CONTRIBUTIONS TO THE STUDY OF SPIKE DISEASE OF SANDAL (*Santalum album* LINN.).

PART XVIII. SOME FACTORS RELATING TO THE ABNORMAL ACCUMULATION OF CARBOHYDRATES IN DISEASED TISSUES.

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Barber recorded that spiked sandal tissues were packed with starch, resulting from stagnation, induced primarily through defective functioning of the roots (*Indian Forester,* 1903, **29**, 21). According to Butler, this abnormal starch content of diseased leaves and twigs, combined with other morphological changes induced in them, was an indication of forced carbon accumulation by the affected tissues (*Indian Forester,* 1903, **29**, App. Ser.). Coleman confirmed the above observations (*Dep. Agric. Mysore State Mycol. Ser. Bull.* No. 3, 1917) and in a single case studied by him, it was noticed that spiked leaves contained twice as much starch as the healthy ones. A systematic examination of this question was made by the author under more comparable conditions and at different stages of disease (*This Journal,* 1928, **11A**, 93; 1929, **12A**, 295). It was shown there that the starch content of the diseased leaves was significantly high from the period of manifestation of disease and was found to increase with the progress of infection. This abnormality was observed irrespective of season, soil conditions and such other external factors.

Coleman (*loc. cit.*) observed that the diastatic activity of spiked leaves was about half that of the healthy ones and that starch was twice that of the latter. In other words, a reduced enzyme action was apparently responsible for the excess of starch, which was not readily converted into the more assimilable form of carbohydrates. Sastri and Sreenivasaya could not confirm Coleman's observations (*This Journal,* 1928, **11A**, 23). According to those authors, diseased leaf tissues and tissue fluids showed considerably higher amylase action than healthy ones. They could not, however, correlate the high amylolytic action with the presence of any accelerator or absence of inhibitors in the diseased portions (*Ibid.,* 1929, **12A**, 233). Sastri recorded at the same time a greater liquefying component of amylase in the affected tissues than the saccharifying one, while the reverse condition was true of the healthy plants (*Ibid.,* 1929, **12A**, 251). In spite of these interesting observations, the accumulation of starch in diseased tissues has not been explained on any rational basis. It was therefore considered necessary to re-investigate this problem with a view to elucidating the mechanism of excess starch formation. It was also equally important to account for the increase in sugar content of spiked tissues with the progress of the disease. Besides these, there were also other aspects which required careful investigation. The results of such a study are reported in the present communication.

EXPERIMENTAL.

Leaf specimens, both healthy and diseased, were obtained from different localities, such as Bangalore, Noganoor, Aiyur, and Jawalagiri in the manner detailed in an earlier communication (A. V. V., 1928). The collected material was transported to the laboratory in stoppered bottles and dried immediately at room temperature in desiccators over calcium chloride under vacuum. These were subsequently powdered and preserved in air-tight containers.

Diastatic activity of these specimens was determined by adding a weighed quantity of the powder to 40 c.c. of 2 per cent. starch (soluble starch according to Zalkowsky) solution in 100 c.c. flasks to which toluene was added and by incubating the contents at the room temperature (25–27° C.) for 24 hours. At the end of this period, the flask was heated on a boiling water-bath for 5 minutes to arrest enzyme action. The solution was filtered hot into a 100 c.c. measuring flask and washed with distilled water (40 c.c.). On cooling, dialysed iron was added as the clarifying agent, excess being removed by addition of potassium sulphate. The contents were finally brought up to 100 c.c. These were then, centrifuged immediately and the clear extracts filtered through dry filter-paper. Sugars were estimated in the filtrate, according to the method of Bertrand and computed as maltose.

Influence of temperature.—Though Coleman's early observations of reduced diastatic activity were contradicted by Sastri and Sreenivasaya, it is of interest to observe that the two sets of determinations were carried out at different temperatures. Thus, Coleman carried out all his studies at a temperature of 50° C. on the evidence of Green (Quoted by Coleman, *loc. cit.*, p. 32) according to whom this is the optimum temperature for translocation diastase. Sastri and Sreenivasaya, on the other hand, employed only room temperature not rising above 30° C. in their determinations. It became necessary therefore to examine whether these variations could adequately explain the difference in their observations. Diastatic activity was therefore estimated at room temperature (25–27° C.) and at 45° C. and 50° C. The results are given in Table I.

TABLE I.

Diastatic activity of healthy and diseased leaves at different temperatures.

Source of Material	Diastatic action (expressed as grams maltose) per gram of leaf powder					
	At 25–27° C.		At 45° C.		At 50° C.	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Bangalore	0·038	0·108	0·151	0·328	0·192	0·398
Ragihalli	0·104	0·484	0·529	0·986	0·588	1·084
Uttrahalli	0·139	0·608	0·588	1·749	0·606	1·968
Aiyur	0·204	0·742	0·714	1·982	0·764	2·147
Jawalagiri	0·084	0·347	0·282	0·995	0·314	1·125
Noganoor	0·130	0·542	0·671	1·188	0·698	1·336

It is clear that diseased leaves show higher enzyme activity at all temperatures. The effect of raising the temperature is more pronounced on healthy specimens than on the affected ones.

Tannins of healthy and spiked sandal and their influence on diastatic activity.—With a view to elucidating the relation of tannins present in the leaves to diastatic activity, the following experiments were carried out. Weighed amounts of healthy and spiked leaf powders were shaken with ethyl acetate for over 8 hours, filtered and washed with the solvent till no more colouring matter was extracted. The spiked leaf extracts were more coloured than the healthy ones. In both cases, the solvent was evaporated at a low temperature and the residue dried over calcium chloride. The tannin contents of the samples were also separately determined (*vide infra*). The percentage of material thus extracted by ethyl acetate is presented in Table II.

TABLE II.

Material extracted from air-dried sandal leaves by ethyl acetate.

(Expressed as per cent. on dry weight)

Source of material	Ethyl acetate extract of leaves	
	Healthy	Diseased
Bangalore	9·46	7·52
Noganoor	8·04	6·17
Aiyur	10·75	9·03

The coloured residues obtained by evaporating the solvent were subsequently shaken thoroughly with small amounts of water to extract the soluble portions (chiefly tannins) and the final volume made up to 20 c.c. The aqueous extracts so obtained with healthy and spiked leaf powders were divided into two equal portions. Each of these was added to starch solutions containing known quantities of the detannised powders (both spiked and healthy) in separate flasks and their diastatic activities determined in the usual manner at room temperature (Table III).

TABLE III.

Influence of ethyl acetate extracts of leaf powders on amylase action.

(Expressed as grams maltose per gram of leaf powder)
Temperature: 26–27° C.

Treatments	Diastatic activity of leaf powders obtained from					
	Noganoor		Bangalore		Jawalagiri	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Untreated (Control) ..	0·255	0·811	0·038	0·408	0·084	0·547
Residue after extraction with ethyl acetate	0·569	1·642	0·088	0·846	0·308	0·908
Do. ½ A ..	0·738	2·136	0·124	1·414	0·592	1·484
Do. ½ B ..	0·659	1·875	0·110	1·152	0·536	1·210

A = Extract from spiked leaf; B = Extract from healthy leaf.

It may be seen that the activity of the detannised residues was higher than that of the original leaf powders. Contrary to expectation, addition of extracts increased the activity, the effect being more pronounced in the case of diseased sample than in that of the healthy one.

The influence of temperature on the diastatic action of the treated powders was next determined. The results are reproduced in Table IV.

TABLE IV.

Influence of temperature on the diastatic activity of extracted specimens.

Source of material	Diastatic action (expressed as grams of maltose) of leaf powders determined at			
	25–26° C.		45° C.	
	Healthy	Diseased	Healthy	Diseased
Noganoor	0·569	1·642	1·838	2·348
Bangalore	0·288	1·210	1·042	1·804
Aiyur	0·384	1·126	1·140	1·982

As observed in the case of untreated powders, temperature exerts a great influence on healthy samples while it is not so effective on diseased ones.

The tannins were then estimated independently by the method of Menaul (*J. Agric. Res.*, 1923, **26**, 257). The results are reproduced below (Table V).

TABLE V.

Tannin contents of healthy and spiked leaves.

Source of material	Tannin as per cent. on dry weight		Ratio $\frac{\text{Spiked}}{\text{Healthy}}$
	Healthy	Spiked	
Bangalore	0·104	0·247	2·39
Noganoor	0·075	0·244	3·14
Jawalagiri	0·089	0·245	2·75
Aiyur	0·092	0·265	2·88

Spiked leaves are found to contain more than twice the quantity of tannin present in the corresponding healthy ones irrespective of locality.

In view of the high tannin values, it is rather difficult to explain the increased diastatic activity of the diseased specimens. Indeed, it is well known that tannins depress amylase action as shown by Brown and Morris (*J. Chem. Soc.*, 1893, **63**, 604). It was therefore of great importance to know whether the tannins present in the diseased specimens differed in any respect, from those in the controls. Aqueous extracts of the leaves were therefore tested with several reagents. The results are presented (Table VI).

TABLE VI.

Some reactions of sandal leaf extracts.

Reagent	Spiked leaf	Healthy leaf
Ferric chloride (strong) ..	Greenish brown	Brown
,, ,, (diluted) ..	Brownish green	Greenish brown
,, ,, + HCl ..	Tarnished brown	No change
,, ,, + Potassium citrate	Yellow	Light green
,, ,, (dilute) + Citric acid	Pink	Yellowish green precipitate
,, ,, ,, + Sulphuric acid	Blue	Greenish blue
Chromic acid	Red brown precipitate	Yellowish brown
Bromine water	Heavy yellow precipitate	Light yellow precipitate
Potassium ferricyanide + Ammonia	Red	Yellow
Sulphuric acid (concentrated)	Red ring	Dark red ; turns green on shaking
,, (on boiling) ..	Turns yellow green slowly	Turns green rapidly
Ferric alum	Dark blue, turns red with ammonia	Greenish black ; turns red with ammonia
Lead acetate	Precipitate becomes brown	Precipitate becomes white
Lime water	Light brown ppt.	Brown precipitate
Iodine with potassium iodide and ammonia	Deep red colour	Light brown colour
Ammonium molybdate ..	Red colour (no ppt.)	Red colour (yellow in transmitted light)
Fehling's solution	Reduction	Feeble reduction
Permanganate	Rapid discharge of colour	Colour slowly reduced

A study of the above would show that in sandal leaf, both pyrogallol and catechol types of tannins are present. With the onset of the disease, it would appear that the pyrogallol group of tannins is largely present while in the healthy condition the catechol type predominates.

Effect of added tannins on the diastatic activity of sandal leaf.— In view of the difference in the nature of tannins noted above, it was of considerable significance to determine whether by the addition of these classes of substances to the original and extracted leaf powders, their diastatic activities could be increased or decreased as the case may be. To this end, weighed quantities of catechin and pyrogallol were dissolved in water, made up to 100 c.c. and preserved under toluene. Aliquots of these were added to the different leaf powders and their diastatic activities estimated as before. The results are presented in Table VII.

TABLE VII.

Diastatic activity of leaf powders in presence of added tannins.

Treatment	Diastatic activity (in grams of maltose) per gram of dry leaf powder			
	Original		After extraction with ethyl acetate	
	Healthy	Diseased	Healthy	Diseased
Leaf powder (0·25 gram) + 10 c.c. water (Control) ..	0·445	1·018	0·569	1·642
Leaf powder (0·25 gram) + 10 c.c., 0·3 per cent. pyrocatechin	0·558	1·562	0·604	1·368
Leaf powder (0·25 gram) + 10 c.c., 0·3 per cent. pyrogallol	1·547	2·216	0·826	1·554

It will be clear from this that pyrogallol accelerates the diastatic activity of healthy leaves to a greater extent than that of the diseased ones. The untreated spiked leaves are also stimulated by the addition of both groups of tannins and particularly by pyrogallol. In the detannised residues this substance has a slightly inhibiting action on the spiked specimens while the corresponding healthy ones are activated by about 50 per cent. With pyrocatechin, this action is less pronounced.

Accumulation of soluble sugars in spiked leaves.—It has already been shown by the author (*This Journal,* 1929, **12A**, 295) that in partially diseased trees, the carbohydrate content of the affected leaves is generally low while starch is high. In an earlier communication (*Ibid.,* 1928, **11A**, 93) it was shown that in advanced stages of the disease spiked leaves contained more total soluble sugars as well as starch than the corresponding controls. It is rather difficult to explain these variations on the basis of increased enzyme activity. It was considered necessary therefore to re-examine this progressive increase of sugars with the advance of disease. To confirm this, some critical estimations of total sugars were carried out on leaves derived from plants at different stages of disease, with necessary controls. The specimens were collected from Jawalagiri (Table VIII).

TABLE VIII.

Soluble sugar content of sandal leaves at different stages of disease.

(Expressed as percentages on dry weight)

Condition of the plant	Reducing sugar as glucose	Total acid hydrolysable sugars as glucose
Healthy	0·018	0·108
Initial stage of spike ..	0·012	0·112
Fully spiked	0·082	0·664
Final stage of spike ..	0·120	0·826

The increase in soluble sugars with the advance of disease is really significant. This has been confirmed by analyses of specimens derived from other localities also.

No explanation has been found possible to account for this on the basis of amylase activity alone. Since carbohydrates in plants are utilised for the formation of proteins and fats, it was thought necessary to compare the fatty matter content of healthy and diseased leaves. The relation of nitrogen to health and disease will be considered elsewhere. Specimens collected and treated in the manner detailed in the earlier part of this paper were employed for this purpose. Weighed amounts of dry powders were extracted in a Soxhlet with petrol ether (light petroleum, 35–50° C.) and subsequently with ethyl ether. The extracts were separately evaporated to dryness in each case and the residues weighed. The results are given in Table IX.

TABLE IX.

Petroleum ether and ether extracts of healthy and spiked leaves.
(Expressed as per cents. on moisture-free material)

Source of material	Petrol ether extract		Ether extract	
	Healthy	Spiked*	Healthy	Spiked*
Bangalore	5·6	2·1	9·2	3·4
Noganoor	4·7	1·5	8·1	2·8
Aiyur	4·4	1·7	8·3	3·1
Denkanikota ..	5·3	2·2	9·1	3·3
Uttrahalli ..	3·7	1·4	8·2	2·9

* Advanced stage of spike.

While healthy specimens are characterised by a high fat content, diseased ones have comparatively low values which are even less than half of those of the controls.

It was thought best to examine the residues for free acid content. To this end, the extracts after evaporation and weighing were taken up in rectified alcohol and titrated against standard alkali with phenolphthalein as indicator (Table X).

TABLE X.

Free acidity of the extracts of sandal leaves.
(Expressed as c.c. N/10 alkali per 100 gms. of dry material)

Condition of plant	Source of material		
	Noganoor	Bangalore	Aiyur
Healthy ..	13·6	14·1	14·8
Spiked ..	18·8	21·4	24·8

The free acidity of the diseased tissues is greater than that of the healthy ones.

DISCUSSION.

The accumulation of starch is a typical biochemical symptom of virus infection in plants. It is also a recognised fact that both synthesis and degradation of starch through sugars are carried out in the green leaf through the action of diastase. Hence any abnormality in carbohydrate metabolism of plants should be traceable to a disturbed diastatic action. Thus in tobacco mosaic, Woods (*Centralblatt. Bakt.*, Abt. II, 1899, **5**, 745) ascribed starch accumulation to an inhibitory action of oxidases on diastase. It was Neger (*Ztschr. pflanzenkrank*, 1919, **29**, 27) who first observed a high diastatic action in potato leaf infected with Leaf-roll disease, besides an increase in starch. A similar observation has been made on other virus-diseased plants including sandal spike. An attempt is now made to offer an explanation for the abnormality noticed in spiked leaves.

The diminished activity of translocation diastase recorded by Coleman through experiments conducted at 50° C. on the evidence of Green (cited by Coleman) could not be confirmed in our experiments (*vide* Table I). The more significant fact observed therein relates to a differential action of temperature on healthy and diseased leaves. Thus with a rise in temperature of 20–25°, the ratio $\frac{\text{diastatic action per gram of diseased leaf}}{\text{diastatic action per gram of healthy leaf}}$ drops to less than 60 per cent. of the value at 25° C. In other words, a higher temperature has an apparently inhibitory action on diseased leaf enzyme.

The relation of tannins and tannin-like substances to health and disease was investigated. A study of the detannised leaf powders (with ethyl acetate) with and without the addition of aqueous extracts of ester-dissolved material has served to differentiate between healthy and diseased conditions. While the controls (*i.e.*, detannised powders) from both healthy and spiked leaves manifest higher diastatic action than the corresponding untreated ones, the addition of diseased leaf extract referred to above, to both healthy and affected control specimens (detannised) results in considerably accelerating the potency of these powders (*vide* Table III). On the other hand, a similar treatment or application with healthy leaf powder does not augment the activity so much. It would appear from this that spiked leaves contain an activator of diastase or that healthy ones contain more of a diastase inhibiting substance which gets probably diminished in amount or disappears with the onset of disease. An examination of the aqueous extract of the original leaf powders throws some light on the nature of tannins in them (Table VI). While other substances might interfere with or modify these reactions, the available evidence shows that the pyrogallol group of tannins appears to predominate in the diseased condition. On the contrary the catechol group is chiefly noticed in the healthy state. The darkening of the spiked leaf juice may then be due to this factor. In other words, pyrogallol group of tannins extractable by ethyl acetate can be considered as accelerators of diastatic activity in plant parts. Experiments with pyrogallol and pyrocatechin appear to support this view (Table VII). Thus pyrogallol enhances the activity of both healthy and spiked leaves, whether detannised or not. The comparatively feeble action with pyrocatechol on healthy samples cannot be easily accounted for. With detannised material, the pyrocatechin is a poor activator. It is of significance therefore to examine this question with pure diastase derived from different sources. Such a study is in progress. In view of the variations in the tannin content of leaves with the onset of spike, it is interesting to determine the conditions under which one type of tannins changes to the other. Again, whether the one or the other group arises in the process of defensive mechanism of the plant following infection, as shown by Cook and Taubenhaus (*Delaware Agr. Exptl. Stat.*, Bull. No. 97, 1912), is a problem which requires further investigation.

In spite of these observations the total tannin content of spiked leaves is more than twice that of healthy ones (Table V). On the other hand, ethyl acetate extracts of diseased specimens are less than those of the controls (Table II). It may be observed here that the water-soluble portion of this extract was more in the spiked leaves only. Hunger (*Bull. Inst. Buitenzorg*, 1903, **17**, 1) explained the low diastatic action in tobacco mosaic as being due to the presence of tannins. That tannins also play a part in oxidation processes has been shown by Rose (*Bot. Gaz.*, 1919, **67**, 105). In the present experiments, in presence of pyrogallol the reacting starch solution developed a dark colour with healthy and spiked leaf powders. It may be remarked here that the hydrolysed starch solution containing pyrogallol and healthy leaf powder was difficult to filter. The reaction with pyrocatechin was strikingly in contrast.

The cause of sugar accumulation.—The increased diastatic activity of spiked leaves may account for the starch storage noticed in them on the assumption that the reaction proceeds in the direction of synthesis of starch from sugars which should correspondingly be reduced in amount. While this is the case in the incipient stages of disease (A. V. V., *This Journal,* 1929, **12A**, 295) an abnormally high value for soluble carbohydrates is obtained with the progress of disease and in advanced stages of spike (Table VIII). It may be argued that the system $\text{Sugar} \underset{\text{(diastase)}}{\rightleftharpoons} \text{Starch}$ works as though in a 'fever' raising its equilibrium to a value out of proportion to the normal, a condition which should result in the death of the plant at once. The increased diastatic action appears to be due to the activators present rather than to a higher enzyme unit. If it is assumed that starch formation is only a mechanism on the part of the plant to diminish the osmotic action of sugars beyond a limit, the increase in the sugar content must be looked for elsewhere. The reduced fat content of spiked leaves forming as it does, only to the extent of 30–40 per cent. of healthy ones (Table IX)—both light petroleum and ether extracts—may account for the high sugar content, either through poor synthesis of fats from carbohydrates or through the conversion of fats already present into sugars. In the latter case, the free fatty acid liberated might serve as material for the high respiratory activity noticed in an earlier communication (A. V. V., *This Journal*, 1933, **16A**, pt. XII). In fact the free acidity is higher in the diseased condition (Table X).

The cause of starch accumulation.—While Quanjer (*Die Nekrose des Phlœms der Kartoffelpflanze die Ursache der Blatrollkrankheit Wageningen*, 1913) accounted for the storage of starch in potato Leaf-roll disease through defective translocation of starch arising from a necrosis of the phlœm tissues, Neger (*loc. cit.*) considered starch accumulation as being bound up with the mineral composition of diseased plants particularly lime, because "Für die Ableitung der Bildungstärke aus den Blättern scheint kalk von Grösser Bedeutung zu sein". According to Esmarch (*Ztschr. pflanzenkrank*, 1919, **29**, 1) storing of starch is due to little or not sufficient active diastase present in these tissues. In Tollenaar's view (*Omzettingen van koolhydraten in het Blad van Nicotiana tabacum Linn. H. Veenman and Zonen, Wageningen*, 1925) such storage in Tobacco mosaic arises in one of two ways: (*a*) the virus influences the reaction Starch $\rightleftharpoons$ Sugar, in the direction of starch synthesis, or (*b*) the virus arrests respiration which consequently impairs the breakdown of starch. Since respiration and starch derivation in the diseased tissues were considered to be normal, the conversion of sugars into starch was believed to result through a process of desiccation. In the case of spike a variety of factors are found to operate. A systematic microchemical examination of twigs and leaves at incipient stages of the disease reveals the interesting fact that the medullary rays of the twigs were first filled with starch, the ones farthest from the shoots having the greatest amount. On the other hand, in the corresponding leaves, the quantity of starch was considerably greater than that of the healthy ones although considerably less than that of spiked twigs. It may therefore be assumed that storing of starch in the cells commences from the lower portions and progresses upwards. With the advance of disease both leaves and twigs are found packed with starch. Thus, starch accumulation of spiked tissues is a progressive phenomenon independent of seasonal and local conditions which are on the contrary found to influence the composition of healthy ones. Diseased twigs cut and kept in darkness do not show even after 24 hours any derivation of starch. Thus, a primary defective translocation seems to be responsible for starch accumulation (A. V. V., D.Sc. Thesis, University of Madras, 1934).

Such defective translocation leading to starch storage ensues when calcium is not made available to the growing plant. This has been well substantiated by Boehm (*Sitz. Akad. Wiss. Wein,* 1875, 71, 287) and by Raumer and Kellermann (*Landw. Versuchsstat,* 1880, **25**, 25). Grüss observed that the hydrolytic action of diastase was ten times great in presence of calcium salts (*Wiss. Beilage. z. Jahresberichte d VIII. Stadt. Realschule, Berlin,* 1895). Schimper (*Flora,* 1890, 73, 207) recorded disease symptoms in plants growing without calcium besides an increased starch content in such tissues. In this case the inactivation of diastase was believed to be due to "Spaltungsprodukten" (sugars). In spike disease of sandal, the evidence so far obtained goes to show that lime is deficient in stems and leaves following the manifestation of disease symptoms. On the other hand, in predisposed plants an abnormal lime content (150 per cent. of that contained in the healthy ones) has been observed. The relation of lime to disease will be considered elsewhere. It appears possible to postulate tentatively, that barring infection the nature of which is yet obscure, the primary reaction is noticeable in the disturbed calcium metabolism of the plant.

SUMMARY AND CONCLUSIONS.

1. Spiked sandal leaves evinced higher diastatic activity at both high and low temperatures and also after being detannised with ethyl acetate.

2. Though the total quantity of material extracted from spiked specimens is less than that from the controls, the actual tannin content of diseased leaves is more than twice that of healthy ones.

3. The extract from affected specimens was also found to enhance amylase activity of both healthy and diseased detannised residues while similar extract of healthy ones was found to be poor in action.

4. Spiked leaves were found to consist largely of pyrogallol group of tannins, while healthy ones reacted chiefly to pyrocatechin class.

5. Pyrogallol was found to accelerate amylase activity of both the types of leaves, whether detannised or not. On the other hand, pyrocatechin showed a tendency to inhibit the diseased specimens more than it did the controls.

6. The high diastatic activity of spiked leaves results in the synthesis of more starch from the sugars present therein.

7. Although starch accumulation is evident from the period of manifestation of disease symptoms, sugars increase in concentration only in advanced stages of spike.

8. The mechanism of this increase of sugars is not through the degradation of starch which is also found to be stored in the tissues to an abnormal extent with the progress of disease, but they may arise from the decomposition of fatty material.

9. The total fat content of diseased leaves is considerably lower than that of the controls. Further, the free acidity of ether extracts was higher in the former.

10. Evidence is adduced to show that this starch accumulation commences from below and hence is due to defective translocation. Though infection is the primary cause of the disease, it would appear that the immediate effect would lie in the non-availability of calcium.

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