SAP TRANSFUSION, A NEW DEVISE FOR VIRUS TRANSMISSION TRIALS IN PALMS

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THE Wilt or the 'Root disease' of coconut in India, the 'Kadang-Kadang disease' in the Philippines, the 'Unknown disease' in Jamaica and other similar diseases have been responsible for heavy reduction in coconut yields. Some of these diseases are suspected to be of virus origin, though none as yet has been authentically proved to be a virus disease. Investigations on the virus aspect of coconut diseases have been rendered difficult by the perennial nature of the crop and its large size which make it a difficult crop to work with under controlled conditions.

Although transmission by grafting is a sure test for most virus diseases, the application of this method in coconut is not likely to be practicable since it is a monocot. The inability to establish grafts in coconut has necessitated the adoption of some new techniques as substitutes for grafting. These methods particularly suited for coconuts, but applicable to other palms as well were devised by one of the authors (T. A. Davis) and are described in this paper.

The Wilt Disease of Coconut

The Wilt or the 'Root disease' in India is the major disease affecting an area of about 1,500 square miles in the central part of Travancore-Cochin State. Menon and Nair (1949, 1951) assessed the loss and have described the symptomatology of the disease. The characteristic symptoms of the disease are, a general wilting of the leaves, flaccidity and ribbing of leaflets and necrosis of leaf-tips. In many cases a general chlorosis is also prevalent on the older leaves. On close examination of the inner leaves, in most of the diseased palms, the leaflets show a large number of chlorotic streaks which later become brownish and necrotic. As the disease advances, the whole crown gets narrowed due to a reduction in the number and size of the leaves. Shedding of immature nuts, if the tree is bearing at the time of infection, is an initial symptom in some cases. The vitality of the reproductive system is affected, with the result that the diseased tree produces smaller spathes with fewer female flowers and consequently smaller number of nuts. The quality of the nuts in every respect deteriorates. In an advanced stage, the crown gets very much reduced in size, ceases to produce any flower-bunch and finally succumbs to the disease.

The root system of the affected palms manifests a characteristic deterioration. 'The cortex develops a deep brown discoloration and dries up in flakes' (Menon and Nair). Most of the rootlets and the main roots start dying from their tips backwards. Cracks, blotches, etc. are also visible on older regions and the entire root ceases to function and crumbles. In some diseased trees, however, even at a very advanced stage of infection the roots remain apparently

healthy, but their absorbing region gets covered with a hard hypodermis revealing a cessation of normal functions. As the disease advances the production of new roots decreases.

Virus Nature of the Wilt Disease

From the foregoing it would appear that the disease is systemic in nature like most of the virus diseases. The universal symptom of viroses, that is, a reduction in the size of the affected plant as well as reduction in its reproductive capacity are fully met with in the diseased coconut palms. The chlorosis and necrosis of the foliage and the necrosis of the root are also suggestive of a virus disease. There is an accumulation of major nutrients in the diseased leaves (Sankarasubramony et al, 1952). This may be due to necrosis or blockage of vascular tissues (phloem necrosis is a characteristic symptom of virus diseases like potato leaf-roll, bunchy-top of banana, etc.). The Wilt disease has been spreading and appears to be infectious.

Some diseases in other coconut growing areas of the world, which are similar to the Wilt disease in India have also been suspected to be of virus origin. Of these the 'Kadang-Kadang disease' in the Philippines is very much similar to the Wilt disease, the major difference being that the former is more virulent and brings death to the affected palms within a shorter time (Ocfemia, 1937 and De Leon, 1951 and 1952). Ocfemia suspected the 'Kadang-Kadang' to be a virus disease. Celino (1947) reported that he transmitted the disease to coconut seedlings by pin-prick inoculation. The 'Unknown disease' of coconut in Jamaica has also been suspected to be of virus origin (Martyn, 1945). In order to investigate the possible virus nature of the Wilt disease of coconut, transmission trials are being conducted at the Central Coconut Research Station, Kayangulam, S. India.

Bawden (1950) classifies the various methods of transmission of virus diseases into three large groups:—(1) Inoculation of plant extracts, (2) Insect transmission and (3) Grafting and the use of dodder.

1. *Inoculation of plant extracts.*—Under this group are included the carborundum method of Rawlins and Tompkins (1936) and the pin-prick inoculation method of Sein (1930). These methods are being tried here and the results are awaited. However, the Wilt may be among the virus diseases that are not transmissible by inoculation of plant extracts, which may be either due to a low concentration of the virus in the plant sap or due to the presence of certain substances in the sap that inhibit infection or inactivate the virus.

2. *Insect transmission.*—This is the most common method of transmission in nature. However, for certain virus diseases no vectors have as yet been found. In such cases, grafting seems to be the only method of transmission.

3. *Grafting and the use of dodder.*—The use of dodder for transmitting virus diseases is of recent origin, first reported by Bennet (1940) and Johnson (1941). This method, however, does not seem to be applicable to coconut as preliminary attempts to parasitise *Cassytha Sp.* and dodder on coconut have failed.

Various methods of grafting such as wedge or cleft-grafting, inarching, bottle-grafting, core-grafting, budding, etc. have been utilised for virus transmission in plants. Grafting is a sure method of transmission for, 'once union occurs between stock and scion, transmission is an inevitable sequel to the ability of the virus to move through vegetative tissues',

(Bawden). However, this universal method of transmission is not likely to be successful in coconut, although Munzik and La Rue (1952) state that grafting is not impossible in some large monocots such as Sugarcane, Maize, etc.

The Wilt or 'Root disease' has been named so because of the pronounced symptoms manifested in the root system of the affected palms and so it is possible that the causal virus may be present in the roots. Even if the virus is present only in the aerial parts but is capable of getting into the system through the roots, as has been shown by Roberts (1950) to be the case with potato virus X, these methods described below will be effective.

Sap Transfusion

Effecting transfusion.—When a functional coconut root is cut off obliquely at a convenient distance from the bole, and the two cut portions are connected by means of tight rubber tubing filled with water, a continuous flow of sap from the lower to the upper portion is still maintained. The lower part of the root, though cut off absorbs soil moisture normally and pumps it up into the connecting tube by root pressure; the pumped up sap is sucked in by the upper part most probably by the transpiration current. This pumping and sucking by the cut roots continue for a considerably long time. Even if such connections are effected between two different roots of the same palm or of two different palms, the connections continue to function as in the previous case. This interesting phenomenon has been made use of in devising the sap transfusion method for virus transmission.

For the purpose of establishing these direct root connections between diseased and healthy coconut palms, pairs of palms (one diseased and the other healthy) growing side by side, within about twenty or twenty-five feet, are selected. Fig. I shows in the foreground a pair of such palms, H and D. The diseased palm D is about forty years and the healthy about thirty years old. The soil between them is excavated to expose some roots that

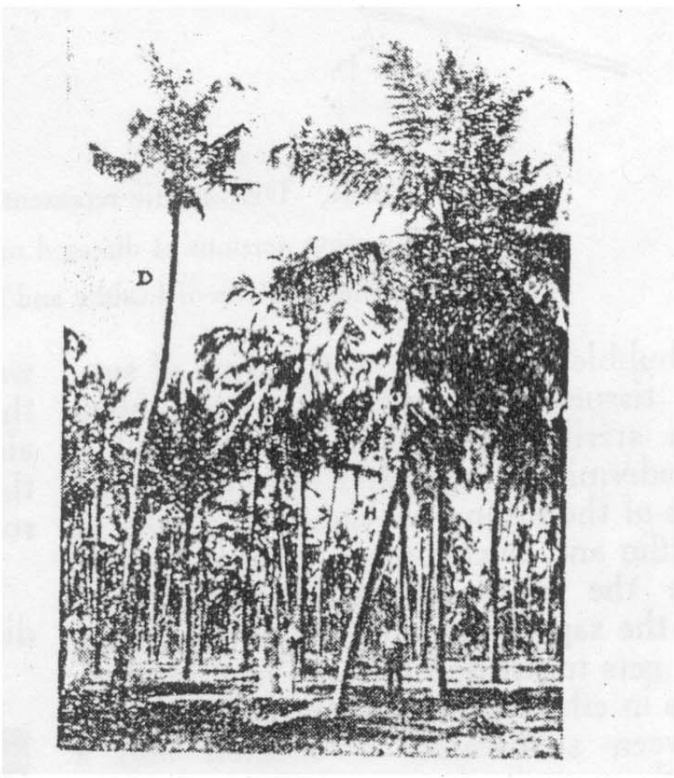

Fig. I. A pair of palms, one healthy (H) and the other diseased (D).

grow almost parallel to each other. Convenient pairs of roots, each pair consisting of a root from each tree, are selected and connections effected between them. As shown in Fig. II, (p. 94) connection A, the roots D 1 and H 1 form a convenient pair. They are cut at a common place and the upper end in contact with the bole of the tree H, is connected to the farther end of the root D, by means of sterilised rubber tubing. The cutting of the roots is effected under water to avoid letting in

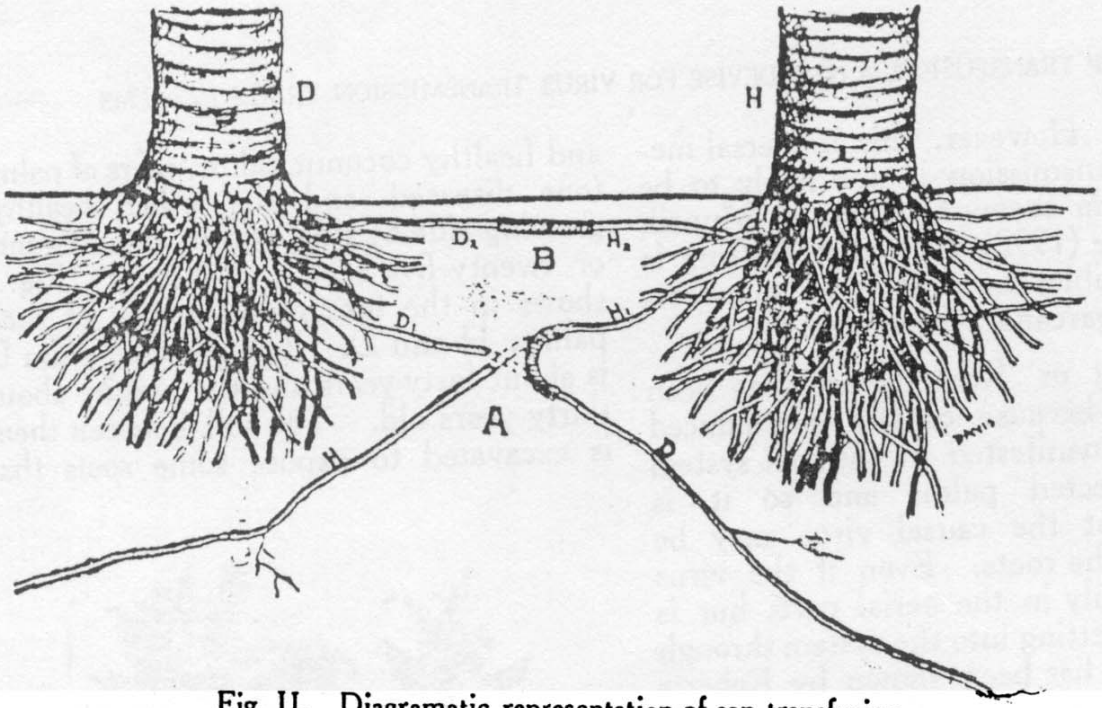

Fig. II. Diagramatic representation of sap transfusion.

A. Pumping portions of diseased root connected to sucking portion.

B. Sucking portions of healthy and diseased roots connected.

air bubbles and to prevent drying of surface tissues. The rubber tubing is filled with sterile water with the help of a hypodermic syringe after making the two ends of the tubing air tight by means of paraffin and or collodion. Within a short time the connection starts functioning and the sap pumped up by the diseased root gets transfused into the healthy tree. This in effect simulates an organic union between a diseased root scion and a healthy root stock.

In order to ascertain whether a connection is functioning or not, a glass tube, preferably a 'T' tube, is intercepted in the connecting rubber tubing. The limb L of the 'T' tube is again connected to a rubber tubing which is fitted with a pinch-cock as shown in Fig. III. Through this limb water is poured into the brim and the pinch-cock closed. An air bubble is let in at the centre of the glass tube by means of a hypodermic syringe, the upward movement of which indicates that the connection is properly working. The air bubble is removed before it reaches the end of the severed tip of the healthy root.

The sap transfusion method ensures a direct entry of sap from living diseased

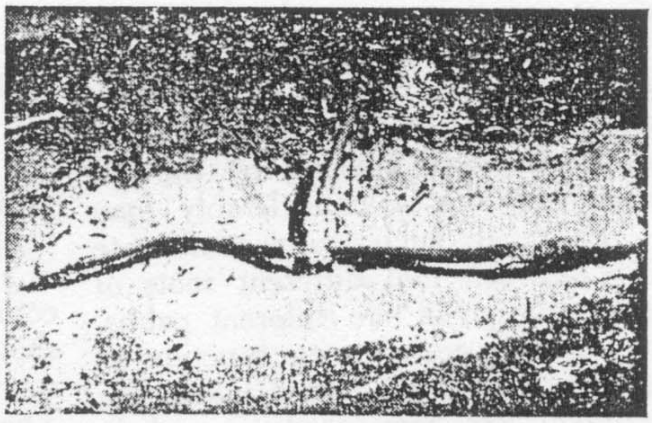

Fig. III. A root connection with a T. tube inserted.

tissues into the system of a healthy tree. Since the transference of sap is direct, crushing of tissues which might cause inactivation of the virus is avoided. The tissues of coconut have a high tannin content. Interaction of free tannin with a virus might cause inactivation as has been shown to be the case with tobacco mosaic virus in strawberry leaf extracts (Bawden and Kleczkowski, 1945). As the entire transfusion system is made air-tight and covered with soil to keep away from light, the adverse effects of atmospheric agents are prevented.

Some viruses are restricted to the phloem tissues and are picked up only by insects that feed on these tissues (Cook, 1947). Since in the transfusion method the diseased sap is brought in contact with all the tissues of the healthy root, unlike the condition in root inoculation by abrasion wherein only the cortical tissues receive the inoculum, even viruses that inhabit the phloem can be expected to be transmitted, other conditions being favourable.

Apart from the type of connection mentioned above, wherein the sap pumped up by a severed diseased root is transfused into a healthy tree through its roots, there is another way of transfusing sap from a diseased to a healthy tree through roots. A pair of roots (one from a healthy tree and the other from a diseased one) are selected as in the previous case and are cut at convenient distances from their boles. The upper end of root D2 (connection B of Fig. II) is connected to the upper end of the cut root H2 with rubber and glass tubings as already described. The connections are effected under aseptic conditions and made air-tight by sealing with paraffin and or collodion.

These cut roots are devoid of root pressure and are governed only by the upward suction from the crown. Thus the suctional pressures of the roots D2 and H2 work along opposite directions. It has been observed that the suctional pressure of a healthy tree is greater than that of a diseased tree. Hence the root H2 overcomes the pressure of root D2 and thereby a slow movement of sap from D2 to H2 is established. Further, the phloem, as well as the Xylem (Strasburger, 1891 and Dixon, 1914, 1924) can conduct sap downwards and this downward flow in the root D2 could also be sucked up by the root H2.

This method of transfusion, however, does not in practice work very satisfactorily, since, often the roots chosen do not possess a workable difference in suctional powers. Anyhow, for virus transmission even a small amount of sap absorbed by the root of the healthy tree from the diseased root will be sufficient. Even if the virus is present only in the phloem in which it moves downwards, its entry into the healthy tree is secured by this method.

Feeding Infective Extracts through Roots

The sap transfusion method described above is workable only when both the trees (healthy and diseased) are standing close to each other. Further, if the virus is present only in the aerial parts, its entry into the healthy tree cannot be secured by this method. Also, infective extracts from hosts other than coconut cannot be administered. Hence for these purposes, feeding of extracts through roots was thought of.

Feeding through root tips.—In older coconut palms it may be rather difficult to reach the tips of roots due to their

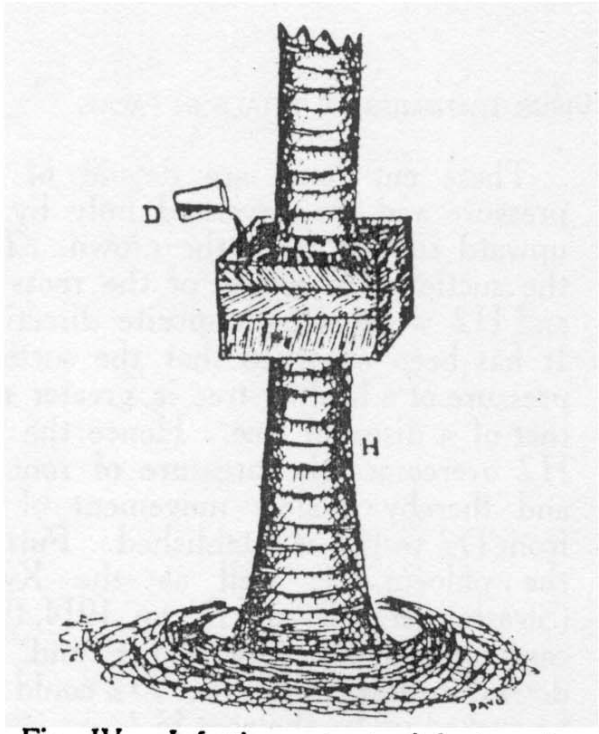

Fig. IV. Infective extracts fed through root-tips induced in a wooden box.

(roots') length which has been found to extend to even above seventy-five feet. For the sake of convenience, therefore, roots are induced from aerial portions of the stem into wooden boxes filled with soil-manure mixture and the extracts can be fed through unsevered root-tips.

It was observed that the unsevered root-tip has a low absorptive power. Further since it is selective in absorption, all the substances in the extracts, for instance the nucleo-proteins, may not be taken in. But, a cut mature root has been found to suck in solutions of even high concentrations and does not exercise selective action on substances.

Feeding through cut ends of roots.— Injection through roots appear to have been first tried by Goff (1897) for injecting certain solutions into freshly transplanted trees. Brooks and Bailey (1919) adopted Goff's method for injecting fungicides into plum trees. Systemic insecticides were administered into plants through cut tap-roots by David and Gardiner (1951). Roberts (1950) has used the method of root injections for injecting infective extracts from aerial portions of plants infected with potato virus X. The cut root method of injection is in use at Central Coconut Research Station, Kayangulam, for injecting certain curative substances into coconut trees (Davis *et al*, 1954).

The method of feeding through cut roots is simple. A mature functioning root of a healthy tree is cut at a convenient distance from the bole and the upper cut end is connected to a rubber tubing which in turn is connected to a graduated cylinder (as shown in the Fig. V) in which is kept the extracts to be fed. The extract is rapidly sucked in. This method, apart from the advantages already mentioned, is of help in feeding the infective extracts to seedlings of coconut and other palms kept in an insect-proof greenhouse.

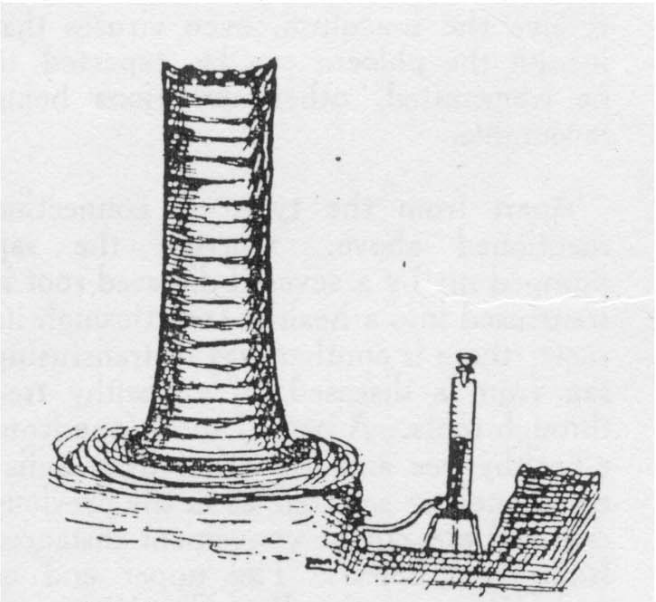

Fig. V. Injection of infective extracts through cut-roots.

Summary

The symptomatology of the Wilt disease of coconuts in India has been briefly discussed, a study of which points to the possibility of this being a virus disease.

Preliminary attempts at grafting in coconut failed. As a substitute to grafting a new technique, *i.e.*, sap transfusion has been adopted for virus transmission trials and the method has been described in detail. This method in effect simulates an organic union between a diseased root scion and a healthy root stock. Of the two possible ways of effecting transfusion of sap from a diseased to a healthy tree, the one wherein the sap pumped up by a severed diseased root is transfused into the system of a healthy tree is more practicable. However, this method is not utilisable for feeding infective extracts of aerial parts of infected coconut palms or other hosts. Therefore, feeding extracts through tips of roots induced in boxes for the sake of convenience was attempted. The absorption of extracts through uncut roots being slow and selective, these were fed through cut ends of mature roots of healthy palms.

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