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

Part V.—Transmission of Spike by Budding.

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The development of a suitable and reproducible technique for artificial transmission of the disease usually forms an important step in the investigation of infectious diseases of plants. Virus diseases have been communicated to healthy stocks in various ways depending upon the virulence of the virus. In the case of several mosaic diseases transmission can be effected through mere pin pricks with an infected needle, while in other cases a hypodermic injection of the infectious sap, or a transplantation of the diseased tissue on to the susceptible region of the healthy stock is necessary to induce the disease. In yet other cases, an actual grafting or budding of the diseased tissue on to the healthy stock may be necessary. Coleman has transmitted spike disease of sandal to healthy stocks through grafting. According to him an intimate organic connection of the tissues is necessary for the disease transference to occur. In his Bulletin (Spike Disease of Sandal, Bulletin No. 3, 1917, Mycological Series, Department of Agriculture, Mysore), he does not, however, mention the percentage of success he was able to achieve by his method of grafting nor does he refer to the behaviour of any controls that may have been run. His experiments appear therefore to be mainly qualitative in character and the effect of hosts on the susceptibility of sandals to graft-infection is not considered. The graft-infections were carried out on stocks growing under natural, i.e., uncontrolled conditions in the soil.

With regard to budding, however, he says, 'Butler, in 1904, following the work of Smith on "Peach Yellows" attempted to produce the disease by budding. For this purpose he took buds from diseased trees and grafted them on to healthy ones. Although he tried some one hundred and fifty budding experiments, none of the buds grew, so that the results of the experiments gave no information of value. In 1914, similar experiments were commenced by us in Bangalore, but it was soon found that budding was not a suitable method of grafting for sandal as only one of the buds formed in connection with the healthy stock and even this one died soon after.' So far the disease has not been communicated by an injection of the maceration extract of the diseased tissues.

Results of experiments on the artificial transmission of spike by budding are recorded in this paper.

EXPERIMENTAL.

The experimental stocks were raised from seeds collected from one of the plantations near Chickballapur. *Acacia farnesiana*, nine months old was the host provided to the sandal after about eight weeks' growth. The plants were grown in a cast iron pot with an internal diameter of 85 cms. and a depth of 40 cms. (see Plate I). The soil consisted of equal parts of red-earth, sand and well-rotted horse manure.

Buds were derived from a scion brought on 13th February, 1928 from the diseased portion of a partially spiked plant in the Uttarahalli area. This plant showed the external symptoms of spike about the middle of February. The scion was about 1 cm. in diameter and 30 cms. long. The scion was first used for a bottle-graft on a different stock and in the course of four days commenced to send forth the dormant buds on both sides of the point of grafting. On 20th February, one of the buds below the grafting point was taken and budded on to one of the lower branches of the experimental stock. The usual mixture of clay and cowdung was not used to dress the wound, but only sterilised wax cloth. The terminal foliage of the branch was cut down while the rest of the plant was left undisturbed.

Four days after the operation, the bud burst and measured 1·5 mm. in height. Subsequently it began to make slow growth. The following are the dates on which various operations were carried out :—

Seed sown	7—3—1927
Germination occurred ...	6—4—1927
Host given	30—4—1927
Transplanted	1—9—1927
Budded	20—2—1928
First photograph taken	20—4—1928
Symptoms of spike observed on stock ...	1—7—1928

As a control, a healthy bud was grafted upon a healthy stock, the same technique being employed. The bud was derived from a healthy plant brought from the Uttarahalli area which was planted in mixed earth in a pot. Six days after, the dormant buds began to burst from several portions of the plant. One of these was used for the budding experiment.

The stock was a comparatively small one growing in association with an *Acacia farnesiana* plant in a flower pot. Budding was carried

out on 26th April, 1928 and the first photograph taken on 19th May, 1928. The period under experiment had the following average values for temperature and humidity in the greenhouse where all the buddings were conducted.

1928	Temperature, F.	Humidity, per cent.
February ...	79·6	54·8
March ...	81·8	44·6
April ...	85·0	53·0
May ...	...	...
June ...	78·6	66·0

Percentage of Success.—In the case of spiked buds about 10 per cent. succeeded while in the case of healthy specimens the figure was only 8 per cent. The number of spiked buds grafted was 79 and healthy buds 75. The dry heat during the early part of the period under experiment was chiefly responsible for the high mortality of the buds concerned. There is no doubt that with the advance of the season, the percentage of success will increase.

Type of Growth.—The growth of a spiked bud is entirely different from the corresponding growth of a healthy bud. The rate of growth in the case of spike is much slower than with a healthy bud. Plate V clearly shows the difference between a spiked bud which has grown for one hundred and twenty-eight days and a healthy natural bud allowed to grow for only 10 days.

The healthy leaves are broad and light green, internodes longer, and the healthy shoot does not show any tendency to branch off laterally. The growth of the spiked bud on the other hand, is quite characteristic. The leaves are small and deep green ; the internodes are very short and the branching is proliferous, imparting a typical bushy appearance to the entire growth (see Plate V). The spiked shoot may also be compared with a healthy shoot budded on another stock (see plate VII). This represents a growth of only 68 days.

Transmission of the Infective Principle to the Stock.

The natural bud on the branch of the stock used for budding, which had been allowed to grow since 5th June, 1928, ceased growing after a fortnight. On 1st July the internodal buds began to burst, though the apical bud did not grow any further. The pale green

colour of the leaves on the shoot intensified to a dark green during the course of the week, and on 7th July the characteristic growth of spiked leaves was first observed, i.e., one hundred and thirty-one days after the bud was inserted. During the weeks that have followed the spike has taken full possession of the shoot (see Plate VIII) and thus the original suspicion has been fully confirmed. It is possible that infection might have been observed at an earlier date if the normal shoot had been allowed to grow earlier than 5th June.

SUMMARY.

Buds derived from spiked stems can be successfully budded on to healthy stocks growing in pots under controlled conditions.

This method provides us with a technique by which the transmission of spike can be effected in young sandals 6–9 months old. Controls can also be run with equal success.

The stock under experiment caught the infection 131 days after the spiked bud was inserted in the stock.

The susceptibility of sandals growing under different controlled conditions to bud infection is being investigated.

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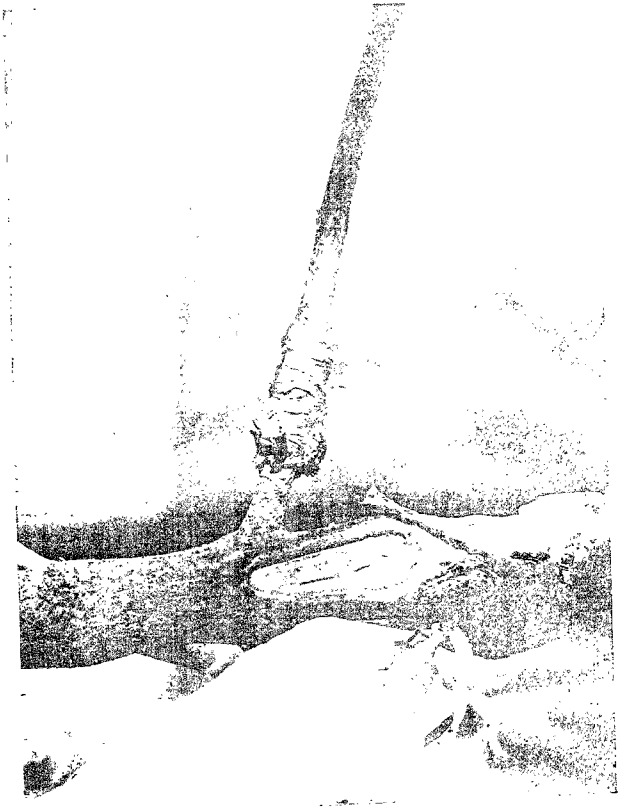
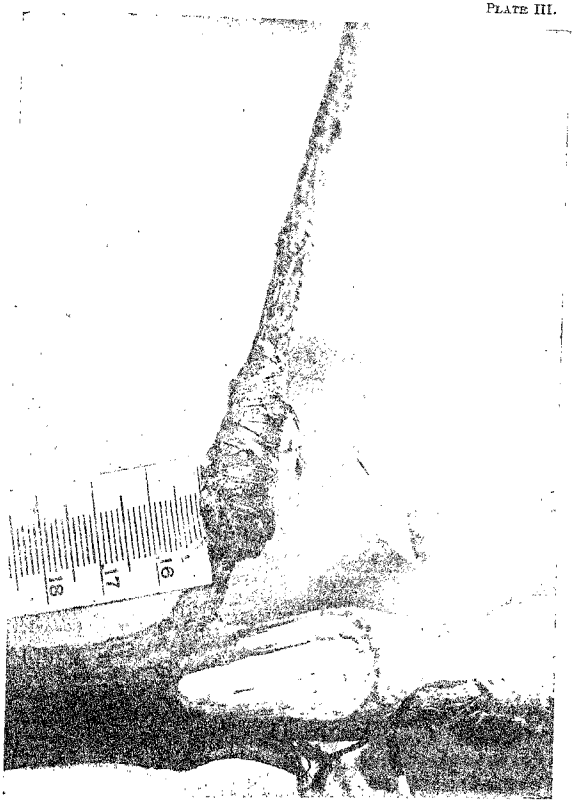
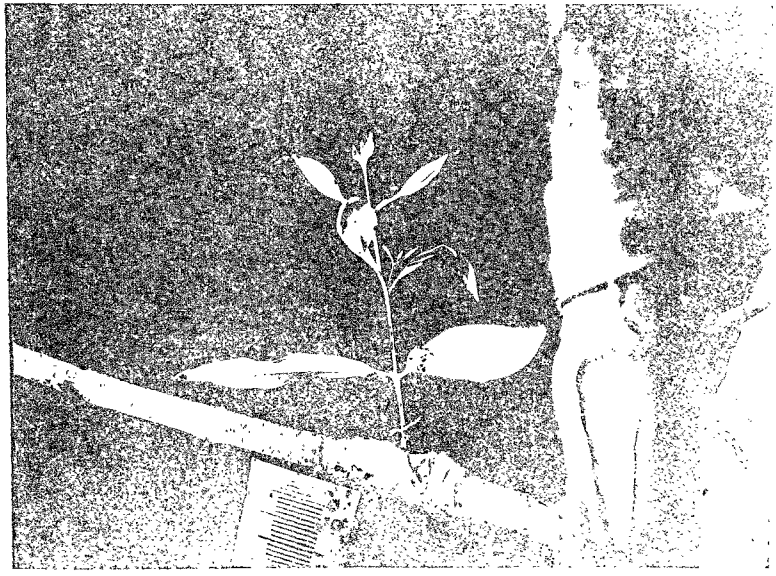
The experimental stock growing with *Acacia farnesiana* as host. Observe the buds, healthy and spiked, enclosed in the marked rectangle and marked A and B.



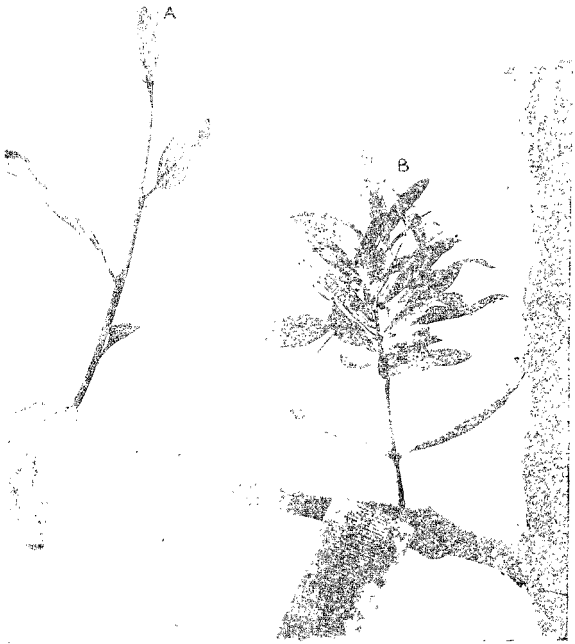
Spiked bud, two months after grafting.



The spiked bud at a later stage, three weeks later than in Plate II.

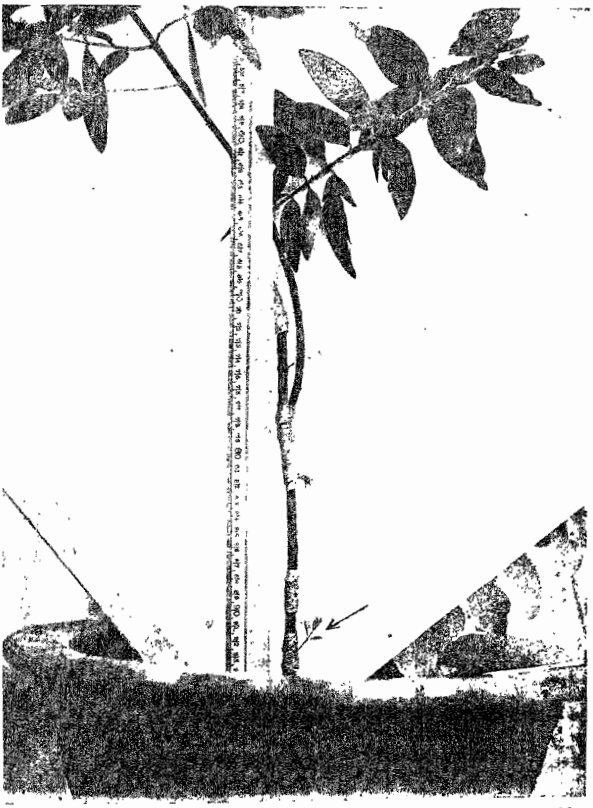


The spiked bud, three months after grafting.

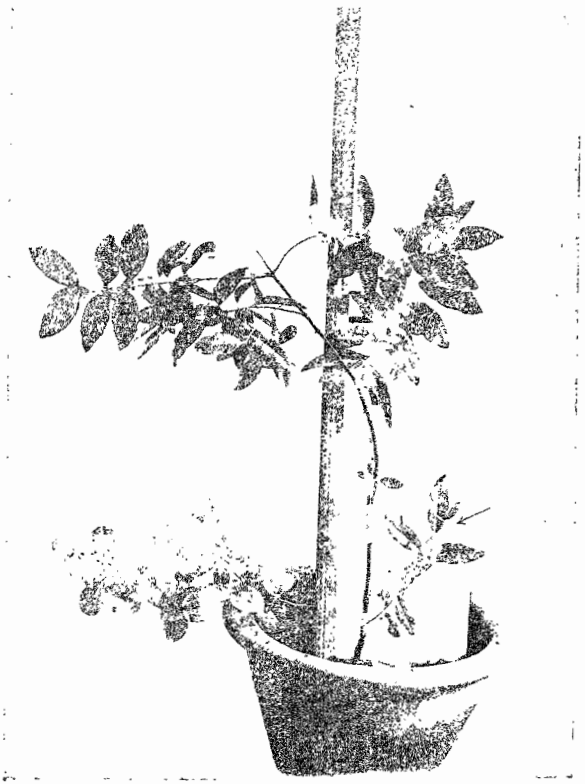


A healthy natural bud A after ten days' growth and the grafted spiked bud B after 128 days growth.

PLATE VI.



Control experiment with a healthy bud. Photographed three weeks after grafting.



Control experiment. The healthy bud 2½ months after grafting.

Compare Plate V. The normal bud A has now become infected.