,, 21 ...	0·68	...	0·08	..	6·25	...

specimens, indicating a diluted condition of the sap, and this is confirmed by the freezing-point determinations.

The specimens having been taken from branches at similar heights, the osmotic concentrations measured on the same dates were comparable with each other, and showed (Table IV) that the concentrations were invariably lower in the spiked than in the healthy specimens. In contrast to this, the electrical conductivity figures for the diseased specimens were higher than those for the healthy ones, pointing to a higher content chiefly of dissociated ions (electrolytes) in the former. It is possible that the higher value of the ratio K/Δ in the affected specimens indicates a greater power of absorption and retention mainly of minerals, than in the healthy ones, as also suggested by the heavier ash from the spiked leaves (Table VII).

The tissue-fluid reactions of healthy and diseased leaves (Table V) varied only slightly from each other. The titration values either to litmus or phenolphthalein were less for the diseased tissue-fluid, indicating a lower buffering capacity than that of the healthy leaves.

The dry matter of the spiked leaf tissue was less than that of the healthy one (Table VI). The extent to which photosynthetic or respiratory activities are responsible for the diminution in dry weight in both the tissue and tissue-fluids of the spiked leaf of the partially diseased tree is a problem for further investigation. The diseased material gave also a greater ash (Table VII) which was in some samples over twice that for the healthy sample. The various ash constituents were higher in the diseased leaf with the exception of calcium which was much lower than in the healthy ones. The greater ash was largely accounted for by the increase in silica.

In the healthy leaf there was generally more maltose and less cane sugar than in the diseased (Table VIII), which contained more dextrins, starch and hemicellulose than the healthy tissues (Table IX).

There is some apparent contradiction between the present set of data and an earlier one (*J. Indian Inst. Sci.*, 1928, **11A**, 93) for the spiked specimens. In the present investigation these were drawn from partially spiked trees, and in the previous one from completely diseased saplings. In both cases the diseased samples were compared with healthy ones. The chief differences between the data of the two sets of spiked specimens were apparent in dry weight, sugars, ash and some of the ash constituents. In the case of the tissue fluids the order of the results was reversed. The only points of similarity between partially and fully spiked trees were the increase in nitrogen and starch values and diminution in calcium contents of the diseased tissues compared with those for the healthy ones.

It is interesting to note that in both cases the ratio Ca/N was much lower for the diseased leaves than for the healthy ones. Since calcium is necessary for normal leaf development, and nitrogen in large quantities induces vegetative growth, the ratio Ca/N, as observed already, should serve as an index for the diseased condition of the plant. Diminished calcium is associated with reduction in the size of the leaf and increased nitrogen with vegetative activity: deficiency in calcium and increase in nitrogen lead to a restricted root development. All these have been observed in the case of spiked sandal. According to Loew (*U. S. Dept. Agr. Bur. Plant Ind.* 1903, *Bull.*, 45) one of the first disturbances when there is deficiency of lime in a plant, is cessation of transportation of starch. Starch tends to accumulate in the lower parts of the stem so that even its transportation from the storage receptacle to the axial parts may stop ultimately. A histochemical examination of the diseased twigs of a partially spiked tree showed a large accumulation of starch in the twigs and a small amount in the diseased leaves. In the healthy leaves and twigs collected on the same days, no such accumulation was noticed. In the healthy branches of the partially spiked tree, there was no noticed abnormality with regard to starch, nitrogen and calcium.

Except for the higher moisture-content in the samples examined during August, a month that was wet and humid with a minimum of bright sunshine, the effect of season on both healthy and diseased leaf tissues and saps seems to have been almost the same. On the whole, the chemical composition and physico-chemical properties of the sap from the healthy leaf tended to vary within a limited range from a mean value, while the corresponding data for the diseased one changed steadily with the advance of the disease. It may therefore be inferred that the metabolic changes characteristic of the different stages of the disease are determined largely by the progress of time from the onset of the disease and are not much affected by seasonal variations.

SUMMARY.

1. Tissue-fluid from the diseased leaves of a partially spiked tree contained less dissolved matter than that from a healthy one. The reaction of the former tended to become more acid, while that of the latter remained about the same throughout the period of the investigation. The osmotic concentration of the sap from the diseased specimen was less than that from the healthy ones. The electrical conductivity of the sap from the diseased leaf was generally greater than that from the healthy one and tended to increase with time.

2. In the diseased tissues greater moisture, ash and nitrogen, with less calcium than in the healthy ones, were noticed. The ratio Ca/N was markedly lower for affected than for healthy tissue.

3. Season appeared to have had a similar effect on the chemical composition of leaf tissue and on the chemical composition and physico-chemical properties of leaf-sap in health or disease.

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