BIOCHEMISTRY OF THE SPIKE-DISEASE OF *Vinca rosea* LINN.

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Vinca rosea, otherwise known as peri-winkle, is a shrub belonging to the order *Apocyanaceæ* which occurs wild in private gardens, agricultural lands and even forests. Two varieties are generally found, one of which is coloured red in the stem and flowers, while the other is white. It grows normally to a height of about two feet, and possesses simple leaves with entire margin. No practical importance is attached to this species. It is not even known whether it can serve as a host to sandal.

It is of considerable practical significance, however, to find that this species is subject to a disease similar to the spike of sandal. Spiked vinca plants have been observed even in localities where there is no sandal or, at any rate, where there is no diseased sandal. The malady is characterised by a bushy appearance in which the leaves are diminished in size and the nodes are closer. In advanced stages, an excessive branching is noticed, so that a diseased plant appears quite stunted. An increased vegetative activity of the diseased plant is one of its significant features. In all stages of disease, flower production has been noticed though it is common that such flowers appear green, forming phyllody. Although it was considered for a long time that only the white variety is affected by the disease, recent observations have shown that even the red one suffers occasionally from it.

Narasimhan has recently drawn attention to the occurrence of X-bodies in the leaf tissues of diseased *Vinca rosea* (*Phytopath.,* 1928, **18**, 815). In view of this further evidence of the virus nature of the disease, it was considered desirable to determine whether the attendant physiological changes are similar to those observed in sandal spike. The results of such a study are reported in the present communication.

EXPERIMENTAL.

Representative specimens of healthy and diseased vinca plants were obtained locally as also from Denkanikota, Sanamavu, Noganoor and other forest areas. After washing to remove the adhering soil particles, the plants were dried within folds of blotting paper. These were then divided into roots, twigs and leaves. The samples were first dried over sulphuric acid at the laboratory temperature to minimise enzyme action (Link, *J. Amer. Chem. Soc.*, 1925, **4**, 470) and subsequently at 40°. The tissues were powdered to pass through the 40-mesh sieve and preserved in glass-stoppered bottles.

Analytical methods.—Moisture was determined by drying known quantities of material to constant weight at 105–107°. Total Nitrogen was estimated according to Kjeldahl-Gunning method to include nitrates and proteins by Stutzer's Copper Hydroxide method (*Methods of Analysis, A.O.A.C.*, 1930, pp. 21 and 279). Total ammonia in the tissues was determined in the water extract by distilling with recently ignited magnesia. Nitrate was estimated according to Scale's method after reducing it with Devarda's alloy (*J. Biol. Chem.*, 1916, 27, 327). Free amino-acid content was determined in the aqueous extract by the usual Van Slyke method. Carbohydrates were determined in the dry powder by extracting it with alcohol. After removal of alcohol under reduced pressure, the residue was taken up in hot water and clarified with basic lead acetate. After de-leading, the filtrate was used for sugar estimations according to the method described earlier by the author (*J. Indian Inst. Sci.*, 1928, **11A**, 103). The residue left after alcohol extraction was used for the estimation of starch by the Taka-diastase method. Ash and ash constituents were determined according to the A.O.A.C. methods (*loc. cit.*). The results have been presented in the following tables:—

TABLE I.

Nitrogen and ash constituents in the leaf tissues of Vinca rosea.

Locality	Percentages (on dry weight)											
	Total ash		Total N.		P_2O_5		CaO		K_2O		Fe_2O_3 and Al_2O_3	
	H	D	H	D	H	D	H	D	H	D	H	D
Bangalore ..	8·48	10·30	3·24	3·86	0·05	0·07	1·88	1·03	3·49	3·40	0·39	0·84
Denkanikota	8·98	10·68	3·64	4·31	0·07	0·09	1·54	0·98	4·21	4·06	0·42	0·86
Sanamavu .	9·65	9·93	3·65	3·97	0·06	0·07	1·62	1·14	4·41	4·02	0·47	0·72

While a slight increase is noticed in the total ash and nitrogen values of diseased specimens, the reduction in calcium is somewhat striking.

TABLE II.

Mineral analysis of stem and root of Vinca rosea.

Locality	Percentages (on dry weight)									
	Total ash		Total N.		P_2O_5		CaO		K_2O	
	H	D	H	D	H	D	H	D	H	D
Bangalore										
Stem ..	5·48	2·83	0·89	0·45	0·16	0·13	0·36	0·17	1·19	0·81
Root ..	4·81	2·48	1·12	0·12	0·15	0·18	0·38	0·60	0·58	0·82
Denkanikota										
Stem ..	5·79	2·70	1·04	0·74	0·17	0·11	0·42	0·21	1·14	0·68
Root ..	5·66	3·15	1·53	0·10	0·17	0·19	0·54	0·62	0·66	0·77

It will be seen that the ash content of the diseased plant is always low. Although the stem resembles the root in its composition in the diseased condition, such roots are characterised by significant reduction in nitrogen and increase in calcium and potassium contents.

TABLE III.

Distribution of Nitrogen in the different parts of Vinca rosea.

Form of nitrogen	Percentages (on dry weight)					
	Leaf		Stem		Roots	
	H	D	H	D	H	D
Total Nitrogen ..	3·24	3·86	1·04	0·74	1·53	0·10
Protein Nitrogen ..	3·02	2·94	0·88	0·50	1·26	0·03
Non-protein N. ..	0·218	0·92	0·16	0·25	0·26	0·07
Nitrate ..	0·010	0·002	0·008	0·003	0·004	0·003
Total ammonia .	0·008	0·013	0·003	0·008	..	..
Free amino N. ..	0·031	0·082	..	..	..	..
Ratio Ca/N	0·44	0·29	0·40	0·20	0·25	4·38

The protein content diminishes with the onset of disease. While the nitrate content is decreased in spiked tissues, ammonia and amino-acid values are increased. Again, the low Ca/N in diseased leaves is in striking contrast with the abnormally high value for the roots.

TABLE IV.

Carbohydrate distribution in healthy and diseased Vinca rosea.

Constituent	Percentages (on dry weight)					
	Leaf		Stem		Roots	
	H	D	H	D	H	D
Free reducing sugars ..	1·23	2·54	0·41	0·40	0·31	0·27
Sucrose	0·81	1·24	0·36	0·31	0·25	0·20
Total acid-hydrolysable *sugars*	0·69	0·89	0·49	0·62	..	..
Starch	0·42	2·14	1·32	2·79	2·30	2·91

A higher soluble sugar content is generally noticed in diseased leaves. Starch accumulation is seen in all parts of the plant. This is borne out by the observations on specimens derived from different localities.

TABLE V.

Diastatic activity of healthy and diseased Vinca *leaves.*

Locality	Sugar produced as mg. of maltose				Reaction of sap (pH)	
	For 1 c.c. of sap		For 1 g. of dry leaf powder			
	H	D	H	D	H	D
Bangalore	54·1	65·8	143	217	5·64	5·76
Denkanikota	16·3	41·8	64	183	..	..
Sanamavu	8·3	38·6	44	194	..	..

It may be noticed that the diseased material is characterised by increased diastatic activity.

TABLE VI.

Carbohydrate distribution in healthy and diseased leaves of Z. œnoplia.

Locality	Percentages (on dry weight)					
	Free reducing sugars		Sucrose		Starch	
	H	D	H	D	H	D
Aiyur	0·18	0·21	0·35	0·49	1·21	2·74
Noganoor ..	0·24	0·29	0·41	0·72	1·04	4·19

As has already been observed by the author (*loc. cit.*) in the cases of sandal spike, an increase in total soluble sugars and starch contents has been noticed.

DISCUSSION.

The physiological studies reported above have clearly shown that the spike of *Vinca rosea* bears close analogy to the similar disease in sandal. It will thus be seen from Table I that the diseased leaf is characterised by increase in total ash, total nitrogen, iron and alumina and decrease in calcium as compared with the healthy one. The difference in calcium content is not so marked as in the case of sandal, but the ratio Ca/N in spiked *Vinca rosea* (leaf and stem) is consistently lower than that in the healthy plant.

In view of the change in mineral composition of leaves following infection, it was considered necessary to determine the mineral matter distribution in stem and roots of such plants. The results presented in Table II show that, irrespective of locality, all the inorganic constituents are less in diseased stems than in the healthy ones. In the case of the roots, some abnormality is noticed, particularly with reference to total nitrogen and calcium. Extremely low values are recorded for nitrogen while lime shows an increase in diseased specimens. It would appear, therefore, that mobilisation of nitrogen takes place from the roots to the other parts of the plant such as stem and leaves. On the other hand, calcium does not seem to be translocated to the different organs where it is required. Thus, apart from the disease being considered infectious, the physiological disturbance starts from the roots which fail to respond to the requirements of other parts.

The high nitrogen content of diseased leaves was further sought to be explained through analysis of the tissues for diverse constituents. Thus, it will be seen from Table III, that the disease is characterised by a diminution in protein and an increase in non-protein, the latter being made up of polypeptides, amino-acids, amides and similar forms, which arise through degradation of proteins. An examination of the leaf tissues showed a characteristic reduction in nitrate accompanied by a significant increase in ammonia. Similar observations have been recorded by Narasimhamurthy and Sreenivasaya (*J. Indian Inst. Sci.*, 1929, **12A**, 153) in the case of sandal spike.

It will be seen from the carbohydrate distribution data (Table IV), that diseased vinca leaves contain more soluble sugars and starch than the corresponding healthy ones. In stems and roots, however, starch alone is found to be increased in plants manifesting the disease, while soluble sugars are comparatively low. This accumulation of starch is probably due to a disturbance in the calcium metabolism as in the case of sandal-spike, since according to the investigations of Boehm (*Sitzber. Wien. Akad. Wiss.*, 1875, **71**, 287), Grafe and Portheim (*ibid.*, 1906, **115**, 1003) and Nightingale and others, a deficiency in the supply of calcium to a developing plant is accompanied by an excessive storing up of starch in the different organs. The diastatic activity of diseased leaves and leaf saps (Table V) has, on the contrary, been shown to be higher than in the controls. This is in agreement with what has been observed by Boas in Leafroll disease of potato (*Zeits. Pflanzenkrank*, 1919, **29**, 171), by Tollenaar Dirk (*Omzettingen Van Koolhydraten in het Blad Van Nicotiana tobacum*, L., 1925, **21**) in tobacco mosaic and by Sastri and Sreenivasaya in sandal spike (*J. Indian Inst. Sci.*, 1928, **11A**, 23). It is rather difficult, however, to explain how starch storing takes place in the cells when the amylase activity is high. There does not appear to be any significant change in the reaction of sap consequent on the disease.

Z. œnoplia is another plant which occurs largely in South Indian forests and is found to be spiked even in localities where no sandal exists. Since its mineral composition has been examined by Sastri and Narayana (*J. Indian Inst. Sci.*, 1930, **13A**, 147), it was considered to be of interest to determine the starch content of diseased leaves in which it is higher than in healthy specimens, irrespective of locality (Table VI). Quantitative observations have also shown that spiked *zizyphus* has a greater diastatic activity than the healthy one.

SUMMARY AND CONCLUSIONS.

1. A study of the distribution of minerals in healthy as well as spiked *Vinca rosea* has shown that, as in the case of sandal spike, both the total ash and the calcium contents of the stem and leaf tissues are reduced and the nitrogen content increased as the result of the disease. In striking contrast with this, is the diminished nitrogen and increased calcium content of the affected roots, suggesting a rapid mobilisation of the former constituent to the aerial parts.

2. The protein contents of the different parts of the diseased plant are generally less than those of the healthy ones. The reverse is observed in the case of ammonia.

3. As observed in the case of sandal-spike, the ratio, Ca/N in the diseased stem and leaves is distinctly lower than that in the healthy ones.

4. The diseased stem and roots contain more of starch and the leaves more of starch and sugars than the corresponding healthy specimens. The diastatic activity of the diseased parts is, nevertheless,

higher than that of the healthy ones. Similar observations have been made in regard to the starch and soluble sugar contents and diastatic activity of healthy and spiked specimens of *Z. œnoplia*.

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