# PHYSIOLOGY OF THE SPIKE DISEASE OF SANDAL.

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## *Introduction.*

FROM a physiological point of view, virus diseases can be classified into two groups (1) the *mosaics*, characterised by an increase in the total nitrogen and a decrease in the total carbohydrates of the foliage, and (2) the *yellows*, distinguished by a decrease in the total nitrogen and an increase in the total carbohydrates in the foliage as compared with normal plants. The spike disease of sandal, which is generally grouped under the latter, shows an increase not only in the carbohydrates as in the *yellows* but also in total nitrogen. Among other characteristic features of spike may be mentioned the exclusive occurrence of mannitol (Sreenivasaya, 1930)—which has not so far been recorded in any other virus affected plant—and succinic acid (Iyengar, 1933) in the leaves. The spike tissues also possess higher acidity (Iyengar, 1928) and are poorer in calcium (Sreenivasaya and Sastri, 1929). Nitrates which are present upto a concentration of 7 per cent. of the total nitrogen of the leaves of healthy plants, are present in much smaller quantities (about 1·8 per cent.) in the leaves of the diseased plants.

The intensive work carried out at the Indian Institute of Science during the period 1927–1933 by Sreenivasaya and his colleagues has resulted in the accumulation of a large volume of data which can be utilised for obtaining a fair idea of the nature of the metabolic disturbances consequent on the invasion of the virus principle; an attempt in this direction is presented in this paper.

### *Carbohydrate metabolism — accumulation of starch.*

The incidence of spike brings about an abnormal accumulation of starch, the most conspicuous change occurring in the leaf. This was first noticed by Coleman (1917) and subsequently confirmed by the Institute workers. Such accumulation has also been recorded in *Dodonæa viscosa* (Sastri and Narayana, 1930), *Vinca rosea* and *Zyzyphus aenoplia*, all suffering from a malady resembling spike of sandal in its external manifestations. Besides starch, there is accumulation of the starch degradation products and of sucrose.

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The earlier workers sought to explain starch accumulation by postulating a poor diastatic activity of the diseased tissues (Coleman, 1917). Later workers showed that the diastatic activity, as measured by saccharifying power, was actually very much higher (2 to 4 times) in the tissues and tissue fluids derived from the spiked plants (Sreenivasaya and Sastri, 1928). The diseased tissues also show a feeble maltase activity, and a pronounced sucrase activity, factors which favour the production of reducing sugars. An enquiry into the factors contributing towards the enhanced diastatic activity revealed, that such activity was partly due to the optimal pH conditions prevailing in the spike tissues, and partly due to the presence of activators whose specific nature was not investigated (Sastri and Sreenivasaya, 1929). The phenomenon of activation of enzymes accompanying various plant diseases is a fruitful line of enquiry. Such studies in the case of animal diseases, such as cancer, have yielded very valuable results (*cf.* Purr, 1934).

The accumulation of starch, which has been observed in the potato leaf-roll, is accompanied by phloem necrosis (Quanger, *et al.*, 1913). A similar phenomenon has not been reported in the case of spike of sandal. The cause of delayed or inhibited translocation of photosynthesised starch is, perhaps, due to the poor liquefying power of the diastase in the diseased leaves (Sastri, 1929). The enzyme from the healthy leaf tissue, on the other hand, possesses a high liquefying activity (nearly two times that of the diseased). The diseased tissues are also poor in calcium and chlorides, two constituents which help the translocation of starch (*cf.* Loew, 1903 ; Tottingham, 1919). The general dwarfing and stunting of the tissues impose mechanical barriers. The equilibrium

$$\text{Starch} \rightleftharpoons \text{products of hydrolysis}$$
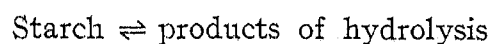
which in the healthy organism leads, through the removal of the products of hydrolysis, to the depletion of starch, is disturbed ; the products of hydrolysis accumulate and as the spike tissues are poor in water content, conditions, most favourable for the accumulation of starch are obtained.

### *Protein Metabolism.*

As physiological characteristics of the disease, may be mentioned an increase in (1) total water-soluble nitrogen, (2) basic nitrogen and (3) total amino nitrogen (Narasimhamurthy and Sreenivasaya, 1929). In the basic nitrogen fraction, a high histidine content has been recorded (Sreenivasa Rau, 1933). There is very little nitrite and ammonia in the healthy and diseased leaves ; this is in strange contrast to the higher proportion of nitrite and ammonia found in the case of spinach blight (Jodidi, 1920),

which is considered to be due to a process of denitrification. A fairly detailed investigation of the nitrogen metabolism has already been published (Narasimhamurthy and Sreenivasaya, 1924). The spike tissues also show a high proteoclastic activity. The disappearance of nitrates and their fate after the virus attack, yet remain unelucidated ; a probable course is the reduction to nitrites which immediately react with amino acids leading to the production of hydroxy acids.

*Respiration and accumulation of succinic acid, hydroxy acids and mannitol.*

As in the case of other virus-affected plants, the tissues of the spiked sandal also show a high oxidase and peroxidase activity (Sastri, 1929). The activity of the cell sap is enhanced (pH $4 \cdot 7 - 5 \cdot 0$ in spike ; and $5 \cdot 2 - 5 \cdot 7$ in healthy). Iyengar (1933) has shown that succinic, malic and oxalic acids are present in the diseased leaves while in the healthy tissues, succinic acid is either absent, or if it occurs at all, it is present only in traces. Further the oxalic acid and malic acid contents are lower in the healthy leaf. The ratio $\dfrac{\text{malic acid}}{\text{oxalic acid}}$ is nearly $0 \cdot 35$ in the case of healthy leaf while in the case of spike leaf, the ratio is more variable, ranging from $0 \cdot 5$ to $1 \cdot 0$. It is regrettable that figures are not available for free acids. It may, however, be assumed that in the healthy plant, the acids are more or less in a neutralised or combined form as the cell sap has a nearly neutral reaction. In the spiked tissues, on the other hand, these acids must be free, at least in part, as the cell sap is highly acid. It has been reckoned (Bennet-Clark, 1933) that at pH $5 \cdot 0$, 3 per cent. of the total malate is present in the acid form, while at pH $3 \cdot 95$, about 20 per cent. of the total malate is free acid.

To understand the mechanism of the production of these acids, it is necessary to study the respiration of the sandal leaf in the healthy and diseased conditions. Only a beginning has been made in this direction. According to the unpublished results of Keshava Iyengar (1932), the oxygen uptake of the diseased leaf, whether calculated on the surface area basis or weight basis, is only about half of that of the healthy tissue. The oxidase and peroxidase activities, on the other hand, are approximately twice as high. These observations indicate that the courses of respiration in the normal and diseased tissues are different.

In considering the respiration it is necessary to remember that in the affected tissues, there is a high concentration of sugars. It will therefore be unnecessary to consider other alternative substances, proteins or fats, which function as substrates when the tissues are carbohydrate-starved. Any scheme of respiration must also account for the production of mannitol
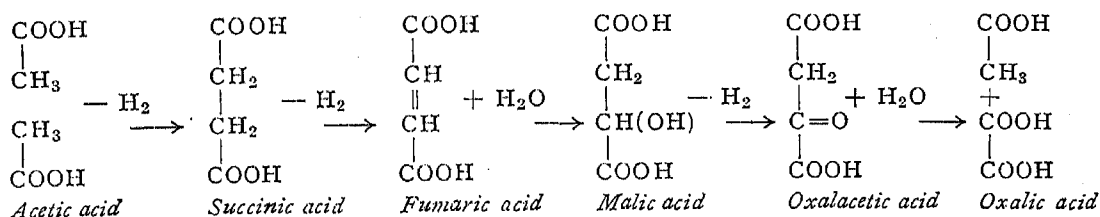
which as Sreenivasaya has shown is exclusive to spike and has not, so far, been recorded in any other virus-affected plant.

The formation of mannitol in fermenting mixtures of plant origin is known to be due to a group of bacteria. They are usually associated with souring materials (Stiles, *et al.*, 1925) such as saurkrout and silage. Mannitol is also produced from sugars by mould fungi, and Raistrick (1931) from his work on the sugar metabolism of mould fungi concluded that the first stage in the sugar breakdown is a Cannizaro reaction involving the production from two molecules of glucose, one molecule of gluconic acid and one of mannitol.

$$2\,C_6H_{12}O_6 + H_2O \rightarrow C_6H_{12}O_7 + C_6H_{14}O_6.$$
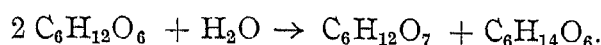
Depending on the requirements of the organisms, mannitol or gluconic acid, or both are destroyed. Raistrick made the significant observation (1931) that the quantity of mannitol produced is, to a large extent, controlled by the degree of aeration to which the fermenting media are subject. Under restricted aeration, mannitol accumulates, and the yield is considerably decreased and mannitol may even disappear under conditions of unrestricted aeration. It has already been mentioned that oxygen absorption of the spike leaves, under comparable conditions, is only half that of healthy ones. This is perhaps a factor favouring hexitol production. The products obtained by the breakdown of mannitol, gluconic acid or glucose, are qualitatively the same although slight quantitative differences exist due to their varying oxygen contents (Kay, 1920). Further decomposition of the substance might be assumed to occur according to the Neuberg scheme, as the production of 4-C acids can take place only through 2-C acids. The reaction may thus be assumed to proceed according to the following scheme :—

Mannitol $\rightarrow$ Methyl Glyoxal $\rightarrow$ Pyruvic acid $\rightarrow$ Acetaldehyde $\rightarrow$ acetic acid

$$
\begin{array}{cccccc}
\text{COOH} & \text{COOH} & \text{COOH} & \text{COOH} & \text{COOH} & \text{COOH} \\
| & | & | & | & | & | \\
\text{CH}_3 & \text{CH}_2 & \text{CH} & \text{CH}_2 & \text{CH}_2 & \text{CH}_3 \\
\;\;\;\xrightarrow{-\,H_2} & | \;\;\;\xrightarrow{-\,H_2} & \| \;\;+H_2O & | \;\;\;\xrightarrow{-\,H_2} & | \;\;+H_2O & + \\
\text{CH}_3 & \text{CH}_2 & \text{CH} \;\xrightarrow{\quad} & \text{CH(OH)} & \text{C}=\text{O} \;\xrightarrow{\quad} & \text{COOH} \\
| & | & | & | & | & | \\
\text{COOH} & \text{COOH} & \text{COOH} & \text{COOH} & \text{COOH} & \text{COOH} \\
\textit{Acetic acid} & \textit{Succinic acid} & \textit{Fumaric acid} & \textit{Malic acid} & \textit{Oxalacetic acid} & \textit{Oxalic acid}
\end{array}
$$

Thunberg has shown that by the dehydrogenation of two molecules of acetic acid, one molecule of succinic acid is produced. Dehydrogenase activities have been shown to be present in virus-affected tissues. Thus in the case of the animal virus, bouvine pleurine pneumonia, Holmes and Pirie (1932) showed the presence of a dehydrogenase. Further reactions are self-explanatory.

The accumulation of acids is determined by the velocities of production of these acids from their respective parent substances. In the spike tissues, owing to the small concentration of calcium, the neutralisation of these acids or their removal by precipitation is hindered, thus leading to their accumulation.

The presence of de-aminase in spike tissues has recently been demonstrated by Iyengar (1935). It is assumed that the production of malic acid is due to a process of oxidative de-amination. This, however, appears untenable as numerous analyses of healthy and spiked leaves from various localities and under varying silvicultural conditions have shown that acid amides and dicarboxylic acids are present only in very small concentrations in spiked tissues and that ammonia is practically absent. The process of de-amination leading to the accumulation of hydroxy acids, appears to be of comparatively little importance. A more plausible mechanism appears to be an interaction between amino acids and nitrous acid. It has already been mentioned that in the spiked tissues the nitrates occcur only in traces. The nitrates are reduced to nitrites, which immediately react with amino acids in the acid medium leading to the production of hydroxy acids.

### Conclusion.

The study of physiological characteristics of virus diseases will be found useful in (1) diagnosing the disease at an early stage and (2) in discovering resistant strains. Such studies have hardly been begun with sandal. Holmes (1932) has recently developed a technique of iodine staining which renders local lesions, due to virus, conspicuous and reveals the points of infection. Such studies may be profitably extended to sandal spike. Resistant varieties are generally characterised by low acidity and poor oxidase activities. Other factors of disease resistance, such, for instance as, phenols, glocosides, alkaloids, etc., have to be investigated. The study of active groups in tissue fluids for which a scheme was recently outlined (Sastri and Sreenivasaya, 1934) may usefully be undertaken in this connection. The possibility of nurturing sandal through appropriate hosts to impart resistance to disease has been amply demonstrated by the work of Sreenivasaya and the study of physiological characters of such plants would give results of great significance.

## REFERENCES.

| | | |
|---|---|---|
| Bennet-Clark | .. .. | *The New Phytologist,* 1933, **32**, 39. |
| Coleman | .. .. | *Spike-disease of Sandal,* Bull. No. 3, Entomological Series, Dept. of Agric., Mysore, 1917. |
| Holmes | .. .. | *Contrib. Boyce Thomp. Institute,* 1932, **4**, 297. |
| Holmes and Pirie | .. .. | *Brit. J. Expt. Path.,* 1932, **13**, 364. |
| Iyengar | .. .. | *J. Ind. Inst. Sci.,* 1933, **16A**, 139. |
| ———— | .. .. | *Ibid.,* 1928, **11A**, 103. |
| ———— | .. .. | *Ibid.,* 1933, **16A**, 139. |
| ———— | .. .. | *Nature,* 1935, **135**, 345. |
| Jodidi, Moulton and Markley | .. | *J. Amer. Chem. Soc.,* 1920, **42**, 1061. |
| Keshava Iyengar | .. .. | *Thesis for the Degree of Master of Science,* Bombay Univ., 1932. |
| Kay | .. .. | *Biochem. J.,* 1926, **20**, 321. |
| Loew | .. .. | *U.S. Dept. Agric. Bureau of Plant Industry,* 1903, Bull. No. 45. |
| Narasimhamurthy and Sreenivasaya | | *J. Ind. Inst. Sci.,* 1929, **12A**, 153. |
| Purr | .. .. | *Biochem. J.,* 1934, **28**, 1907. |
| Quanjer | .. .. | *Meded Landbwhoogesch.,* Wageningen, 1913, **6**, 41. |
| Raistrick | .. .. | *Phil. Trans.,* **B220**, 153. |
| Raistrick, *et al.* | .. .. | *Phil. Trans.,* 1931, **B220**, 9. |
| Sastri | .. .. | *Proc. Ind. Sci. Cong.,* 1929, p. 242. |
| | .. .. | *J. Ind. Inst. Sci.,* 1929, **12A**, 25. |
| Sastri and Narayana | .. .. | *J. Ind. Inst. Sci.,* 1930, **13A**, 147. |
| Sastri and Sreenivasaya | .. | *J. Ind. Inst. Sci.,* 1929, **12A**, 233. |
| Sreenivasa Rau | .. .. | *J. Ind. Inst. Sci.,* 1933, **16A**, 91. |
| Sreenivasaya | .. .. | *Nature,* 1930, **126**, 438. |
| Sreenivasaya and Sastri | .. | *J. Ind. Inst. Sci.,* 1928, **11A**, 23. |
| ———— | .. .. | *J. Ind. Inst. Sci.,* 1929, **12A**, 239. |
| Stiles, Peterson and Fred. | .. | *J. Biol. Chem.,* 1925, **64**, 643. |