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CONTENTS

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

Part XIII. Investigation of the Hexone Bases.

By Y. V. Sreenivasa Rao.

It has been shown by Narasimhamurty and Sreenivasaya (*J. Indian Inst. Sci.*, 1929, **12A**, 153) that with the onset of spike there is distinct increase in the basic nitrogen content of the leaves of sandal. The basic fraction has now been partitioned into individual amino-acid components by Van Slyke's method.

The preparation of the experimental material has been fully described in the previous communication. The leaf material (40 g.) thus obtained was repeatedly extracted with boiling ammonia-free distilled water and filtered. After treatment with 2-3 c.c. of glacial acetic acid, the combined filtrates were passed through paper pulp and the total nitrogen content determined on aliquot portions thereof. The bulk of the filtrate was then hydrolysed with 20 per cent. hydrochloric acid for one hour. After removing the acid-insoluble humin, the hydrolysate was distilled under reduced pressure to remove hydrochloric acid. Calcium oxide was then added in slight excess and the ammoniacal nitrogen distilled under reduced pressure. The residue was filtered with frequent washings and the combined filtrates concentrated to a small volume (50 c. c.).

Hydrochloric acid was added to this solution until the concentration was 1N and 10 grams of phosphotungstic acid were stirred into the liquid.. The mixture was then placed on a boiling water-bath until most of the precipitate dissolved. It was taken out and, after cooling to the room temperature, kept in the ice chest for 24 hours. The phosphotungstic acid precipitate was separated by centrifuging and washed repeatedly with ice-cold 1 : 10 hydrochloric acid. The centrifugate and the washings made up to known volume constituted the non-basic fraction. The precipitate was dissolved in the minimum quantity of 1 per cent. sodium hydroxide and made up to known volume.

The basic and the non-basic fractions obtained in the above manner from the four specimens (two healthy and two spiked) from two different areas (Uttarahalli and Ragihalli) were analysed according to the Van Slyke method as modified by Plimmer and his co-workers. (*J. Biol. Chem.*, 1911, **10**, 15 ; *Biochem. J.*, 1925, **19**, 1004 ; *Ibid.*, 1927, **21**, 247). The results are shown in Tables I and II.

TABLE I

Percentages on moisture-free basis.

Form of Nitrogen	UTTARAHALLI AREA		RAGIHALLI AREA	
	Healthy	Spiked	Healthy	Spiked
Total	1.77	2.21	1.94	1.43
Water-soluble ...	0.56	0.78	0.37	0.39
Basic	0.09	0.19	0.08	0.12
Amino ...	0.05	0.10	0.04	0.07
Non-amino ...	0.04	0.09	0.04	0.05
Non-basic	0.38	0.44	0.24	0.28
Amino	0.18	0.20	0.13	0.16
Non-amino ...	0.20	0.24	0.11	0.12

TABLE II

Distribution of nitrogen in the basic fraction.

	PERCENTAGES			
	UTTARAHALLI AREA		RAGIHALLI AREA	
	Healthy	Spiked	Healthy	Spiked
Arginine	66.7	26.3	50.0	33.3
Histidine	3.3	42.1	0.4	16.7
Cystine	22.2	5.3	12.5	25.0
Lysine	11.1	21.1	25.0	25.0

It may be noted that basic fraction forms a greater proportion of the water-soluble nitrogen in the case of the spiked leaves than in the healthy ones. The occurrence of a high percentage of histidine fraction in the diseased condition is also significant and the necrosis of the root ends of the diseased plant is explained by the assumption that histidine is converted into histamine. Attempts to detect histamine in the diseased tissues have not, however, so far been successful.

SUMMARY.

The distribution of hexone bases in the water extract from the leaves of healthy and spiked plants from two different sources has been determined.

It has been observed that the basic nitrogen content of the spiked leaf is greater than that of the healthy one. Particular mention should be made of the histidine fraction which occurs in large quantities in the former while being present only in small amounts in the latter.

My thanks are due to Mr. M. Sreenivasaya for many useful suggestions.

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

Part XIV. Study of Mosaics associated with spiked areas.

By Y. V. Sreenivasa Rao.

It was observed in a number of spiked areas that many of the weeds like Aegeratum and Gysekia Spp. were affected with mosaic. The absence of this disease from healthy areas was rather striking and in view of its similarity to and possible association with the incidence and spread of sandal spike, an investigation of the mosaic of aegeratum was undertaken. The present paper relates to the distribution of inorganic constituents and nitrogen metabolism in conditions of health and disease.

The inorganic constituents were estimated according to the A.O.A.C. (1925) methods. Nitrogen distribution was determined by the method of Van Slyke modified by Plimmer and his co-workers (*Biochem. J.*, 1925, **19**, 1004; *Ibid.*, 1927, **21**, 247). The results have been presented in Tables I and II respectively.

TABLE I

Expressed as percentages on moisture-free material.

	AREA			
	JAVALGIRI		AIYUR	
	Healthy	Diseased	Healthy	Diseased
Total nitrogen ...	3.92	4.53	4.65	3.40
Phosphoric acid (as P_2O_5)	0.78	0.43	1.88	0.89
Calcium (as CaO) ...	3.97	3.74	3.14	2.45

TABLE II

Percentages of total nitrogen

	AREA			
	JAVALGIRI		AIYUR	
	Healthy	Diseased	Healthy	Diseased
Insol. Humin	7.84	7.92	2.53	7.46
Soluble Humin	1.91	1.42	0.78	1.23
Amide N	8.91	12.55	5.88	9.07
Free ammonia* ...	1.2	3.4	0.9	2.8
Total basic	13.84	14.53	16.50	19.73
Non-basic	60.10	60.50	67.89	61.18
Amino	48.67	56.0	65.33	57.51
Non-amino ...	11.39	4.5	2.56	3.67

* Estimated according to Grafe's method (*Z. Physiol. Chem.*, 1906, **48**, 300).

Inorganic constituents (Table I) do not show any striking differences. Tissues of spiked sandal are characterised by a low content of calcium while aegeratum affected with mosaic contains about the same percentage of calcium as the corresponding healthy leaves. Diseased aegeratum leaves have, on the other hand, lower phosphorous contents than the healthy ones.

The distribution of nitrogen (Table II) shows that the disease is more allied to tobacco mosaic and spinach blight than to sandal spike, if the ammoniacal nitrogen is taken into consideration. There is a striking increase in the ammoniacal nitrogen in the diseased aegeratum leaves, while in the case of the spike-disease of sandal the ammoniacal nitrogen is not appreciably affected. The higher basic nitrogen characterising spiked sandal is absent from the diseased aegeratum.

The author's thanks are due to Professor V. Subrahmanyan for his keen interest in this work.

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