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

PART XIX. PHYSIOLOGICAL AND PHYSICAL METHODS OF CHARACTERISING THE DISEASE.

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Spike disease in sandal is characterised morphologically by a diminution in the size of leaves and by a shortening of the internodes of the twigs bearing such leaves. A second distinguishing feature of this disease lies in the inability of the affected branches or trees to produce flowers and fruits, while even healthy portions of infected plants might bear flowers as well as fruits. A chemical examination of representative specimens collected from different localities revealed a striking deficiency in lime in the diseased leaves and twigs combined with an abnormal accumulation of starch and a significant increase in nitrogen content of such specimens (*This J.*, 1928, **11A**, 93; 1929, **12A**, 295). These observations were found to be unaffected by season, soil and such other factors which generally influence the composition of healthy plants. In view of the fact that sandal is found to grow under diverse conditions of soil, host associations, etc., considerable difficulty has been experienced by investigators when sometimes the shape and size of leaves of sandal resemble those of spiked ones. A typical instance of such a plant was first noticed in Noganoor, and is dealt with later. In the course of this investigation, several enquiries relating to such doubtful cases have been referred to the author for detailed examination and report. It was therefore necessary to work out a simple method for identifying the disease correctly.

PHYSIOLOGICAL INDEX.

For quite a long time, such suspected specimens were microscopically examined for the excessive presence of starch, as compared with normal ones collected from the same locality. It may at once be remarked that this procedure is quite misleading and therefore useless, in so far as the starch content of sandal leaves has been found to be influenced by season, associated host plants, etc., and also by the physiological state of the plant, such as flowering, resting, or vegetative phases. Other diagnostic features such as the

ability to transmit infection by grafting, etc., or the presence of X-bodies in spiked leaves as shown by Narasimhan (*Phytopath.*, 1928, 18, 815) are extremely useful for demonstrative purposes, but these will not serve for immediate identification. Further, they are also time consuming, besides being conditioned by many factors. Hence an attempt was made to secure more ready and easy methods towards this end. Since the diseased leaves and twigs were found to differ in their ash, nitrogen and calcium contents from typically healthy ones, it was discovered that the ratio Ca/N would serve as a more useful index for characterising the infection. A comparative idea of this ratio for specimens derived from different localities is presented in Table I, for early and advanced stages of spike.

TABLE I.

Ratio Ca/N for healthy and spiked leaves.

Locality	Partially spiked condition		Advanced stage of spike	
	Healthy	Spiked	Healthy	Spiked
Uttarahalli ..	0·84	0·23	1·32	0·29
Ragihalli ..	..	..	1·01	0·35
Noganoor ..	0·72	0·19	0·96	0·31
Javalagiri ..	0·78	0·21	0·94	0·28
Aiyur ..	0·80	0·22	1·28	0·27

The significance of the ratio Ca/N as an indicator for the presence of infection is established beyond doubt. In each case, a dozen trees were examined in addition to routine analytical estimations made in the course of this investigation.

With a view to rendering this observation more useful, the ratio N/Ca was estimated of specimens collected over long intervals. These values are strikingly brought out in Fig. 1.

It is well known that sandal derives its mineral supply including nitrogen through its roots. Since the intake of calcium and nitrogen is seriously disturbed, it was of interest to find whether the entire ash content of the spiked specimens bore similar relation with reference to nitrogen. The ratio N/Ash was calculated for leaves from different

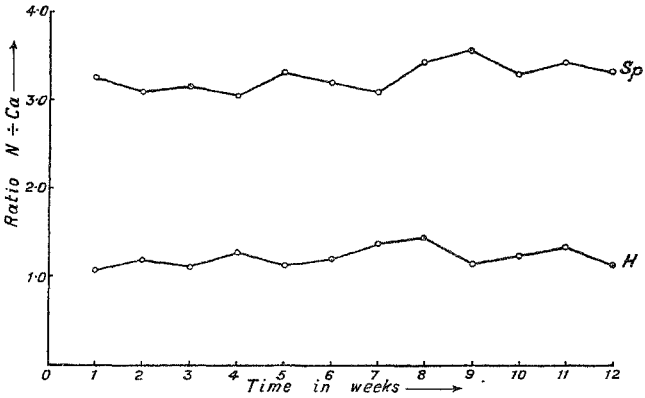

Fig. 1.

N/Ca in healthy and diseased sandal leaves.

areas and at different stages of spike, with a view to finding any apparent differential absorption in the assimilation of these two groups, as a characteristic of disease. The results are presented in Table II.

TABLE II.

Ratio N/Ash in healthy and diseased sandal leaves.

Condition of the plant	Uttarahalli		Ragihalli		Noganoor	
	I	II	I	II	I	II
Healthy ..	0·24	0·23	0·22	0·26	0·22	0·26
Partially Spiked ..	0·19	0·16	..	..	0·13	0·15
Healthy ..	0·20	0·25	0·25	0·19	0·26	0·21
Fully Spiked ..	0·36	0·32	0·40	0·46	0·51	0·48

In the advanced stages of the disease, the ratio N/Ash is nearly 1½ times that for the controls. In the incipient condition of spike, however, this feature is reversed. It is doubtful therefore whether

it can serve as a useful index for the purpose of diagnosis. It is possible that the same is considerably modified by the ash content, which in turn is a function of the stage of infection (*This J.*, 1929, **12A**, 295).

Although no physiological significance can be attached to the ratio Ca/N, it has nevertheless helped to distinguish healthy plants

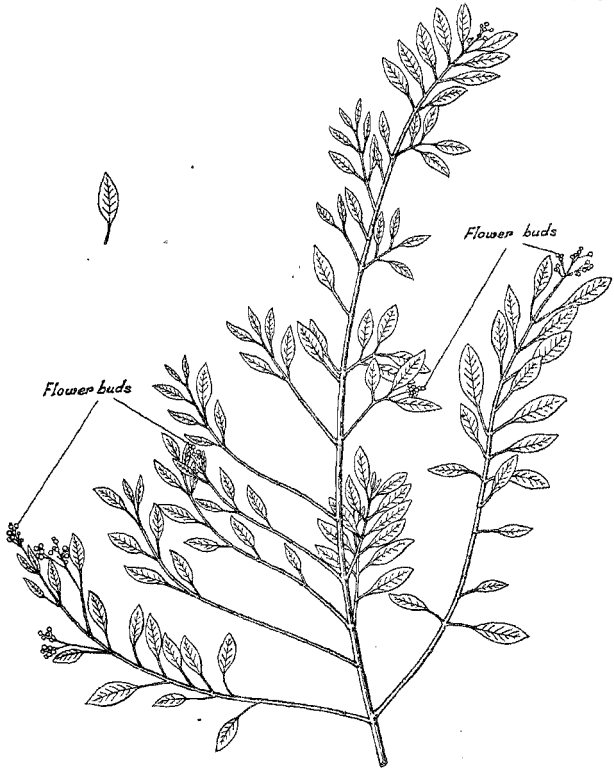

Fig. 2
Abnormal sandal plants apparently resembling spike
Coorg specimen.

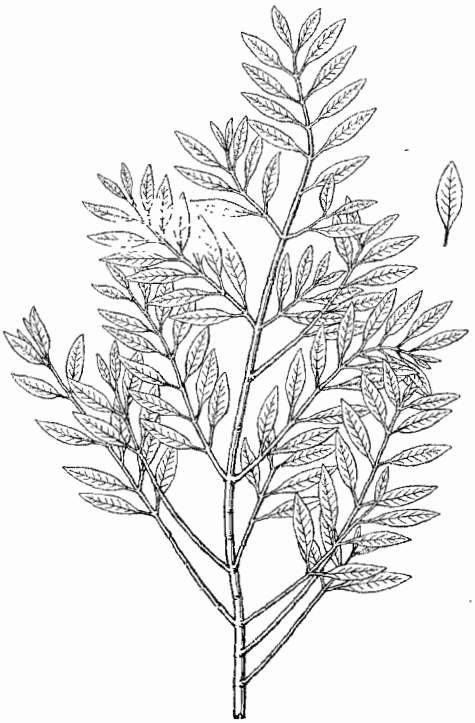

Fig. 2.

Abnormal sandal plants apparently resembling spike. Noganoor specimen.

from suspected cases of spike, as will be indicated later on. Some of these cases have been collected in areas which lie 50–100 miles away from the nearest spiked locality. But all of these were characterised by short leaves, and were examined for their mineral composition. Two typical instances which were found in heavily infected localities will be specially considered here. One of these was in Noganoor situated only a few furlongs from the nearest spiked area. No one

could fail to declare it diseased at first sight. Specimens from this plant are reproduced in Fig. 2. The leaves were extremely small but not characteristically narrow as in the case of spike. Again, the plant had only a few flowers, and this also is not typical of disease. A second instance was from Coorg, where it was designated as 'flowering' type of spike, because the branches had plenty of flower buds, etc. Here too, no other abnormality was noticed except the strikingly small size of the leaves. Specimens from this plant also are reproduced in Fig. 2. Leaf and other specimens from both of these plants were examined chemically and physically, besides testing them for their starch content. Further, grafting experiments were carried out with a view to testing their possible infective power. It may be remarked here that the grafting tests were negative, not a single case out of the ten tried having shown positive transmission. With regard to calcium and nitrogen contents, there was no abnormality in either of these constituents, as will be seen later (Table V). All these help to a correct diagnosis of the disease in control operations, so as to eliminate healthy trees.

PHYSICAL INDEX.

It is evident that the above technique is reliable being dependent on the analytical estimations made in the laboratory. But this becomes tedious for the investigation of a large number of samples as a routine. A more simple but fairly reliable method—a rough and ready one—was necessary to enable the field worker to determine the healthiness or otherwise of a given tree, reserving of course the chemical process in cases of grave doubt, and controversy. It was considered possible to define the well-established symptoms of spike in a more precise and mathematical way. To this end, biometric measurements were tried early by the author ("Reports on Spike Disease Investigation," Parts I–VII, *Proc. Indian Sci. Congr.*, 1931, 18, 284) and were further developed to cover a large field. By this means, it was intended to convey a fairly accurate and quantitative idea of the abnormalities referred to previously. The results are summarised in Table III.

In spite of the wide variations in the individual values for the healthy specimens, the ratios were found to be fairly close and agreeing. The most striking feature of spike is visible in the reduction of both the width of the lamina and also of the petiole length The same is significantly brought out in Fig. 3. The ratios are considerably higher in the diseased specimens with little overlapping.

TABLE III.

Biometric measurements on healthy and diseased sandal leaves.

Condition of the plant	Linear measurements in mm.			Ratios	
	L	B	P	L/B ± S.D.	$\frac{L-P}{B}$ ± S.D.
Healthy (Bangalore) ..	63·2*	23·1	9·4	2·84 ± 0·3	2·4 ± 0·2
„ (Uttarahalli) ..	94·4	38·8	13·2	2·50 ± 0·3	2·4 ± 0·2
„ (Aiyur) ..	91·7	36·9	12·9	2·30 ± 0·3	2·0 ± 0·1
Spiked (Early stage) ..	36·3	7·6	2·8	4·90 ± 0·6	4·5 ± 0·6
„ (Advanced) ..	23·5	6·2	2·3	4·62 ± 0·5	3·9 ± 0·5

* Each is an average of the values secured on over 2,500 leaves collected at different periods.

In the above table, L is the entire length of the leaf from petiole to the tip of the leaf blade,
B is the maximum breadth of the leaf blade perpendicular to the length,
P is the length of the leaf stalk,
and S.D. is the standard deviation by Fischer's method.

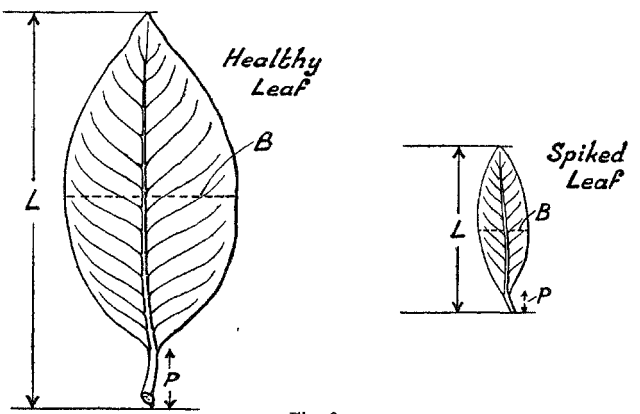

Fig. 3.
Reduction in leaf size following infection.
L = Entire length of leaf blade.
P = Petiole length.
B = Maximum breadth of blade.

Internodal distance in relation to health and disease: That spike disease is characterised by a shortening of the internodes does not signify much except in a qualitative manner. In healthy twigs, the internodes are situated at irregular intervals, varying normally between 5–35 mm. while in the diseased condition, this distance is considerably diminished, besides being set at quite regular intervals, beginning from the shoot. These values are reproduced in Table IV.

TABLE IV.

Internodal distances in healthy and diseased twigs.

Condition of specimen	Average value for internodal distances in mm.*				
	I	II	III	IV	V
Healthy (Bangalore)	22·8	23·9	16·5	28·2	21·7
„ (Uttarahalli)	26·9	24·4	26·5	16·4	29·8
Spike (Incipient stage, Uttarahalli)	5·8	5·6	6·8	7·2	5·2
„ (Advanced, Ragihalli) ..	3·9	4·3	3·5	3·1	4·1

* Each figure represents the average of 500 twigs individually examined.

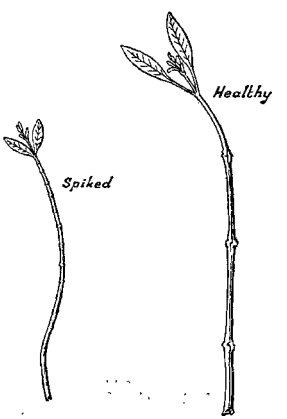

Fig. 4.
Internodal distances in healthy and spiked twigs.

The striking reduction in the setting of internodes as a result of spike infection is worth noting. But the disposition at regular intervals is a feature exclusively selective of spike, and this is significantly brought out in Fig 4. It will be clear from this that in place of a few internodes in healthy specimens, in consequence of infection the number of dormant buds increase giving rise to vegetative development and the characteristic branching. In the controls such regularity with regard to internodes has not so far been recorded, even under adverse conditions of drought or poor host associations. The difference between healthy and spiked plants with the respective leaves is significantly brought out in Fig. 5. The execessive branching in the latter case may be noted.

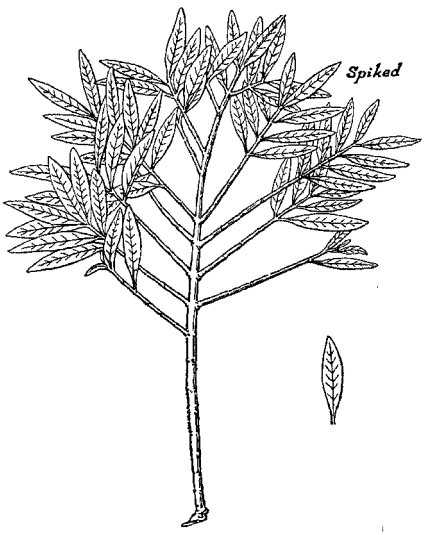

Fig. 5.

Excessive branching indicative of increased vegetative activity.

In view of the fact that the biochemical index as represented by the Ca/N ratio, and the physical index as indicated above are clear cut in healthy and diseased plants without any overlapping, and thus serve

for diagnostic purposes, it became of interest to find whether any correlation could be traced between the two indices to a better advantage. A resume of the two sets of indices for sandal in health and disease from different localities is presented in Table V.

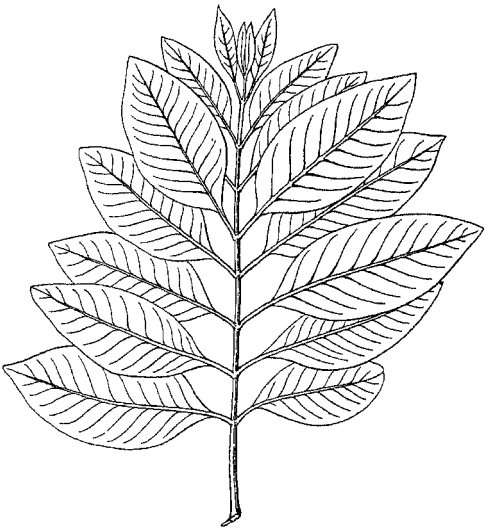

Fig. 5.
A corresponding healthy specimen.

The increase in the ratio of L/B as a result of infection may be roughly deemed to correspond qualitatively with the increase in N/Ca ratio. Apart from this apparent relation, there does not appear to be any other correlation between the physical and physiological indices.

TABLE V.

Comparative values of biometric data with the corresponding biochemical index.

Source of Material	LEAVES			TWIGS	
	Ratio L/B	Petiole length in mm.	Ca/N	Internodal distance in mm.	Ca/N
Bangalore (Healthy)	2·2	11·3	0·95	16·5—29·0	0·94
(Spiked)	4·1	2·5	0·28	2·1— 5·0	0·31
(Partial Spike) ..	4·4	5·1	0·23	9·0—10·0	0·32
Noganoor (Healthy)	2·2	8·3	1·08	11·0—23·0	0·89
(Spiked)	3·6	3·0	0·31	4·0— 6·0	0·29
Denkanikota (Healthy)	2·40	10·0	1·25	4·0—12·0	0·85
(Spiked)	3·95	4·6	0·27	2·0— 5·0	0·24
Fraserpet (Healthy)	2·43	10·0	0·82	10·0—15·0	0·98
(Spiked)	4·1	2·6	0·29	2·0— 4·0	0·25
Javalagiri (Healthy)	2·1	12·2	0·99	9·0—16·0	0·94
(Spiked)	4·0	2·4	0·24	4·0— 6·0	0·30
Ragihalli (Healthy)	2·3	15·2	1·02	9·0—15·0	0·97
(Spiked)	3·7	3·5	0·34	4·0— 5·0	0·29
Uttarahalli (Healthy)	2·05	13·3	1·04	7·0—14·0	0·22
(Spiked)	3·94	3·1	0·24	2·0— 5·0	0·40
*Noganoor (Suspected plant) ..	2·75	5·0	0·79	1·0—30·0	0·81
*Fraserpet (Suspected plant) ..	2·50	8·0	0·84	1·0—25·0	0·87

* Both were found to produce flowers and hence were referred as *flowering types*.

DISCUSSION.

The early observations on the chemical composition of sandal in health and disease (*This J.*, 1928, **11A**, 93) indicated that calcium nitrogen and ash contents were primarily affected by the disease. Since the total mineral content was found to be influenced by the stage of disease, it cannot adequately serve for diagnostic purposes. Although nitrogen relation of sandal is not very abnormal as is the case with lime, the increase in this constituent in spiked specimens is quite significant. It thus became possible to combine these two and to derive the ratio Ca/N to identify the presence of spike. It will be clear from Tables I and V that this ratio is nearer to unity in healthy ones and nearly a third of it in affected cases. Even though nitrogen and ash contents are absorbed through roots, the rate of intake of the

former is different from that of the latter (Table II). The ratio N/Ash is not really significant for reasons indicated before.

The biometric measurements are fairly accurate and serve as reliable guide for the fieldman in the selection of trees for treatment in disease control. This test is quite simple and easily carried out. Healthy plants under certain adverse conditions produce narrower leaves, but these also have shown a ratio closely agreeing with that for controls. As typical instances, two prominent cases were examined and reported (Table V). Reference to Fig. 2 will explain the apparent differences. While these data are useful in diagnosing doubtful cases of spike, it must be pointed out that none of the three individually will serve to distinguish healthy trees from spiked ones. Each one may not be specific, but when combined, all these, *viz.*, (*a*) the ratio of length to bredth of the leaf blade, (*b*) the length of the petiole and (*c*) the regularity in the disposition of the internodes with their respective distances in the twigs, have so far not failed in application. A successful case was the claim made by Chatterjee of transmission of disease through infection with the insect, *Petalocephala uniformis*, subsequent to their feeding on spiked plants ("Reports on Spike Disease Investigation," 1932, Pt. III). The relevant data are reproduced below. The biometric measurements as well as the Ca/N ratio tallied with those from healthy samples. The suspected plants had the figures for L/B 2.80, for P 6.0 mm., and for the internodal distance 11 mm., while the corresponding data for controls collected from other pots were 2.2, 10.0 and 6.12 respectively. In the same way, the ratio Ca/N was 0.64 as compared with the value 0.85 for the healthy and 0.26 for diseased samples. Thus, the claims of this beetle as a vector of spike were tested, with negative results.

A criticism of the above technique was made by Dover (*Indian Forest Records*, 1933, 20, Pt. I) when he claimed positive transmission with *Moonia variabilis*, as vector of spike. He relied for his support on the nitrogen content and on the presence of X-bodies in the leaves. It will be clear from this contribution that nitrogen values are not reliable as a diagnostic index, as it is considerably influenced by the host plant. Dover did not, however, carry out an examination of the calcium content, which alone would have provided him with the necessary clue. At a later date, the suspected plant developed healthy shoots. In his opinion, the ratio L/B was useful in typical healthy and spiked plants. He cited instances of a few healthy specimens where the ratio was as high as 5 and of some spiked ones in which the ratio was less than 3. He however argued that the insect fed plants evinced a ratio of over 4 which led him to conclude that they were really spiked! In fact, the author's observations on

these, tallied with those of the healthy ones. Moreover, he had not presented data relating to petiole length and internodal distance in the twig. These three in combined form would have yielded sufficient clue to the claims. Thus, where the physical index was not definite in its conclusions, the physiological or biochemical index was the surest indication.

SUMMARY AND CONCLUSION.

1. A simple method was evolved for the identification of spike disease in sandal based on the uniformly low values for the Ca/N ratio for the affected specimens.

2. The values for the ratio N/Ash was found to be influenced by the stage of disease. It cannot therefore be employed successfully for diagnostic purposes.

3. A simpler and more useful technique, though somewhat less accurate, was devised for field purposes, involving biometric measurements on leaves and twigs.

4. The ratio L/B is significantly higher in the diseased leaves.

5. The leaf stalk is characteristically short in length, adding to the stiffness of spiked leaves.

6. In the diseased twigs, the internodes are set at regular but short intervals, unlike in the controls.

7. Even though each of the above three physical tests may be occasionally encountered in sandal plants growing under peculiar conditions, the above technique has never failed to identify the disease when combined together.

8. Two interesting cases naturally occurring in the field have been examined in detail and proved to be healthy by these tests.

9. In two other instances of reported transmission of spike by artificial means, these tests have again proved invaluable.

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