THE SPIKE-DISEASE OF *DODONAEA VISCOSA*.

By B. N. Sastri and N. Narayana.

Dodonæa viscosa (Kanarese—*Bandarike*) is a shrub occurring wild in the *maidan* forests of Mysore. It grows to a height of about 4 ft. and possesses stipulate, lanceolate leaves with entire margin. The plant is regarded as a febrifuge in Ayurvedic medicine, but has no economic importance otherwise. It has been known to occur as a host for sandal.

This plant suffers from a disease similar to the spike of sandal (Coleman, *Dept. of Agriculture in Mysore, Myc. Bull.*, 1917, 3, 19), prevalent in areas where sandal spike occurs. As in the case of sandal, the diseased leaves diminish in size and the internodes become shortened giving the whole plant a characteristic bushy appearance: flowering and reproduction cease and vegetative growth is accelerated.

In view of observations previously made on healthy and diseased tissues of sandal (*J. Indian Inst. Sci.*, 1928, **11A**, 23, 97 and 241), it was considered that a similar study of *Dodonæa viscosa* might throw light on the nature of sandal spike. The investigation was expected to be less complicated than in the case of sandal because the plant is not a parasite and being a shrub, presents no difficulties in the collection of samples for study.

EXPERIMENTAL.

Representative specimens of healthy and diseased plants were collected from different areas where sandal occurs, and were separated into leaves, stems and roots after thorough washing. The various tissues were dried quickly over calcium chloride in vacuum desiccators at about 0° to prevent changes due to enzyme action (Link, *J. Amer. Chem. Soc.*, 1925, **47**, 470). The dry tissues were powdered to pass a 40-mesh sieve and preserved in glass-stoppered bottles.

Methods of analysis.—Total nitrogen was determined by Gunning's modification of Kjeldahl's method to include the nitrogen of nitrates, proteins by Stutzer's copper hydroxide method and free and saline

ammonia by Graffe's method (*Z. Physiol. Chem.*, 1906, **48**, 300). For the last determination, 20 g. lots of the material were treated with 100 c.c. of ammonia-free water, 100 c.c. of 95 per cent. alcohol, and 25 c.c. of saturated sodium chloride solution. Saturated sodium carbonate solution (25 c.c.) was added to the mixture which was then distilled *in vacuo* at 37° for 4 hours, ammonia being determined in the usual way.

Nitrate was estimated by Scale's method (*J. Biol. Chem.*, 1916, **27**, 327). The material (40 g.) was extracted with 80 per cent. alcohol for 36 hours and the extract after removal of alcohol, clarified with lead acetate and made to a definite volume. The nitrate in aliquot parts was reduced by a zinc-copper couple and the ammonia estimated. The Griess-Ilosway method (qualitatively) was used for nitrites.

Distribution of non-protein nitrogen.—Preliminary experiment showed that the material contained large quantities of mucilaginous matter hindering filtration of the hot-water extracts; 60 per cent. alcohol was therefore used. Weighed quantities were extracted repeatedly with boiling alcohol and the combined extracts concentrated by distillation under reduced pressure at a temperature not exceeding 40°. The concentrate was taken up with water, filtered and freed from fat by extraction with ether; it was then treated with dialysed iron, centrifuged, and the clear extract made up to a known volume.

The extract contained polypeptides, amino-acids, amides, ammonia and other forms of non-protein nitrogen. A definite volume was hydrolysed for 12 hours with sufficient hydrochloric acid to bring the concentration to 20 per cent. The insoluble melanin was separated and its nitrogen estimated. The filtrate, after distilling the hydrochloric acid was diluted, and the distribution of nitrogen determined by Plimmer's modification of the Van Slyke method (*Biochem. J.*, 1925, **19**, 1004).

Carbohydrates.—Alcoholic extracts of the material were clarified with basic lead acetate and used for the estimation of sugars. Sucrose was determined after hydrolysis with 10 per cent. citric acid, reducing sugars by Bertrand's method and starch in the residue after alcoholic extraction by hydrolysis with taka-diastase according to Davis and Daish (*J. Agric. Sci.*, 1914, **6**, 152).

Ash constituents were estimated by the A.O.A.C. methods (1925). The results of analysis are shown in Tables I–VII, the specimens having been collected from the Laggare forest area on 14th August, 1928, unless otherwise stated.

TABLE I.

Percentage of Dry Weight

	Leaves		Stems		Roots	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Total nitrogen ..	1·86	1·86	0·38	0·51	0·37	0·17
Protein nitrogen ...	1·76	1·58	0·36	0·48	0·32	0·14
Non-protein nitrogen (by difference) ...	0·10	0·28	0·02	0·03	0·05	0·03
Ash ...	5·75	6·34	3·91	3·80	6·15	2·49

TABLE II.

Percentage of total Nitrogen

Form of Nitrogen	Leaves		Stems		Roots	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Nitrate ...	0·16	0·38	2·37	4·38	1·40	2·00
Free ammonia ..	0·40	0·45	1·29	1·58	0·50	2·64
Nitrite ...	Nil.	Traces	Nil.	Nil.	Nil.	Traces

TABLE III.

Distribution of Non-protein Nitrogen.

Percentage of total Non-protein Nitrogen

Form of Nitrogen	Leaves		Stems		Roots	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Melanin { Insoluble ...	10.02	11·12	8·14	11 57	9·37	14·45
Melanin { Soluble ...	5·56	4·25	4·07	4·63	4·48	1·53
Amide ...	15·50	20·54	17·66	19·50	13·75	15·16
Basic ...	25·41	19·33	24·53	19·17	15·00	14·85
Non-basic ...	47·50	47·59	44·75	43·87	55·90	55·45

TABLE IV.

Carbohydrates.

Percentage of Dry Weight

	Leaves		Stems		Roots	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Free reducing sugars as—						
Glucose ...	0·78	2·34	0·24	0·49	0·41	0·27
Sucrose ...	0·50	0·60	0·48	0·26	0·23	0·17
Starch ...	2·49	4·61	3·76	3·81	2·71	2·86

TABLE V.

Ash Constituents.

Percentage of Dry Weight

Date of collection	Locality	Tissue—condition	Ash	Total nitrogen	P_2O_5	Ca	K_2O
14—11—27	Laggare ...	Leaf, H	6·20	1·85	0·10	0·38	0·47
,,	,, ...	,, D.	9·65	1·76	0·15	0·15	0·55
15—4—28	Denkanikota ...	,, H.	4·96	1·95	0·11	0·77	0·45
,,	,, ...	,, D.	6·27	2·29	0·15	0·10	0·46
15—7—28	,, ...	,, H.	6·09	2·36	0·52	0·95	0·32
,,	,, ...	,, D.	6·43	1·91	0·53	0·77	0·48
23—8—28	Uttarahalli ...	,, H.	6·57	2·41	0·37	0·59	...
,,	,, ...	,, D.	5·17	1·82	0·39	0·56	...
14—9—28	Laggare ...	,, H.	5·75	1·86	0·52	0·56	0·14
,,	,, ...	,, D.	6·34	1·85	0·32	0·37	0·12
,,	,, ...	Stem H.	3·91	0·38	0·39	0·32	0·04
,,	,, ...	,, D.	3·80	0.51	0·29	0·27	0·03
,,	,, ...	Root H.	6·15	0·37	0·25	0·63	0·19
,,	,, ...	,, D.	2·49	0·17	0·24	0·36	0·15

H=healthy; D=diseased; .... not determined.

Discussion.—It may be seen from Tables I, II and III, that the different forms of non-protein nitrogen, such as nitrate, amide and ammonia are higher in the diseased tissues than in the healthy ones. Bourquet obtained similar results in his study of the virus diseases, the mosaic of tobacco, the curly-top of sugar beet and the leaf-roll of potato (*J. Amer. Chem. Soc.*, 1916, **38**, 2572). The present observations suggest that the spike of *Dodonæa viscosa* is allied to the above and also caused by a virus.

Table IV shows that, as in the case of sandal spike (*J. Ind. Inst. Sci.*, 1928, **11A**, 99) diseased leaves have higher sugar and starch contents than the healthy ones. Similar results were obtained in another series of experiments in which tissue-fluids were expressed from the leaves, as may be seen from Table VI. The diastatic activity of the juice was measured and found to be higher for the diseased plant as in the case of sandal.

TABLE VI.

Specimens collected from the Uttarahalli forest area on 23-8-1928.

Carbohydrate	Healthy	Diseased
Free reducing sugars in 100 cc. of fluid ...	301 mg.	341 mg.
Sucrose ,, ...	114 ,,	225 ,,
Diastatic activity mg. of maltose*	6·7 ,,	24·0 ,,
Starch in residue ...	6·1 per cent.	8·4 per cent.

* Vide *J. Ind. Inst. Sci.*, 1928, **11A**, 25.

The figures for nitrogen, phosphorus and potassium (Table V) do not appear to depend upon the condition of the plant. Those for calcium are, however, more significant, being invariably lower in the case of diseased tissues. It is well known that calcium assists the hydrolysis and translocation of starch and prevents the accumulation of organic acids such as oxalic, which would otherwise prove poisonous. The growing parts of plants require large quantities of calcium, and serious metabolic disturbances are caused by a shortage of the element. The high N/Ca ratio in the diseased tissues is indicative of pathological disturbances (Parker and Truog, *Soil Sci.*, 1920, **10**, 49).

To determine whether the differences in mineral composition observed in the cases of healthy and diseased tissues of sandal and *Dodonæa viscosa* are borne out in the case of *Zyzyphus œnoplia*, known also to suffer from a similar disease, specimens of the latter were analysed for their nitrogen, phosphorus and calcium contents, percentage on the weight of dry leaves being shown in Table VII.

TABLE VII.

Analysis of Leaves of Zyzyphus œnoplia.

Date	Locality	Condition of the leaf tissue	N	P_2O_5	Ca
5— 1—28	Aiyur	Healthy	2·47	1·53	0·89
,,	,,	Diseased	3·68	1·87	0·38
5— 3—28	Denkanikota	Healthy	3·20	1·07	1·15
,,	,,	Diseased	3·87	1·28	0·47

Calcium is again lower in the diseased tissues, and measurements of diastatic activity showed that this factor was higher than in the healthy leaf fluid.

SUMMARY.

The non-protein nitrogen constituents of the tissues of *Dodonæa viscosa* in healthy and diseased conditions were determined. The nitrate, nitrite, ammonia and amide contents of the diseased tissues were invariably higher than those of the corresponding healthy ones.

As in the case of sandal, sugars and starch tend to accumulate in the diseased tissues. The diastatic activity of the latter was also higher than that of the healthy tissues.

The diseased condition is further characterised by deficiency in calcium. This is true also of *Zyzyphus œnoplia* which suffers from a similar disease.

In conclusion, our thanks are due to Prof. Roland V. Norris, for his keen interest in the work and to Mr. M. Sreenivasaya for many helpful suggestions.

Department of Bio-chemistry,
Indian Institute of Science,
Bangalore.

[Accepted, 25-9-30.]