STUDIES ON SANDAL SPIKE

Part II.* Physiological Significance of the Disturbed Iron Balance in the Spike Disease of Sandal (*Santalum album* Linn.)

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IT has recently been shown from this Laboratory that there is a distinct difference in the phenol content of the healthy and spiked sandal leaves.[1] In continuation of our studies on the sandal spike problem, the distribution of iron in the healthy and diseased sandal trees has been investigated.

Iron plays an important role in plant metabolism. In the leaves it exists in two forms: the mobile or active and the stationary.[2] The latter constitutes the major part and is found in chloroplasts, firmly bound to phosphoproteins[3] and is trivalent.[4, 5] According to Noack and Liebich[5] this stationary iron is possibly of importance in photosynthesis. Recently Boichenko and Zakharova, while studying the role of iron in photosynthesis, have found evidence to show that an iron-containing polyhydroxy acid is a primary product of carbon dioxide assimilation,[6] and in this acid too iron is in trivalent form and appears to correspond to the stationary phase. Possibly on account of this stationary iron, which is not so much affected as the mobile or active iron in the spike-affected sandal trees and which increases in some cases where abnormal accumulation of iron takes place, the photosynthetic efficiency of spiked leaves is on the increase and this may be the reason for the increased carbohydrate content noticed in spiked leaves.[7, 8] On the other hand, the mobile form, which is very active physiologically, contains divalent iron.[4, 5] This mobile iron is supposed to take part in the production of chlorophyll by bringing about the formation of an intermediate compound of the type of the magnesium salt of α-pyrrol carboxylic acid[9] (*cf.* Chernavina *et al.*[10] who are of the opinion that the biosynthesis of chlorophyll may be connected with the activity of the iron-protein group of enzymes) and in support of this a good proportionality between the iron content and chlorophyll formation has been noticed.[11, 12]

* The article entitled "The Role of Phenolic Bodies in the Metabolism of Sandal (*Santalum album* Linn.) in Health and Disease" (*J. Sci. industr. Res.*, 1961, **20 C**, 273–75) is Part I of this series.

Iron-chlorosis in normal plants is a deficiency disease caused by non-availability of appropriate amounts of iron in the soil and although the deficiency of iron in the soil is made up at a later stage, the disease, if in an advanced stage, cannot be remedied.[12, 13] It means that much of the iron, entering the leaf after a preliminary period of chlorosis, is present in bound-form and not in the mobile form which alone takes part in chlorophyll formation. In spike disease the sandal leaves also suffer from chlorosis, and it would be of importance to investigate whether the cause of this chlorosis is the same as that in other normal plants, because it may throw some light on the factors causing or helping spike disease.

For the present experiments the leaves were collected from trees of nearly the same girth and height (age: 10–15 years) from different areas as was done in the earlier investigations: from the nursery of this Laboratory and Lal Bagh, Bangalore (non-spiked areas) and Kenchanahalli, Dobbspet and Narasimha Devara Betta (spiked areas). Kenchanahalli is 8 miles south-west, Dobbspet 37 miles north-west and N.D. Betta 43 miles north, from Bangalore. The leaf material was collected between 9 A.M. and 10 A.M. The leaves were dried in a ventilated oven at 85–90° C. and were powdered using a porcelain mortar and pestle. This powder was used for analysis. To follow the trend of accumulation of iron in different parts of the plant, the stems and roots were also collected in some cases for analysis. They were first cut into bits of convenient size and were washed first with dilute hydrochloric acid (0·5 N) and then in running water. They were then dried, chipped and coarsely powdered using a porcelain mortar and pestle. Representative samples (10–15 gm.) were taken, ashed and extracted with hydrochloric acid according to the A.O.A.C. procedure.[14] After making up the solution to 250 ml., the iron content was estimated using *ortho*-phenanthroline reagent.[14] The results are represented in Tables I and II and the Histogram I. Percentages of iron were expressed on dry weight basis which was found to be most suitable as a basic reference factor for iron in plants.[15] Since the iron content of the soil and its pH naturally affect the iron content in the plant, these factors were also studied with the soil in each locality and the results are given in Table III.

It is clear from the results in Table I that the iron content of spiked sandal leaves is lower than that of healthy leaves, the figures for the partially affected plant samples also showing the same trend; the actual content naturally varies with the locality and the season. These observations are at variance with the results already reported by Coleman[7] and Iyengar[8] who did not find any significant difference in the distribution of this element in

TABLE I

Iron content of healthy, spiked and partially spiked sandal (Santalum album *Linn.*) *leaves*

Locality	Healthy			Spiked		
	Date	Plant No.	Iron %*	Date	Plant No.	Iron %*
Kenchanahalli ..	23– 8–1960	8	0·01551	23– 8–1960	10	0·01055
Do. ..	,,	9	0·01536	,,	11	0·01067
				,,	12	0·00909
Do. ..	15–11–1960	28	0·01200	15–11–1960	29	0·00951
					30	0·00821
Do. ..	9– 8–1960	15†	0·01327	..	..	0·01014
Do. ..	15–11 1960	13†	0·01000	..	..	0·00607
Lal Bagh ..	16– 8–1960	5	0·02595	Non-spiked area		
..	,,	6	0·02029			
Dobbspet ..	31– 8–1960	20	0·01468	31– 8–1960	17	0·00925
				,,	18	0·00993
				,,	19	0·01071
F. R. Laboratory Nursery	3–10–1960	1	0·02404	Non-spiked area		
	,,	2	0·01563			
	,,	3	0·01637			
	3– 2–1961	31	0 02502			
N. D. Betta ..	6–10–1960	24	0·01460	6–10–1960	21	0·00821
..	,,	25	0·01460	,,	22	0·00800
..				,,	23	0·00924
..	6– 2–1961	32	0·01614	6– 2–1961	22	0·03656

* All percentages on zero-moistuie basis.

† Partially spiked.

TABLE II

Iron content of the leaf, stem and root of healthy and spiked sandal and Ca/Fe ratio in the roots

Locality	Date	Plant No.	Condition of plant	Iron content %*			Calcium content %* in the root	Ca/Fe in the root
				Leaf	Stem	Root		
Kenchanahalli ..	15–11–1960	28	Spiked	0·00951	0·00463	0·01703	0·569	33·4
,, ..	,,	29	,,	0·00821	0·00442	0·01328	0·723	54·4
,, ..	,,	30	Healthy	0·01200	0·00368	0·00710	0·780	109·8
F. R. Laboratory Nursery	3– 2–1961	31	,,	0·02502	0·00198	0·00533	0·521	97·7
N. D. Betta ..	6– 2–1961	22	Spiked	0·03656	0·00190	0·00406	0·315	77·6
,, ..	,,	32	Healthy	0·01614	0·00183	0·00242	0·256	105·8

* All percentages on zero-moisture basis.

TABLE III

Iron content and pH of the concerned soils

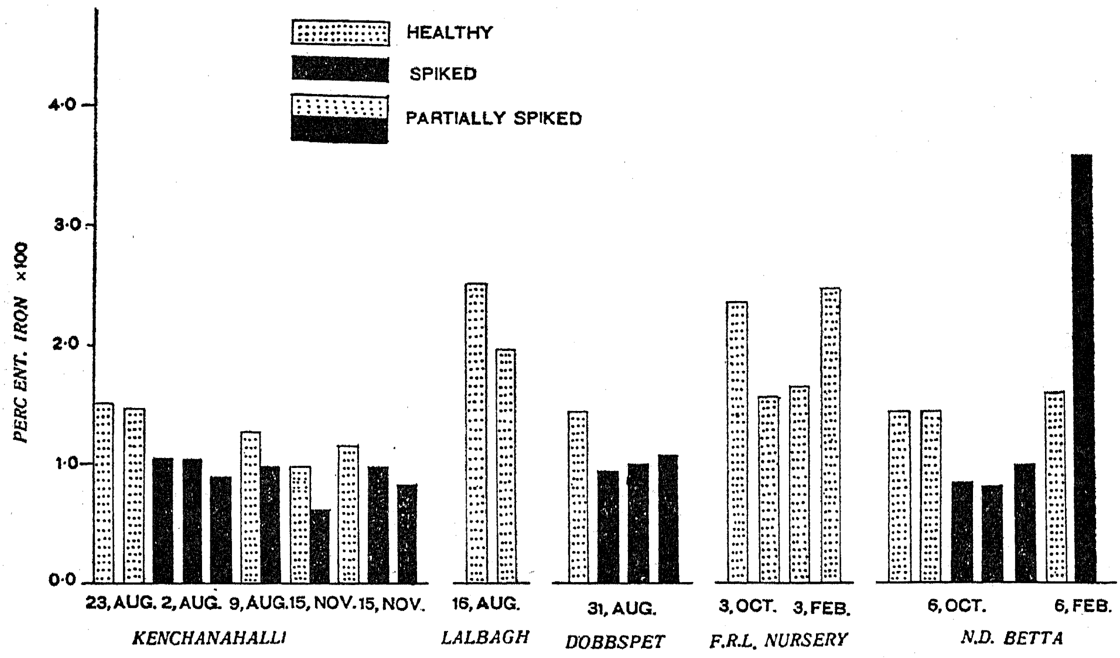
Locality	Iron content %*		pH	
	Healthy	Spiked	Healthy	Spiked
Kenchanahalli ..	2·190	3·225	6·0	6·0
Lal Bagh ..	3·268	..	6·3	..
Dobbspet ..	2·911†	2·911†	6·2†	6·2†
F. R. Laboratory Nursery	3·627	..	6·0	..
N. D. Betta ..	1·806	2·528	6·0	6·1

* Percentages on zero-moisture basis.

† Healthy and spiked plants were collected from the same area.

healthy and diseased leaves. Whether this is due to variations in the age of the plants used by Iyengar[8] for analysis or any other cause, is engaging our attention.

HISTOGRAM I CORRESPONDING TO TABLE I



HISTOGRAM I

The levels of iron in the leaves of healthy plants from non-spiked areas are comparable, provided the soil condition with regard to the iron content is more or less the same. The iron content of the sandal leaves, particularly the healthy, appear to be influenced by the soil iron content as can be seen from the values obtained for leaf samples from the Laboratory Nursery and Lal Bagh (*vide* Tables I and III).

At N. D. Betta the spiked plant showed abnormal accumulation of iron on February 6, 1961 as compared to the normal value on October 6, 1960. This shows that the spiked leaves which are chlorotic accumulate iron abnormally as they approach completion of development. This is in conformity with the observations of Jacobson and Oertli[12] on other chlorotic plants.

Steinberg *et al.*[16] found iron deficiency to lead to an increase in the nitrate level and a decrease in the protein content; this decrease in protein content with deficiency in iron was also noticed by other workers.[17, 18] In the iron-deficient spiked sandal leaves also, Murty and Sreenivasaya found decreased protein content, while, however, the nitrate content was either very low or altogether absent.[19] The characteristically low or nil amounts of nitrate in the spiked sandal leaves, in which total nitrogen was, however, found to increase as a result of the disease,[8] shows the existence of a serious disturbance in nitrate reductase activity in the spiked sandal leaves. This aspect of the study is already in progress in this Laboratory.

From the results in Table II it can be seen that the percentage of iron in the spiked plant roots is more than that in the healthy plant roots at any one time. The stem contains the minimum amount in both the healthy and the spiked plants, while the leaves in the case of the healthy plants and the roots in the case of the spiked plants contain the maximum.

According to Shestakov,[20] iron injures plant roots unless calcium is present in sufficient amounts to exert a protective action, while according to Shcherbakov[21] iron inhibits calcium absorption in the plant, thus bringing out the importance of the calcium/iron ratio in maintaining the normality and function of the plant roots. The percentage of calcium in the roots of the healthy and spiked sandal plants has, therefore, been determined and is given along with the iron percentage in Table II; the Ca/Fe ratios are also given in the table. It can be seen that while the Ca/Fe ratio is fairly high in healthy roots, it is very much lower in the diseased roots, thus showing the relative preponderance of iron over calcium in the latter. This observation is of importance in view of the injurious effect of the spike disease on the normal functions of sandal roots.[22]

CONCLUSION

Chlorosis in spiked sandal leaves, as in the chlorotic eaves of other plants, is caused by a deficiency of iron. However, the deficiency appears to be not on account of reduced availability of this element in the soil, but a direct effect of the spike disease, since the soil iron content is normal and the pH of the soil is on the acid side (Table III). Like other chlorotic leaves,[23, 27] the spiked sandal leaves show an increase of amino nitrogen,[19] oxalic and malic acids,[28] and a decrease in calcium content.[8, 29] Thus chlorosis in sandal leaves, caused by spike-induced iron deficiency, bears resemblance to the chlorosis in other leaves which is caused by the reduced iron availability in soils.

In spike-diseased plants there is a comparatively higher accumulation of iron in the roots than in the leaves, which suggests that probably the mechanism of translocation of iron in the plant is affected. The preponderance of iron over calcium in the spiked sandal roots, as evidenced by the much lower Ca/Fe ratio in them, seems to be responsible at least in part for the death of root ends and cessation of haustorial connections in the spiked sandal.

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