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

PART XI. New Methods of Disease Transmission and their Significance.

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In a previous communication (*J. Indian Inst. Sci.*, 1928, 11A, 244), it has been shown that spike can be transmitted to healthy sandals by budding. The process is, however, rather a troublesome one to conduct and the percentage of buds which grow is very low. Further experiments have now led to the discovery that the disease may be transmitted with much greater certainty by the process of 'patch-grafting' and still more readily by 'leaf insertion.' The latter operation is particularly effective as well as simple to conduct and promises to be of very great value in future investigations.

I. *Transmission by 'Patch-grafting.'*—In the course of several buddings carried out in the laboratory, it was found that some of the stocks operated upon succumbed to the disease although the bud did not grow. On examination, the patch was found to have remained green and to have become intimately fused with the stock, so that it was evidently responsible for transmitting the infection. This observation suggested that an ordinary patch of bark tissue including the cortex and the bast which can easily be skinned from a scion, might, on grafting, induce the disease in healthy stocks. A number of healthy sandals were subjected to this 'patch-grafting' employing three kinds of patches, (1) those with active buds, (2) those with dormant buds and (3) those without buds.

It will be seen from table I, that patches bearing actively growing buds are more virulent and transmit the disease to healthy stocks in a much shorter time than those with dormant buds. Patches completely free from buds are less infective than the other two varieties as shewn by the longer periods taken by the bud-free patches to induce the disease. This confirms the view generally held that young and actively growing tissues are most favourable for the multiplication of the virus.

TABLE I.

Results of patch-grafting under controlled laboratory conditions.

(Sandals nurtured by *Acacia farnesiana* were used as stocks for all the experiments.)

Infective material	Number of stocks treated.	Percentage of fusions	Percentage of transmissions	Minimum period for manifestation of disease symptoms (days)
Active buds ...	29	88·0*	27·5	57
Bud-bearing patches.	35	93·5	11·4	98
Bud-free patches ...	33	9·6	9·1	158

The method of patch-grafting was also tested as regards its capability for inducing disease in stocks growing under sylvicultural conditions. The operation was conducted at two places representing two distinct types of forest. The results are summarised in table II.

TABLE II.

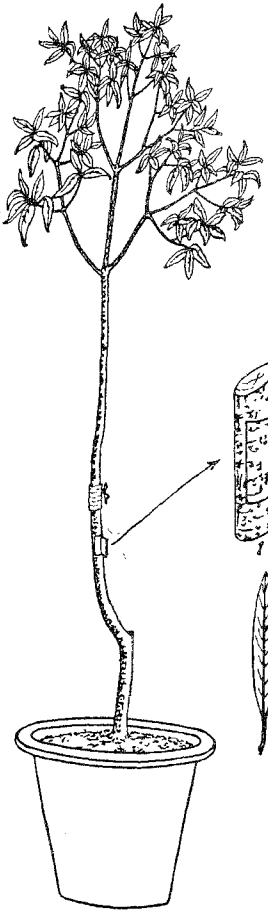
Results of patch-grafting under sylvicultural conditions in Coorg and North Salem.

Area	Kind of Patch-graft	Number of stocks treated	Precentage of fusions	Percentage of disease transmission within 120 days
Coorg	Healthy ...	34	97	6
	Spike ...	39	95	44
North Salem	Healthy ...	19	100	10
	Spike ...	20	90	65

60 trees in Coorg and 39 in North Salem were kept under observation as controls. 10 per cent. of the former and 5 per cent. of the latter became infected from natural causes during the above-mentioned period.

It is clear that a remarkably high percentage of successful fusions can be obtained by this method of disease transmission even in

* The bud grew only in 24 per cent. of these cases.



Transmission by "leaf insertion"

Healthy Stock

1. Incision to Skin out the bark
2. Bank flap opened
3. Leaf inserted
4. Bank flap closed
5. Bandaged
6. Spike leaf
7. Spike leaf trimmed for insertion

sylvicultural conditions which would ordinarily prove fatal to scion grafting and budding operations. The higher percentage of disease transmissions obtained in the North Salem area indicates that the trees in that region are highly susceptible to disease. Sandals in the Coorg area are less susceptible, while the pot cultured plants in the laboratory exhibit a high degree of resistance as shown by table I.

The advantages claimed for this new method of disease transmission are :—(1) the operation is extremely simple, in marked contrast to the delicate and difficult technique of budding; (2) the method lends itself to quantitative treatment as the infective patches can be either measured or weighed; (3) weather conditions do not affect the success of the operation; high percentage transmissions have been obtained in the hottest months of the year; (4) the operation can be successfully applied to infect stocks growing under sylvicultural conditions; (5) there is no need for the buds to burst from the scion which is essential for a successful budding operation; and (6) controls can be run with uninfected patches.

II. *Transmission by 'leaf insertion'*.—It has been shown that the biochemical changes occurring in the plant with the onset of disease, manifest themselves in the leaf tissue in a more intensive and pronounced manner than in any other part of the plant. It appeared likely therefore, that the diseased leaf might be a seat of high virus concentration. In order to test this hypothesis fresh diseased leaves trimmed to the form of a rectangle, were inserted between the wood and the bark of the stem (see plate I) covered with the bark flap and bandaged with wax cloth. If the operation was successful, the injured portion fused by callous formation in the course of 10 to 15 days and the leaf tissue became completely covered.

Under normal conditions, it was found that the disease manifested itself in the course of 45 to 60 days but the period varied widely with the number of leaves inserted, the size of the stock and the individuality of the plant.

Of the fourteen stocks operated upon by this method, ten, or 72 per cent., have already succumbed to the disease, a very high proportion for pot-cultured stocks which, as already pointed out, are more resistant than those growing under natural conditions. This supports the theory of the high concentration of the infective principle in the leaf.

Leaf insertion thus affords a very convenient method of disease transmission. The operation is simple and a large number of leaves can be inserted without seriously injuring the stock, while the percentage

of effective transmissions is very high and the period of infection can be reduced by increasing the number of leaf insertions. The method lends itself to quantitative control because the leaves can be weighed and their areas measured. Moreover, controls may be run by inserting healthy leaves.

III. Transmission by other methods

(*a*) *Patch-grafting of root bark on root.*—The tissue fuses with the stock quite readily but up till now (16 months after the grafting operation), no positive results have been obtained. This indicates that either the root bark does not contain the infective principle or the root is not a region susceptible to infection.

(*b*) *Leaf mutilation.*—Diseased leaves were put on the healthy leaves of a healthy stock and crushed together so as to ensure an exchange of sap between them. No disease transmission has resulted during a period of 19 months. This points to the conclusion that the leaf is not a vulnerable region and confirms the observation that with an abundance of green foliage, an infected stock tends to resist the disease. (*Cf.* Sreenivasaya, *Proc. Indian Science Congress, Section of Botany*, 1930.)

(*c*) *Wood grafting.*—Wood from young diseased scions was taken after removing the bark and carefully inserted into the healthy sandal in place of a corresponding piece which was cut from the stock. The bark flap was then bandaged with wax cloth. Fusion of the bark took place in the course of three weeks.

The operation is a difficult one to conduct but in no case in which it was successful has the disease been transmitted, although the experiments were started in January 1928. The wood tissue consequently does not appear to be infectious, particularly as the corresponding bark tissue produced symptoms of the disease in another batch of stocks in the course of four months.

(*d*) *Transmission by injection of tissue fluids.*—Attempts to induce the disease by injecting stocks with the tissue fluids derived from various kinds of diseased tissues were not successful. Difficulty was found in injecting the juice into young, healthy, pot-cultured stocks as the tissues are too hard to pierce with a hypodermic needle. The method finally adopted was to insert filter papers freely impregnated with the tissue fluid under the bark in the same way as a leaf in the operation of patch-grafting. The fusions were successful but symptoms of disease have not appeared on any of the stocks which were operated upon in June 1929. The tissue fluids from the leaf,

bark and root have been tried but in no case there has been any disease transmission.

The infective principle may become neutralised by other cell contents or rendered innocuous in some way during the operation of extraction. Support is lent to this view by the fact that if the leaf tissue is inserted after fine pulping, it does not transmit the disease so that the mere disintegration of the cells appears to lead to a destruction or neutralisation of the infective agent. Attempts are now being made to isolate the virus without damaging the leaf by methods involving adsorption and cataphoresis.

In the course of these experiments, indications have been obtained that several varieties of sandal exist, differing in their disease-resisting properties. In particular, one very resistant variety appears to be characterised morphologically by a definitely ovate leaf, a stem rich in lenticels and a root with high haustorising capacity. Attempts are now being made to classify other varieties.

SUMMARY.

Several new methods of disease transmission have been described, of which 'patch-grafting' and 'leaf insertion' are the most successful.

Although the transmission of disease has not been so far secured by the other methods, they have led to important conclusions as to the distribution of the infective principle in the plant and the susceptibility to infection of certain tissues in different regions of the plant.

The experiments strongly support the view that laboratory pot-cultured plants are more resistant to disease, than those growing under natural conditions.

Some evidence has been obtained regarding the existence of varieties of sandal differing in their disease resisting properties.

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