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CONTENTS

CONTRIBUTIONS TO THE PHYSIOLOGY OF SANDAL
(*SANTALUM ALBUM*, LINN.)

PART I. NATURE AND EXTENT OF PARASITISM

PART II. INFLUENCE OF HOST ON THE NITROGEN METABOLISM
OF SANDAL

BY

Y. V. Sreenivasa Rao.

JOURNAL
OF THE
INDIAN INSTITUTE OF SCIENCE

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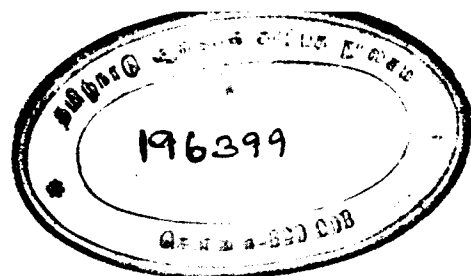
CONTRIBUTIONS TO THE PHYSIOLOGY OF SANDAL
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PART I. NATURE AND EXTENT OF PARASITISM

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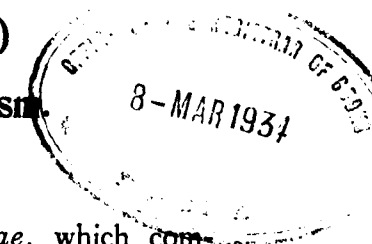


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CONTRIBUTIONS TO THE PHYSIOLOGY OF SANDAL
(*SANTALUM ALBUM*, LINN.)

PART I. Nature and extent of parasitism.

By Y. V. Sreenivasa Rao.



Sandal belongs to the Natural Order *Santalaceae*, which comprises an entire class of root parasites and of which about two hundred species are known to occur widely distributed all over the world, in the temperate as well as tropical forests. Of these, sandal is practically the only economic species, the aggregate annual revenue received from it amounting to several lakhs of rupees.

That sandal is a root parasite was first recognised by Scott in the year 1871, but the practical significance of this fundamental fact is still not adequately appreciated. Fuel exploitation is allowed in sandal-bearing areas which results in gradual depletion of the host plants of the parasitic sandal. Afforestation of sandal areas with suitable species of hosts is essential for successful silviculture of sandal.

Rama Rao (*Indian Forest Record* 1910, 2, Part V, 159) gives a list of some 140 species of plants which he found to have been haustorised by sandal. The fact that sandal has made its haustorial connection with a particular species is not, however, a criterion for the latter being a suitable host plant. The extensive pot culture trials carried out by Sreenivasaya at the nursery of the Indian Institute of Science have, in fact, established that there is great variation in the "host value" of various species of plants. Thus, whereas leguminous hosts generally nourish the sandal to rapid and luxurious growth, plants of the *phyllanthus* species make bad hosts. It is not possible to raise sandal with *Phyllanthus embellica* though a haustorial connection may, with some difficulty, be established between the host and the parasite.

An exhaustive study of the haustorium was conducted by Barber (*Indian Forester*, 1904, 30, 545; *ibid.*, 1905, 31, 189; *Mem. Dept. Agric. India, Bot. Ser.*, Vol. I, No. I, Parts 1 and 2) who also investigated the conditions for its formation by sandal. In view of the economic importance of the plant and the serious nature of the spike disease, a closer knowledge of the physiology of sandal is necessary.

The present communication deals with two aspects of the problem :—
(1) the extent to which sandal is dependent on its host in regard to its mineral nutrients and (2) the physico-chemical relationship existing between the host and the parasite.

EXPERIMENTAL.

Material.—Sandal seedlings numbering one hundred, were transplanted into pots made up with equal quantities of manure and soil. Into one set of pots, *Acacia farnesiana* was introduced as the host, while an equal number (50) were left as controls without any host plant.

It was observed that the seedlings in association with the host soon developed a healthy, green flush and put on rapid growth as contrasted with controls which made no appreciable progress and the leaves of which turned to an unhealthy pale yellow (*vide* photograph).

Six months after transplanting, when the difference between the plants with and without hosts became quite manifest, they were carefully removed from the pots, washed free from mud and adhering dirt and divided into three portions, leaves, stems and roots. The specimens were dried over calcium chloride in a vacuum desiccator. They were then powdered to pass a 40-mesh sieve and preserved in bottles for analysis.

Methods.—Total nitrogen was estimated according to the method of Kjeldahl as modified by Gunning to include nitrates. Total ash, phosphoric acid, potash, calcium, iron and alumina were estimated according to the standard methods of A.O.A.C. (1925). The results have been presented in Table I in which two independent sets of observations showing the possible range of values have been given.

It may be seen from the above that the different parts of sandal nourished by hosts were consistently richer in nitrogen, phosphorus and potash than the corresponding portions of plants without hosts. On the other hand, the latter contained more ash, iron, alumina and calcium than those associated with hosts. The above differences were most prominent in the case of leaves.

Other conditions being alike for the two sets of plants, it may be inferred from the above that better development of sandal raised in association with the host is due to the nutrition supplied by the latter, particularly nitrogen, phosphorus and potash. On the other hand, the values obtained for iron, alumina and calcium would point to those constituents being taken directly from the soil: the lower percentages observed in the case of plants with hosts may be largely

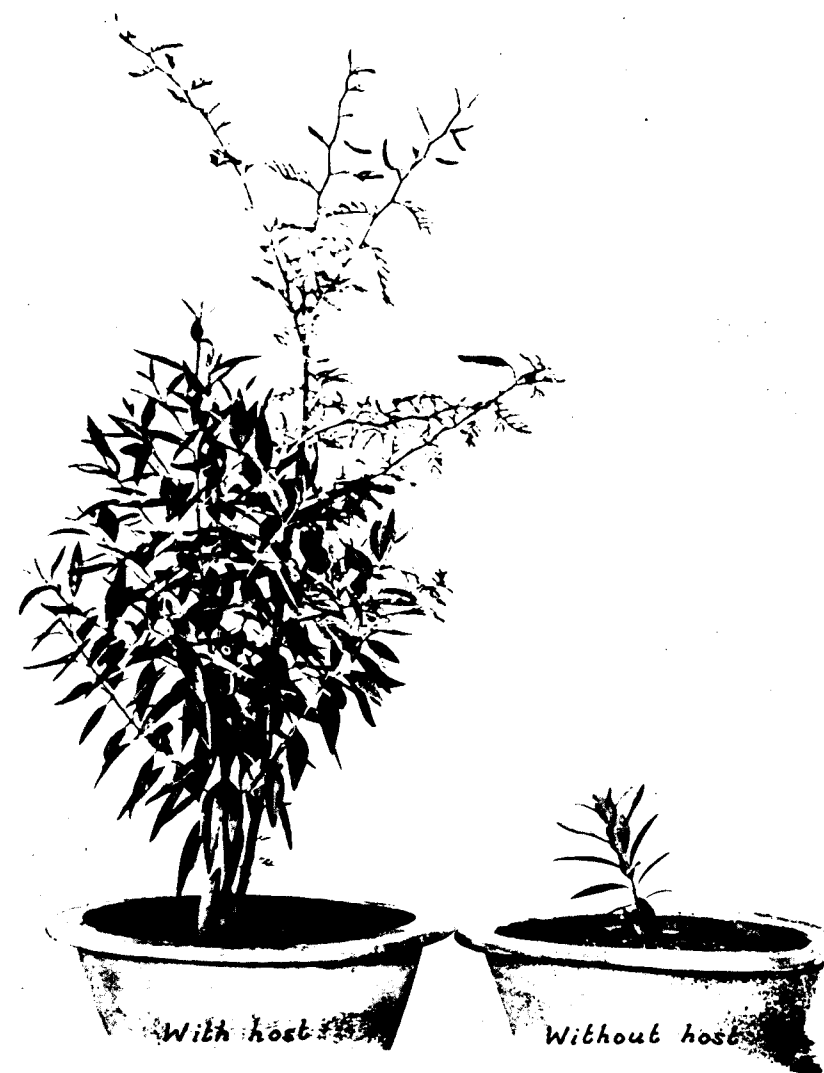


TABLE I
PERCENTAGES ON MOISTURE-FREE MATERIAL

LEAVES				STEMS				ROOTS			
I		II		I		II		I		II	
With host		Without host	With host	Without host	With host	Without host	With host	Without host	With host	Without host	With host
Ash	9.08 ...	13.76	8.22	14.68	3.40	7.22	3.21	7.49	3.09	5.86	6.29
Total N ₂	4.22 ...	1.75	2.69	1.19	1.27	0.98	1.30	0.88	1.18	0.96	0.99
P ₂ O ₅	0.47 ...	0.26	0.41	0.32	0.23	0.21	0.25	0.20	0.34	0.24	0.19
Fe ₂ O ₃ and Al ₂ O ₃	0.30 ...	1.69	0.25	0.70	0.23	0.32	0.30	0.65	0.42	0.78	0.98
CaO	1.32 ...	4.15	1.36	4.23	0.82	2.41	0.69	2.64	0.24	0.36	0.31
K ₂ O	3.49 ...	1.69	2.43	1.38	1.68	0.95	0.94	0.78	1.81	1.03	0.88

explained by the fact that those plants had greatly increased in total weight (made up chiefly of products of photosynthesis) as compared with the controls which made no appreciable growth.

Physico-chemical relationship of sandal and its associated host plant.—In recent years the physico-chemical study of tissue fluids has been receiving an increasing amount of attention. Study of the osmotic concentrations in auto-tropic plants by Dixon and Atkins (*Sci. Proc., Royal Dublin Soc.*, 1912, **13**, 219; 1915, **14**, 374; *ibid.*, **15**, 51) helped to throw fresh light on the problem of the ascent of sap. Particular attention has been paid by them to the effects of environment on osmotic pressure and hydrogen-ion concentration. Study of the tissue fluids has also been extended to plant diseases by various workers and a considerable amount of work is being carried out in this laboratory by Sreenivasaya and his collaborators (*Indian Inst. Sci.*, 1928, **11A**, 23).

The exchange of water between the living cell and its environment depends on the osmotic pressure of the cell. It is this pressure that regulates the flow of vital fluids from one part of a tree to another and serves the entire plant with nutrition. The determination of the osmotic pressure of the host and parasite has thrown considerable light on the mechanism of the supply of nutrition from the host to the parasite (Harris *et al.*, 1914-24). In this laboratory Krishna and Ranga Rao (unpublished work) have also carried out systematic studies on the physico-chemical changes in the tissue fluids of free and attacked lac hosts.

In view of the importance of physico-chemical measurements in determining the relationship between the host and the parasite, some studies were carried out on the tissue fluids of sandal in association with different host plants.

Material.—The samples were all taken from the nursery attached to the department.

Various methods have been proposed for the extraction of plant tissue fluids, but the observations of Dixon and Atkins (*Proc. Roy. Dublin Soc.*, 1913, **13**, (N.S.) 422), and Gortner and his co-workers, (*Plant World*, 1914, **17**, 53; *Biochem. Bulletin*, 1916, **5**, 139) have shown that preliminary freezing of the tissues with either liquid air or solid carbon dioxide render the cell walls permeable so that subsequent pressing brings out all the available tissue fluid. Since our experience in this laboratory also pointed to the same conclusion, the tissue fluids investigated in the present study were obtained by first freezing the material with liquid air and then pressing it out under conditions which were standardised for each material. Thus, leaf tissues were pressed

at one ton per sq. inch for 3 minutes, roots at $1\frac{1}{2}$ tons for 5 mins., and stems at 2 tons for 7 mins. By adhering to this formula fairly uniform specimens of sap were obtained for all the experiments.

Sandal plants with hosts were carefully pulled out and washed free from adhering soil. They were then divided into three portions, stems, leaves and roots as before and the tissue fluids pressed out. The products thus obtained were centrifuged at 3,000 revolutions per minute to free them from suspended impurities. The clear sap was used for subsequent analyses.

Nitrogen and the ash constituents were determined according to the methods already referred to with the difference that when the quantities of specimens were small, the estimation of nitrogen was carried out according to the micro-method of Pregl. Osmotic pressure, electrical conductivity and hydrogen-ion concentration were determined according to the methods described in an earlier communication (*Jour. Indian Inst. Sci.*, 1928, **11A**, 104-106). The results have been given in the following tables.

It will be seen from the above that the values for the osmotic pressure of the tissue fluids from different parts of sandal are invariably higher than the corresponding quantities for the associated hosts. This observation is in conformity with those of Lawrence and Harris (*Amer. Jour. Bot.*, 1916, **3**, 438-455) on the Jamaican *Loranthaceae* and their various hosts. On the other hand, the osmotic pressures of the tissue fluids from different parts of the hosts growing by themselves under similar conditions (*vide* Tables III and V) are consistently higher than those of similar plants serving as hosts; in the case of *Pongamia glabra*, however, the values are higher than those of the associated sandal plants themselves. This is a rather striking observation as it would appear to contradict the prevalent opinion that a higher initial osmotic pressure of the parasite is necessary to cause the flow of the nutrients from the host. The results do in fact show that the diminution in the osmotic pressure of the tissue fluids of both *Acacia farnesiana* and *Pongamia glabra* is the result of parasitisation by sandal so that the flow of sap from the host to sandal would appear to be the result of some other specific attribute of the latter rather than to its tissue fluid possessing a higher osmotic pressure than that of the host. Further work on this aspect of the problem is in progress and it is expected that the results would lead to findings of fundamental importance in the study of factors relating to the nature of parasitism in plants.

The hydrogen-ion concentration of the tissue fluid from different parts of sandal is generally higher than that of the corresponding hosts.

TABLE II
Sandal-*Acacia* combination

PARASITE	Percentage of sap on fresh weight	EXPRESSED AS GRAMS IN 100 C. C. OF TISSUE FLUID						Osmotic pressure in atmos.	Specific electrical conductivity $\times 10^3$	P _H	
		1st SET. Specimens collected on 23rd September 1929									
		Total solids	Ash	Total nitrogen	P ₂ O ₅	CaO	K ₂ O				
Sandal leaves	...	32.84	17.76	3.15	0.37	0.16	0.48	0.82	19.66	14.72	...
" stems	...	25.54	12.50	1.25	0.25	0.14	0.11	0.29	12.66	8.99	...
" roots	...	35.69	10.48	1.07	0.15	0.09	0.08	0.34	10.37	9.70	...
HOST											
<i>Acacia farnesiana</i> leaves	41.19	12.38	1.66	0.31	0.10	0.21	0.47	13.75	9.32	...	
" stems	21.75	9.89	1.42	0.23	0.11	0.08	0.22	10.61	9.29	...	
" roots	29.41	6.43	1.22	0.12	0.09	0.10	0.29	7.89	9.65	...	
PARASITE											
2ND SET. Specimens collected on 12th November 1929											
Sandal leaves	...	33.22	20.43	3.19	0.34	0.19	0.59	1.03	19.05	13.03	4.95
" stems	...	19.56	15.02	1.35	0.21	0.17	0.33	0.49	14.34	8.38	4.90
" roots	...	24.58	14.68	1.14	0.19	0.11	0.27	0.42	12.54	8.03	5.35
HOST											
<i>Acacia farnesiana</i> leaves	47.47	11.15	1.50	0.31	0.11	0.13	0.53	12.66	9.72	5.30	
" stems	19.68	8.62	1.43	0.19	0.11	0.14	0.50	9.77	8.99	4.95	
" roots	28.07	4.17	0.88	0.12	0.08	0.12	0.14	5.31	8.34	5.55	

TABLE III
Acacia farnesiana alone

	Percentage of sap on fresh weight	EXPRESSED AS GRAMS IN 100 C.C. OF TISSUE FLUID						Osmotic pressure in atmos.	Specific electrical conductivity $\times 10^3$	P _H
		1st SET. Specimens collected on 16th September 1929								
		Total solids	Ash	Total nitrogen	P ₂ O ₅	CaO	K ₂ O			
Leaves	27.60	15.07	2.03	0.39	0.12	0.27	0.67	18.76	11.05	5.10
Stems	20.00	8.94	1.44	0.29	0.18	0.06	0.47	12.27	10.40	5.20
Roots	21.30	8.28	1.36	0.26	0.11	0.08	0.45	12.27	13.10	5.65
2ND SET. Specimens collected on 31st October 1929										
Leaves	...	11.06	1.52	0.34	0.13	0.20	0.66	16.36	9.52	5.05
Stems	...	8.84	1.24	0.22	0.16	0.07	0.40	14.44	9.19	4.95
Roots	...	7.04	1.03	0.23	0.11	0.08	0.44	10.11	9.15	5.94

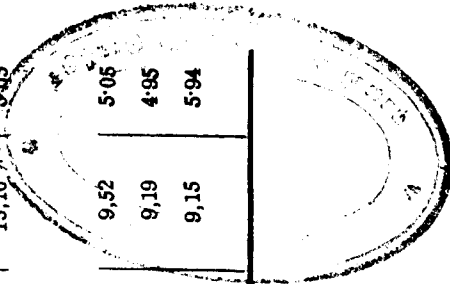


TABLE IV
Sandal-Pongamia glabra combination

	Percentage of sap on fresh weight	EXPRESSED AS GRAMS IN 100 C.C. OF TISSUE FLUID						Osmotic pressure in atmos.	Specific electrical conductivity $\times 10^3$	P _H
		1ST SET. Specimens collected on 27th November 1929								
		Total solids	Ash	Total nitrogen	P ₂ O ₅	CaO	K ₂ O			
PARASITE										
Sandal leaves	35.92	18.91	2.42	0.38	0.07	0.38	0.76	23.99	12.43	4.96
" stems	10.91	13.26	1.36	0.22	0.11	0.08	0.49	13.83	14.55	5.05
" roots	20.41	12.99	1.19	0.17	0.06	0.10	0.39	12.42	8.75	5.40
HOST										
<i>Pongamia glabra</i> leaves	14.77	19.58	2.54	0.38	0.13	0.36	0.89	16.64	9.42	5.30
" stems	25.65	15.09	1.91	0.41	0.20	0.19	0.49	13.93	9.62	5.70
" roots	30.87	9.32	1.56	0.39	0.06	0.07	0.84	9.53	10.84	5.55
PARASITE										
Sandal leaves	39.66	19.70	3.04	0.37	0.09	0.49	...	21.16	14.18	5.18
" stems	12.90	12.61	0.84	0.22	0.08	0.11	...	12.72	8.77	5.05
" roots	17.13	12.26	0.88	0.16	0.06	0.11	...	10.55	9.40	5.50
HOST										
<i>Pongamia glabra</i> leaves	28.15	9.35	1.15	0.40	0.09	0.26	...	17.59	9.68	5.33
" stems	29.41	10.31	1.49	0.70	0.11	0.20	...	10.91	12.46	5.70
" roots	32.43	8.39	1.57	0.46	0.06	0.11	...	9.17	15.10	5.98

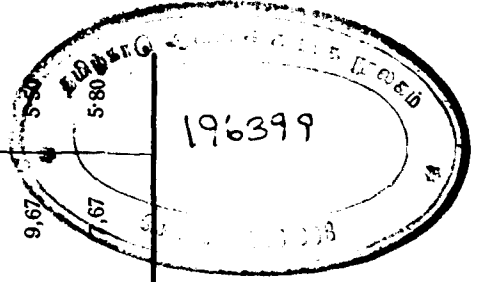
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TABLE V
Pongamia glabra alone

Specimens collected on 11th February 1930

	Percentages of sap on fresh weight	EXPRESSED AS GRAMS IN 100 C.C. OF TISSUE FLUID					Osmotic pressure in atmos.	Specific electrical conductivity $\times 10^3$	P _H
		Total solids	Ash	Total nitrogen	P ₂ O ₅	CaO			
Leaves	43.93	5.47	0.89	0.41	0.16	0.08	25.31	11.18	5.80
Stems	42.31	8.73	1.20	0.75	0.22	0.15	19.11	9.67	5.80
Roots	47.82	8.69	1.40	0.68	0.08	0.11	14.97	7.67	5.80

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The tissue fluid from the leaf would appear to be slightly more acid than that from the roots. The values of specific electrical conductivity throw no light on the nature of the relation between the host and the parasite. The conductivity of the sap generally decreases as we pass from the leaf to the root. A similar observation would also apply to the total solids, ash, nitrogen, calcium and phosphorus.

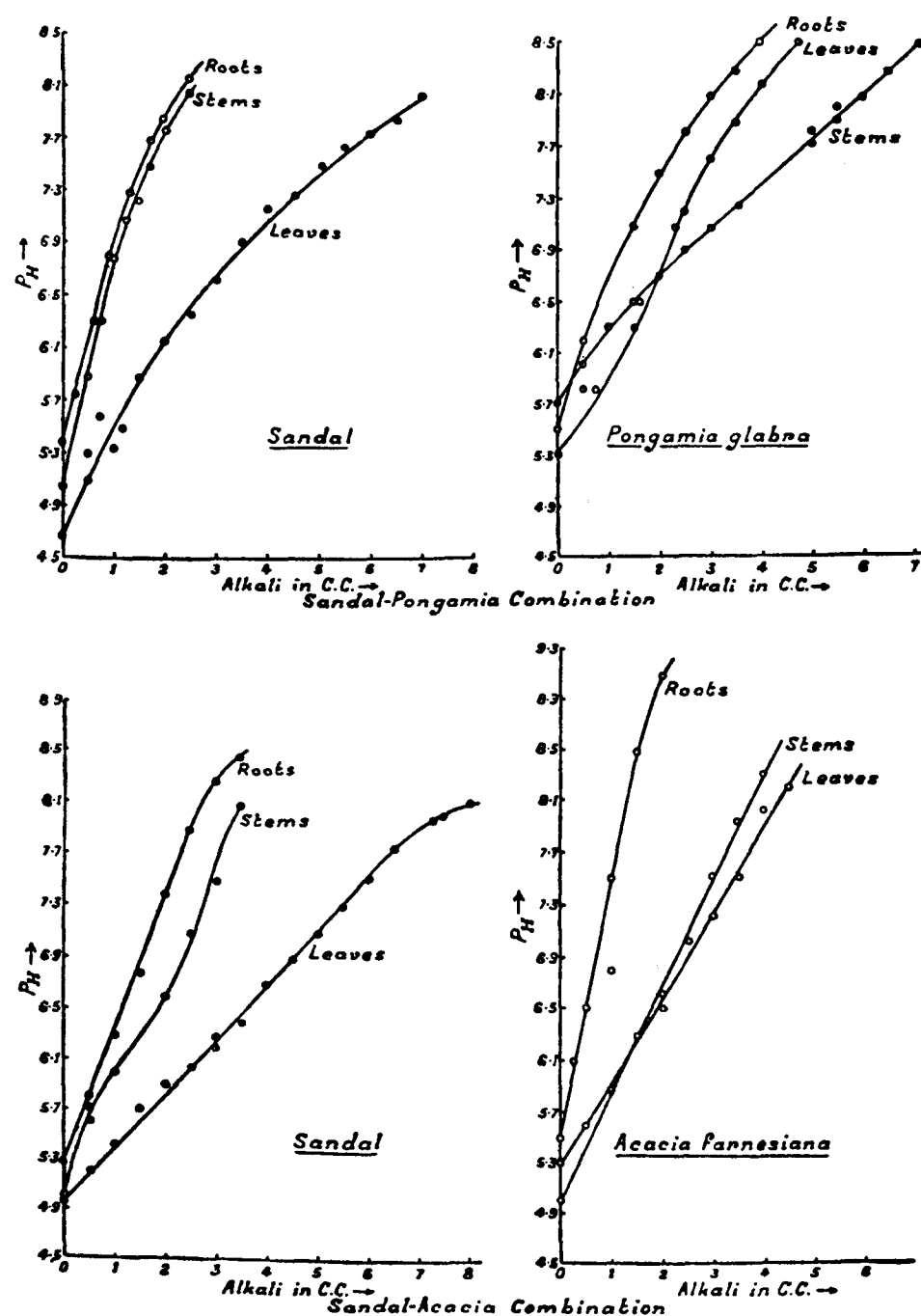
In view of the dependence of sandal on its host for the supply of nitrogen and certain essential mineral constituents, some experiments were carried out to determine the effect of association of sandal with various hosts on the chemical composition and hydrogen-ion concentration of its tissue fluid. The results have been presented in the following table (Table VI).—

TABLE VI
*Chemical composition of the tissue fluid of sandal raised
in association with different host plants*

Host	IN 100 C.C. OF TISSUE FLUID EXPRESSED AS GRAMS					
	Ash	Total solids	Total nitrogen	P ₂ O ₅	CaO	P _H
<i>Melia indica</i> ...	2.83	15.02	0.33	0.09	0.67	5.6
<i>Lantana camara</i> ...	3.96	13.47	0.08	0.08	0.09	4.8
<i>Acacia farnesiana</i> ...	3.15	17.76	0.37	0.16	0.48	5.0
<i>Pongamia glabra</i> ...	2.42	18.91	0.38	0.08	0.38	5.3
<i>Casuarina aquisetifolia</i> ...	1.03	14.81	0.28	0.05	0.35	5.1
<i>Dendrocalamus strictus</i> ...	0.83	12.43	?	0.07	0.18	5.6-5.8

It may be seen from the above that the chemical composition of the tissue fluid of sandal is largely determined by the nature of the associated host. Taking nitrogen, for instance, it may be seen that the plants in association with the leguminous hosts have generally larger quantities of that element than those parasitising on other hosts. On the other hand, legumes render sandal susceptible to spike-disease while several other classes of plants are known to impart resistance. The significance of nutrition in relation to resistance to disease is an important aspect of the physiology of sandal and will form the subject of a later study.

The above observations are also of great importance in relation to the silviculture of sandal. The introduction of the right kind of host



not only enriches the tissue fluid of sandal but also promotes vigorous growth and in some cases imparts resistance to disease. The preponderance of comparatively poor hosts or lack of sufficient number of good hosts impoverishes the tissue fluid, impairs the growth and renders the plant susceptible to disease.

Buffering capacities of the tissue fluids of sandal and its associated hosts.—This was determined by titrating the tissue fluids against alkali and determining the quantities of the latter required for each unit shift of the hydrogen-ion concentration. The results presented in graphs I to IV show that the tissue fluid from different parts of sandal has higher buffering capacity than those from corresponding parts of the hosts. The different parts of sandal in association with *Acacia farnesiana* have higher buffering capacity than those of plants parasitising on *Pongamia glabra*. The possible relation of this observation to the greater susceptibility to spike observed in the case of sandal associated with *Pongamia* is under investigation.

SUMMARY.

1. Sandal depends on its host for its requirements of nitrogen, phosphorus and potash; the other ash constituents including calcium, iron and alumina would appear to be directly derived from the soil.
2. The tissue fluids from different parts of sandal have consistently higher osmotic pressure than those derived from corresponding parts of the associated hosts. The higher osmotic pressure observed in the case of the hosts (particularly *Pongamia glabra*) when growing alone would suggest that the lower values obtained in the previous case are the result of parasitisation by sandal and not a cause as generally believed.

The hydrogen-ion concentration of sandal is higher than those of the two hosts which were investigated. The buffering capacity of the tissue fluid of sandal in association with *Acacia farnesiana* is slightly higher than that of sandal with *Pongamia glabra* as host. The chemical composition of the tissue fluid of sandal is dependent upon the nature of the host with which it is associated.

My thanks are due to Mr. M. Sreenivasaya for many useful suggestions and to Prof. V. Subrahmanyam for his keen interest in the work.

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[Accepted, 12-8-1933.]

CONTRIBUTIONS TO THE PHYSIOLOGY OF SANDAL

PART II. Influence of host on the nitrogen metabolism of sandal.

By Y. V. Sreenivasa Rao.

The study of nitrogen metabolism of plants is of very great importance in view of its intimate relationship with the fundamental activities of life such as growth and reproduction. The metabolic changes proceed in the different parts of the plant but those occurring in the leaf are of special interest since it is the seat of protein synthesis and other related activities. Sandal being a root parasite, the changes taking place in its system would throw light on the nature of the nitrogenous constituents which the plant is capable of synthesising and the extent to which it depends on the host for elaborated food. Since the haustorial connections of sandal die out with the onset of spike-disease, it appeared probable that the conditions in diseased sandal would at least partly correspond to those in sandal when it is deprived of its host. In view of the above and the observation of Sreenivasaya that association with certain plants render sandal susceptible to spike while that with others imparts resistance to artificial infection of the disease, it was considered that the study of nitrogen metabolism of sandal, with and without hosts, would lead to findings of some practical importance.

EXPERIMENTAL.

The experimental plants were raised and the leaf materials prepared in the manner described in the previous part (*Jour. Indian Inst. Sci.*, 1933, **16A**).

Methods.—Total nitrogen was determined according to Gunning's modification of Kjeldahl's method to include nitrates. Protein nitrogen was estimated by the Stutzer's method (*J. Land.*, 1880, **28**, 103). Nitrates were estimated according to the method of Scales (*J. Biol. Chem.*, 1916, **27**, 327) and nitrite by colorimetric method using Griess Ilosway reagent for the purpose. Ammonia was estimated according to Grafe and Erich (*Zeit. Physiol. Chem.*, 1906, **48**, 300). Distribution of water-soluble nitrogen was determined by the methods of Jodidi, Kellog and True (*Jour. Agric. Res.*, 1918, **15**, 385) and amino nitrogen by the Van Slyke method (*Jour. Biol. Chem.*, 1911, **9**, 185; *ibid.*, 1912, **12**, 275). The details of the methods as adopted for

different determinations are similar to those described by Narasimhamurthy and Sreenivasaya in their investigation on the distribution of nitrogen in healthy and spiked sandal (*Jour. Indian Inst. Sci.*, 1929, **12A**, 153).

As mentioned in a previous paper it was observed that sandal in association with the host (*Acacia farnesiana*) had a higher percentage of nitrogen (2.69 to 2.84) than the one without the host (2.03 to 2.19). Nitrite could not be detected in the extracts from sandal growing in association with the hosts while it was present only in traces in the control set of plants. Quantitative determination of nitrites was not therefore carried out.

The other results have been presented in the following tables :—

TABLE I

Distribution of nitrogen in the leaves of sandal with and without host plant.

Form of Nitrogen	PERCENTAGES ON MOISTURE-FREE BASIS			
	Set I		Set II	
	With host	Without host	With host	Without host
Total nitrogen ...	2.83	2.03	2.84	2.05
Protein	2.07	0.86	2.08	0.86
Nitrate	0.07	0.06	0.07	0.06
Total water-soluble N ...	0.68	1.19	0.77	1.20
Ammonia N ...	0.01	0.04	0.01	0.05
Amide	0.08	0.01	0.06	0.01
Humin	0.09	0.20	0.12	0.20
Basic	0.14	0.46	0.19	0.44
Mono-basic	0.19	0.21	0.19	0.21
Non-basic	0.13	0.25	0.18	0.25
Peptide	0.004	0.00	0.00	0.00

TABLE II

Distribution of the water-soluble nitrogen in the leaves of sandal with and without host plants

Material	Percentage of HCl used	Time of digestion in hours	PERCENTAGE OF THE TOTAL WATER-SOLUBLE NITROGEN					
			Ammonia	Amide	Hummin	Basic	Mono-amino	Residual
With host	4	4	1.89	11.76	13.60	20.29	28.11	18.94
Do.	20	8	1.89	11.76	13.60	...	30.01	17.04
Without host	4	4	3.75	0.50	13.49	38.87	17.72	21.16
Do.	20	8	3.75	0.50	13.49	...	21.30	17.58
With host	4	4	1.67	7.91	16.20	24.06	24.02	22.94
Do.	20	8	1.67	7.91	16.99	...	26.04	20.92
Without host	4	4	3.73	0.56	16.63	36.90	17.61	21.01
Do.	20	8	3.73	0.56	16.63	...	20.30	18.30
								Peptide
								...
								0.63
								...
								...
								0.49
								...
								...

TABLE III

Percentages of nitrogen in the leaves of sandal occurring in association with different hosts

Host	Nitrogen	Host	Nitrogen
<i>Lantana camara</i> ...	1.29	<i>Murraya koenigii</i> ...	2.77
<i>Divi Divi</i> ...	2.17	<i>Dendrocalamus strictus</i> ...	1.58
<i>Casuarina aquisetifolia</i> ...	2.91	<i>Pongamia glabra</i> ...	3.23
<i>Melia indica</i> ...	2.78	<i>Acacia farnesiana</i> ...	4.22
<i>Rulea graveolens</i> ...	2.79		

TABLE IV

Form of Nitrogen	RESULTS EXPRESSED AS PER CENT. OF TOTAL N.	
	With host	Without host
Total basic nitrogen ...	5.26	22.87
Amino N. ...	2.45	8.64
Non-amino N. ...	2.81	14.23
Arginine N. ...	3.38	17.07
Histidine N. ...	0.40	2.14
Cystine N. ...	0.41	0.42
Lysine N. ...	1.01	3.23
Nitrogen in filtrate from bases ...	13.40	23.06
Amino N. ...	8.88	12.50
Non-amino ...	4.52	10.56

It may be seen from the above that the nitrogen content of sandal is determined by the nature of the host with which it is associated. Thus, the plants growing in association with the two leguminous hosts, *Pongamia glabra* and *Acacia farnesiana*, have much higher nitrogen contents than those with other hosts. The results, together with pot culture observations, showed that the supply of suitable hosts

particularly those of leguminous type is necessary to promote the rapid vegetative growth of sandal. Sandal without hosts leads a struggling existence and since the improved growth observation in association with a host is accompanied by increase in nitrogen content, it would appear that the supply of nitrogen by the host plant is one of the limiting factors in the growth of sandal, at any rate, in the early stages. The leaves of sandal without host turn yellow and the plants make no appreciable growth but if the host plant is provided, preferably one of leguminous type, the natural green colour is restored and the plant makes very rapid growth.

The nitrate content of sandal with host is slightly higher than that of the one without it. Sandal fed by host is richer in amide nitrogen than the one without it. Since amides play a very important part in transformations of nitrogen into proteins it would appear the conditions are more favourable in sandal with host than in the one without. This observation is supported by the protein contents which show that the host-fed sandal is richer than the one without (Table I). The observations relating to synthesis of proteins are further supported by the comparatively smaller amounts of water-soluble nitrogen in host-fed plants while the corresponding condition in hostless sandal would point to proteolytic degradation taking place therein.

The state of sandal that is deprived of its host corresponds to that of starvation observed by Chibnall in the case of the runner bean plant which showed decrease in protein nitrogen, followed by increase in water-soluble forms with the progress of starvation (*Biochem. J.*, 1912, **16**, 344). Chibnall also observed that the starved plants contained a greater quantity of what he called the 'other N', which corresponds to the 'residual N' in column 10 of Table II in the present paper. Drastic treatment (with concentrated HCl for 16 hours) to effect complete hydrolysis led to increase in ammonia and basic nitrogen followed by decrease in 'other N'. This suggested to Chibnall that 'other N' might consist of ureides like barbituric acid which resist mild hydrolysis, but which, on prolonged hydrolysis, yield bases giving a precipitate with phosphotungstic acid. Basing his conclusions on those of Fosse (*Compt. rend.*, 1913, **156**, 567, 1938; 1914, **158**, 1374) who demonstrated the presence of traces of urea in higher plants, Chibnall drew the conclusion that the catabolic process of the nitrogen metabolism in the leaves proceed along the line leading to increased production of 'other N'. The results presented in column 10 of Table II are similar to those obtained by Chibnall. Although drastic hydrolysis with concentrated acid was not conducted, yet prolonged treatment with 20 per cent. hydrochloric acid led to increase in the mono-amino nitrogen content of the hydrolysate, thereby suggesting that the latter was derived from the 'residual' nitrogen. It would follow therefore

that the values for mono-amino nitrogen given in column 9 of Table II, did not represent the entire amount of mono-amino nitrogen present in the water extract. Another reason for concluding that column 9 does not represent the actual amount of mono-amino nitrogen is that according to Hart and Bently (*J. Biol. Chem.*, 1915, **22**, 477) and Roxas (*J. Biol. Chem.*, 1916, **23**, 71) at least a part of the humin nitrogen formed as a result of hydrolysis with HCl is formed out of the mono-amino nitrogen. For the same reason column 11 cannot represent the actual amount of peptide nitrogen present in the extract as part of the increase of amino-nitrogen due to hydrolysis of peptide by acid may be converted into humin nitrogen. In view of these defects in the method of acid hydrolysis, a development of the method of hydrolysing the peptides with a specific enzyme seems necessary for the estimation of the true peptide nitrogen.

The results given in Table III show that the nitrogen contents of sandal leaves in association with different host plants are dependent on the nature of the hosts with which it is associated.

Table IV gives the results of the distribution of the hexone bases in the leaves of sandal with and without host plant according to the method of Van Slyke as modified by Plimmer and his co-workers (*Biochem. J.*, 1925, **19**, 1004; *ibid.*, 1927, **21**, 247). The proportion of water-soluble nitrogen in the leaves of sandal with and without host is as 1:3 and the amino nitrogen in the filtrate obtained after separation of bases is less in the case of sandal with host plant than in one without. The significance of these observations is still somewhat obscure.

When the hexone bases are expressed as percentages of the total basic nitrogen arginine and histidine are found to exist to the same extent in sandal raised with and without hosts. Lysine is present to a slightly greater extent and cystine very much more so in sandal nourished by hosts than in without. Since cystine is known to be essential for the growth and active functioning of plants, it is probable that the inadequacy of supply of that essential amino-acid may be partly responsible for the poor development of hostless sandal.

SUMMARY

A detailed investigation of the nitrogen metabolism in the leaves of sandal with and without hosts has been made.

Total nitrogen in the leaves of sandal is largely influenced by the nature of the host plant with which it is associated.

A great proportion of the nitrogen in host-fed sandal exists in the form of protein. Absence of host plant seems to bring about a

decrease in protein followed by an increase in the amount of water-soluble nitrogen.

Basic nitrogen content of leaves of hostless sandal is greater than that of the host-fed plant.

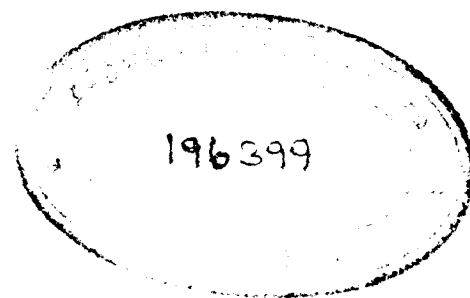
Sandal without host resembles the spiked plant in that its protein nitrogen is low and water-soluble nitrogen (made up chiefly of basic forms) high. On the other hand, the ammonia content of hostless sandal is higher and amide nitrogen lower than that of host-fed sandal. This observation differs from that in the case of spiked sandal, the ammonia and amide contents of which are not significantly different from those of the healthy one.

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