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# Studies on some properties of the coconut wilt virus

*By*

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## INTRODUCTION

THE coconut palm, throughout the world, is subject to four diseases of undetermined cause of which, the Cadang-cadang of Philippines and the root (wilt) disease of South India are highly infectious (Price, 1958; McWhorter, 1959; Menon and Nair, 1951; Menon, 1961). In spite of the economic importance of these, progress of work has been curtailed by the perennial nature of the crop and the unwieldy size of the test material involved. While the aetiology of the other two diseases are still unknown, suggestions have been made that Cadang-cadang of Philippines may be caused by a virus (Price, 1958; McWhorter, 1959).

Investigations on the wilt disease of South India revealed its complex nature, no single factor being found to be responsible for initiating the disease (Menon and Nair, 1951). Recently, a mechanically transmissible virus was found to be involved in this complex disease syndrome (Nagaraj and Menon, 1956; Shanta and Menon, 1960), the virus being transmitted not only to coconut under field conditions but to a number of secondary hosts as well. The following is an account of some properties of the virus *in vitro* and its mode of transmission.

## MATERIALS AND METHODS

A local strain of cowpea, *Vigna sinensis* Endl., found to be very susceptible to infection and producing fairly severe symptoms, was used as indicator plant throughout these investigations. The plants were grown in steam sterilised soil and kept in an insect proof house at an average temperature of 28° to 30° C. Sap inoculation by the method of Rawlins and Tompkins (1936) was adopted, the site of inoculation, the age of test plants used etc. being the same as described

earlier (Shanta and Menon, 1960). The inoculum was sap extracted in 2.5 volumes of 0.05 M phosphate buffer at pH 8.0 from the tender leaves of a naturally infected coconut palm in the advanced stage of the disease. Generally, eight seedlings were inoculated for each treatment under an experiment and the experiment itself was repeated at least thrice. In every experiment, one set of plants was maintained as uninoculated control to check up possible contamination from soil. Isolations from naturally infected plants were made by inoculation of sap from roots or shoots of such plants on the leaves of 8-day-old cowpea seedlings.

The weeds selected were those exhibiting symptoms characteristic of virus diseases in general. However, in order to find out the relationship between coconut wilt virus and the foliar symptoms observed in the weed hosts, plants that looked apparently normal were also used for inoculations.

Of the many plants used in the host range studies, the strains of those obtained from the Agricultural Research Institutions at Coimbatore and New Delhi are specified. Others with unspecified strains were obtained from local farms.

## RESULTS

### *Isolation from infected coconut palms and other natural weed hosts*

The virus was first isolated from the tender leaves of coconut palms in the advanced stage of the disease, in November, 1959 on seedlings of cowpea (Shanta and Menon, 1960). It was later isolated from different tissues of several palms both in the middle and advanced stage of disease showing different types of symptoms (Table 1).

TABLE 1.

*Presence of coconut wilt virus (CWV) in different parts of coconut palm*

| Source of inoculum | Condition of the palm | | | |
|---|---|---|---|---|
| | Healthy | Early stage of disease | Middle stage of disease | Advanced stage of disease |
| Tender healthy root | -/12* | 1/12 | 3/12 | 13/14 |
| Mature decaying root | -/12 | 1/12 | 4/12 | 14/14 |
| Root sap | -/12 | -/12 | 5/12 | 10/10 |
| Tender leaf | -/12 | 22/32 | 21/23 | 14/14 |
| Anther | -/12 | 1/6 | 1/6 | 7/9 |
| Female flower | -/12 | -/6 | -/6 | 3/4 |

* Numerator indicates the number of palms yielding the virus and the denominator the number tested.

The virus, showing the same symptoms on cowpea, was isolated later from leaves of arecanut, *Areca catechu* growing in fields and from the roots of *Ageratum connizoides*, *Cleome viscosa*, *Leucas aspera*, *Hemidesmis indicus*, *Panicum* sp., *Phyllanthus niruri*, *Physalis minima* and *Vernonia cinera* collected from the base of diseased trees and from the bunds of fields. It may be seen from Table 2 that the majority of plants growing at the base of diseased trees as well as hose growing within a radius of 3 to 4 metres, which was the maximum distance tested, harboured the virus in their root system. Generally, the roots of 1 to 2-month-old plants were free from the virus. The virus was restricted to the root system of all plants tested, whether healthy or diseased, with the exception of *Physalis minima* which, when infected showed foliar symptoms similar to those in cowpea (Fig. 1).

The virus was also isolated from soil collected from the base of diseased trees.

*Hosts infected by mechanical inoculation*

A number of plants were found to be susceptible to the virus when mechanically inoculated. These were cross inoculated on cowpea for confirmation of results.

*Leguminosae*

*Crotalaria striata*. Mild chlorotic rings, spots, or streaks developed systemically in the newly formed leaves appearing 15 to 30 days after inoculation. The transmission of the virus from this host to cowpea was effected only with difficulty (Joseph, unpublished).

*Dolichos biflorus* (horsegram). An unspecified strain obtained locally showed 'vein clearing' and malformation in cowpea, when inoculations were done in the two leaf stage (Fig. 2). *Dolichos biflorus* $Co_1$, 213 was resistant.

*Phaseolus mungo* (black gram). 'Vein clearing' as in cowpea caused by the necrosis of parenchyma cells along the main veins, appeared on the first trifoliate leaf of an unspecified strain obtained locally (Fig.3), but *P. mungo* strain 212 from Coimbatore was resistant.

*Tephrosia candida*. Chlorotic spots appeared on the younger leaves with severe stunting of the affected plants, the symptoms appearing 2 to 3 weeks after inoculation. The mechanical transmission of the virus from this host to cowpea was effected only with difficulty (Joseph, unpublished).

TABLE 2

*Details of isolations of CWV from weed hosts*

| Name of host | Symptoms of host plant | Source of the host | Age of the host in months | Source of inoculum | Infection on cowpea |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |
| **Cleome viscosa** | Yellow vein mosaic | base of diseased tree | 3—4 | root | 4/8* |
| | normal | bund - 2.5 to 3.5 m. away from diseased tree | ,, | ,, | 2/8 |
| **Leucas aspera** | curling and paling of leaves | base of diseased tree | 2—3 | root | 4/8 |
| | ,, | bund - 2 m. from diseased tree | 1—2 | ,, | 5/8 |
| | ,, | ,, | ,, | leaf | -/8 |
| | normal | bund - 3 to 4 m. from dis. tree | 2—3 | root | 2/8 |
| **Hemidesmis indicus** | typical mosaic | base of dis. tree | 3—4 | root | 2/8 |
| | ,, | bund - 2 m. from diseased tree | 1—2 | ,, | 2/8 |
| | ,, | ,, | ,, | leaf | -/8 |
| | normal | ,, | ,, | root | 2/8 |
| **Panicum sp.** | yellow mosaic | base of diseased tree | 3—4 | root | 3/8 |
| | normal | bund - 2 to 3 m. from dis. tree | ,, | ,, | 3/8 |
| **Physalis minima** | malformation | base of dis. tree | 2—3 | root | 2/8 |
| | ,, | bund - 2 to 3 m. from dis. tree | 1—2 | ,, | 5/8 |
| | ,, | ,, | ,, | leaf | 4/8 |
| | normal | ,, | 2—3 | root | 2/8 |
| **Ageratum connizoides** | yellow vein mosaic | base of diseased tree | 2—3 | root | 3/8 |
| | ,, | ,, | 1—2 | ,, | 1/8 |
| | ,, | ,, | ,, | leaf | -/8 |
| | normal | 3 to 4 m. from dis. tree | 2—3 | root | 4/8 |
| **Vernonia cinera** | normal | base of dis. tree | 1—2 | root | 4/8 |
| **Phyllanthus niruri** | normal | base of dis. tree | 1—2 | root | 2/8 |
| | ,, | 3 to 4 m. from diseased tree | ,, | ,, | 4/8 |
| **Areca catechu** | slight yellowing of leaves | near diseased tree | 15 to 20 year | tender leaf | 1/8 |
| | flaccidity and drooping of leaves | ,, | ,, | ,, | 4/8 |
| | stunting of leaves | ,, | ,, | ,, | 3/8 |

* Numerator indicates in this and in all subsequent Tables the number of plants infected, and the denominator, the number inoculated, unless otherwise stated.

*Vigna sinensis* (cowpea). All strains of cowpea tested viz., C, 521, C. 57, E. C. 455, Pusa var phalguni, Pusa var bursati and three different unspecified strains obtained locally but named here for experimental purposes as GN 4, 5 and 6 respectively were susceptible producing typical symptoms of 'vein clearing', malformation and necrosis (Fig. 4).

**Solanaceae**

*Capsicum annum* (chilli). Infected plants of strain long red (Pochas', Poona) were comparatively stunted (Fig. 5) with no visible foliar symptoms.

*Lycopersicon pimpinellifolium.* Comparative dwarfing and delayed flowering of affected plants in strain Red EC. 252. Slight flaccidity of leaves also was noticed in the early stages before dwarfing was apparent.

**Palmae**

*Areca catechu* (arecanut). Slight paling of the entire plant followed by severe stunting and necrosis of veins of the youngest leaf (Fig.6). The necrosis was absent in subsequent leaves but stunting persisted.

The following plants were found to be resistant to the virus when mechanically inoculated: *Cajanus cajan* S. A1, *Canavalia ensiformis*, *Cyamopsis tetragonaloba* strains C. P. 78 and Pusa monsin, *Dolichos lablab* strains Co 173, 231, 250, 269, 279 and 1428, *Phaseolus lunatus*, *P. vulgaris*, *P. radiatus* strain Co 1, *P, lathyroides*, *Capsicum fruitescens*, *Datura stramonium*, *Lycopersicon esculentum* varieties red top, prosperity, sanmarzana and sioux and *Physalis peruviana*.

No host developing local lesions has so far been found.

## Properties in vitro

Some properties of the virus, determined by inoculation on cowpea, are described below:

*Thermal inactivation point:* Samples of the sap from infected coconut were held at a dilution of 1/2.5 in 0.05 M phosphate buffer at pH 8.0 for 10 minutes at temperatures ranging from 40° to 90° C. before inoculation on cowpea, It was observed that the sap was completely inactivated by heating at 76° C for 10 minutes but not at 74° C (Table 3).

*Longevity in vitro:* Samples of sap at a dilution of 1/2.5 were infective for 3 weeks when stored at room temperature (28° to 30° C) and for 8 weeks when incubated at 80° to 10° C. (Table 3).

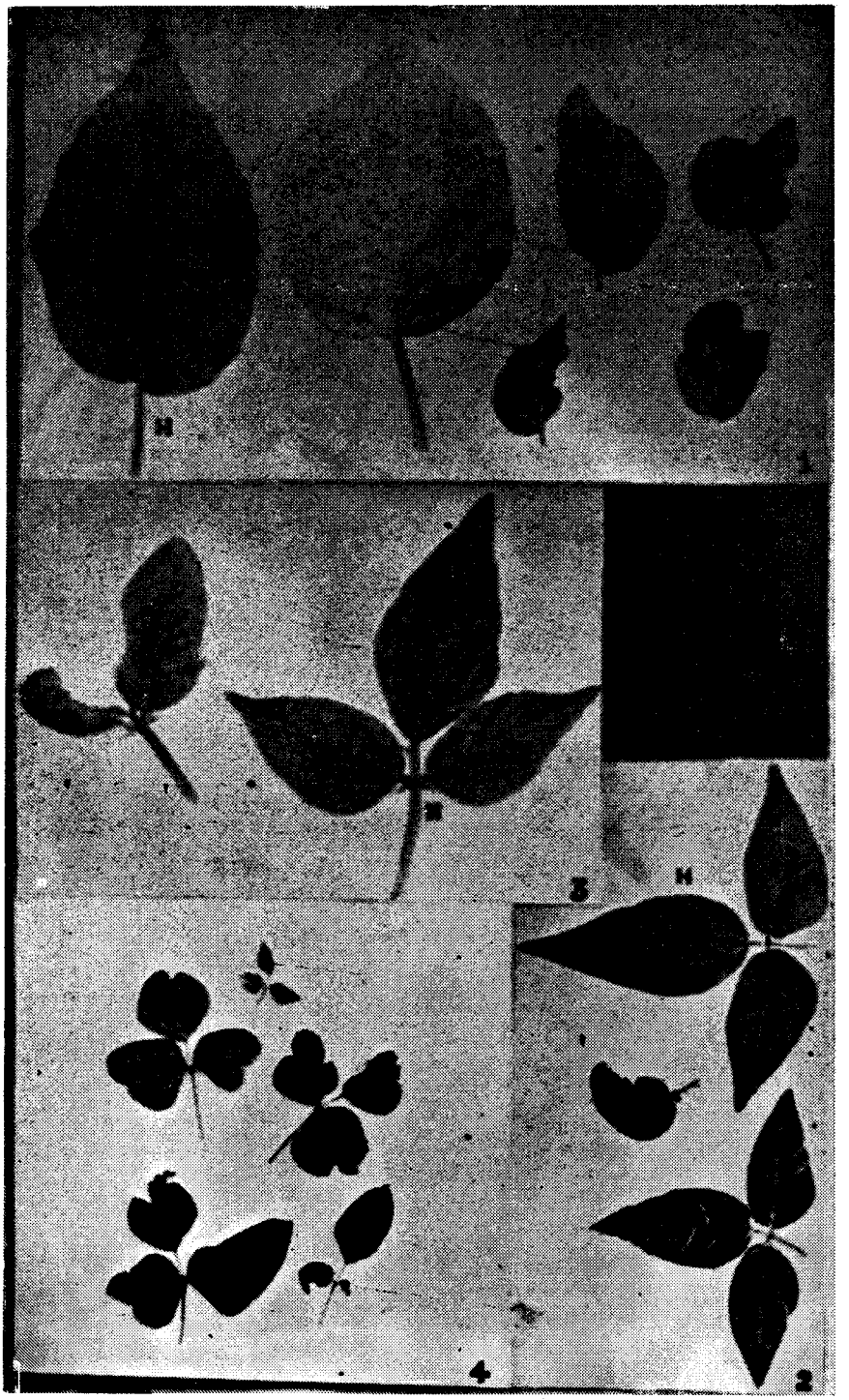


Fig. 1

*Symptoms on Physalis nirui due to natural infection*

Fig. 2, 3 and 4

*'Vein clearing' and malformation on Dolichos biflorus, Phaseolus mungo and Vigna sinensis caused by artificial inoculation of C. WV.*
*(H = uninoculated control)*

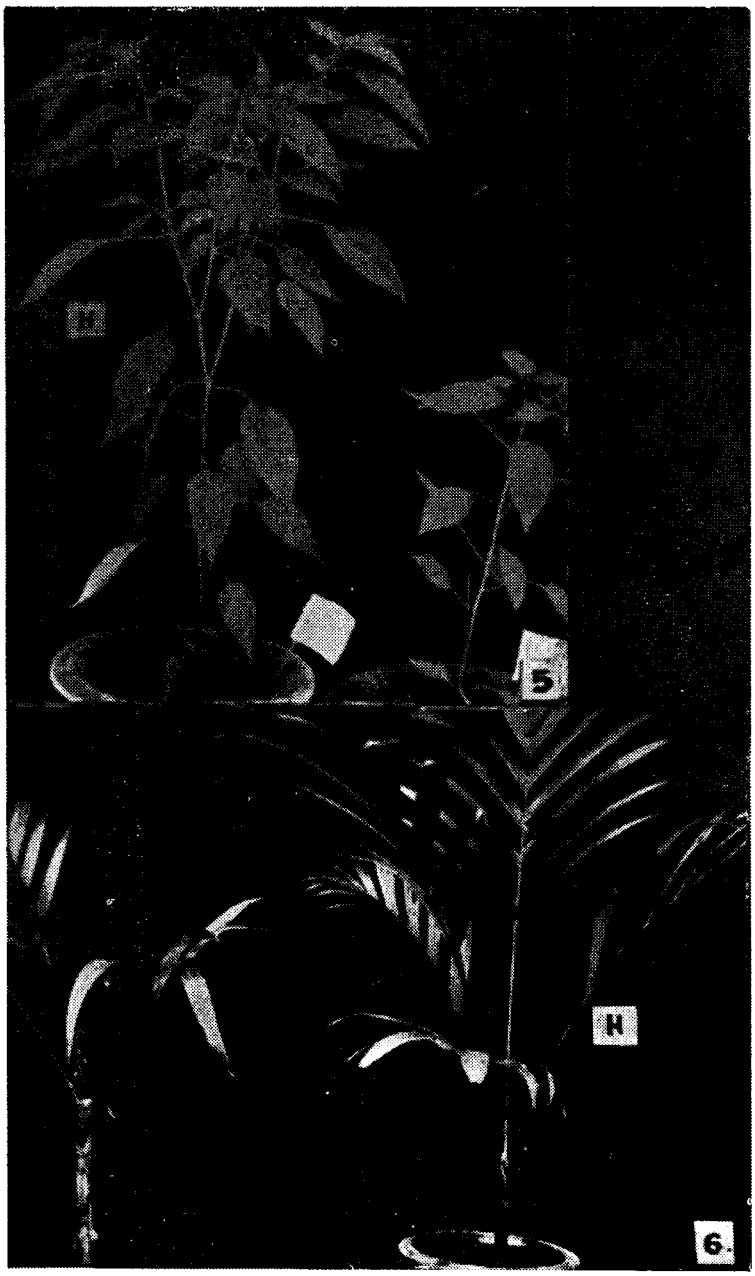


Fig. 5 and 6

*Stunting of Capsicum annum and Areca catechu caused by artificial inoculation of CWV.*
*(H = uninoculated control)*

*Freezing and thawing:* When stored frozen, the virus was active in phosphate buffer (pH 8) extracts of coconut leaf sap at a dilution of 1/2.5 for a period of 8 to 9 weeks but lost infectivity by the end of 10 weeks (Table 3).

TABLE 3

*Thermal inactivation point and longevity in vitro of coconut wilt virus*

| Thermal inactivation point | | Longevity in vitro | | | |
|---|---|---|---|---|---|
| Temperature (°C) | Infectivity on cowpea | Incubation period in days | Infectivity on cowpea — Temperature of incubation | | |
| | | | −4° C | 8–10° C | 28–30° C |
| Unheated | 8/15 | 0 (fresh sap) | | | 10/16 |
| 40 | 8/15 | 6 | 10/16 | 7/15 | 9/15 |
| 50 | 10/17 | 15 | 11/16 | 8/16 | 8/16 |
| 60 | 5/15 | 22 | 8/16 | 6/16 | 8/16 |
| 70 | 7/16 | 29 | 6/15 | 9/16 | −/16 |
| 72 | 6/16 | 56 | 9/16 | 8/16 | −/15 |
| 74 | 8/16 | 63 | 6/15 | −/16 | −/16 |
| 76 | −/16 | 70 | −1/4 | −/15 | |
| 78 | −/15 | | | | |
| 80 | −/16 | | | | |

*Dilution end-point*: It was observed that the concentration of the virus in the leaf varied considerably at different times even when the inoculum was taken from the same tree. For instance, the sap was infective at a dilution of $10^{-2}$ in six out of seven experiments, at $10^{-3}$ in three out of seven experiments and only once at a dilution of $10^{-4}$. Evidently, environmental factors play a considerable role in influencing the susceptibility of the test plants and perhaps the concentration of the virus in the different tissues of the host itself.

*Effect of pH on infectivity*: Clarified infective sap at a dilution of 1/2.5 was adjusted to different pH values ranging from 3 to 10, by the addition of 0.05 M hydrochloric acid or sodium hydroxide. The samples were then centrifuged, the supernatant fluids decanted and the sediments were suspended in distilled water. The pH of all the samples were adjusted to 8.0 before inoculation. It was observed that the supernatant was active in samples adjusted to pH 5.0 to 9.0 (Table 3). Maximum infection was obtained from the supernatants at pH 6.0 to 9.0. At pH 4.0 the supernatant was inactive but the resuspended sediment was slightly active. The virus was completely inactivated at pH 3.0.

TABLE 4

*Effect of pH on the infectivity of coconut wilt virus*

| pH | Infectivity of | |
|---|---|---|
| | Supernatant fluid | resuspended sediment |
| 3.0 | -/8 | -/8 |
| 4.0 | -/8 | 1/8 |
| 5.0 | 1/8 | -/8 |
| 6.0 | 3/8 | -/8 |
| 7.0 | 4/8 | -/8 |
| 8.0 | 2/8 | -/8 |
| 9 0 | 3/8 | -/8 |

*Multiplication and movement of the virus in cowpea*: The simple primary leaves of cowpea were inoculated with the virus at a dilution of 1/2.5 and its further multiplication and movement *in vivo* were tested at intervals of 24 hrs. by inoculation on healthy cowpea seedlings. Quantitative determination of the concentration of the virus was made by inoculating the virus at different dilutions of sap extracted from various tissues of the inoculated plant on healthy seedlings of cowpea. It may be seen from Table 5 that the virus has remained in the seat of inoculation for 24 hours after inoculation and the concentration of it has not increased to the point where it can be transmitted to fresh hosts. This remains so for 48 hours after inoculation but by this time the virus has already moved into the growing shoot apex as evidenced by the symptoms produced on the first compound leaf, even after the removal of the inoculated leaves at this stage. In other words, by 48 hours after inoculation the virus has not only multiplied at the site of inoculation but has also moved out into the growing shoot apex where however, it has not multiplied enough as to cause infection on reinoculation. By 72 hours it has multiplied at the site of inoculation as well as in the

TABLE 5

*Multiplication of the CWV and its movement in cowpea*

| Incubation period in hrs. | Source of inoculum | | | | | Infection on cowpea after removal of the inoculated leaves |
|---|---|---|---|---|---|---|
| | simple leaves | growing shoot apex | stem upto cotyledons | stem below cotyledons | Root | |
| 24 | -/16 | -/16 | -/16 | -/16 | -/16 | -/16 |
| 48 | 4/24 | -/16 | -/16 | -/16 | -/16 | 4/16 |
| 72 | 9/28 | 7/16 | 2/21 | -/16 | -/16 | 9/16 |
| 96 | 13/29 | 13/24 | 11/22 | 7/13 | 6/15 | 8/16 |

growing point to give a titre of 1/10 and 1/2.5 respectively. By this time it has started moving down into the stem although only two out of twentyone plants were infected on reinoculation. By 96 hours, the virus was detected in all parts including the root system. Further studies on movement of the virus on older seedlings of cowpea are in progress and the general trend of movement conforms to what is discussed above.

### *Soil transmission*

Tests conducted so far tend to show that CWV is soil-borne. Samples of soil from the base of severely diseased and healthy trees were collected, air-dried and passed through a 2 mm. seive to remove larger particles of soil and debris. 10 g. samples of these were separated according to Stokes' Law into clay and silt fractions. In these studies, no preliminary treatments such as heating or treatment with chemicals which are intended to obtain a finer dispersion of soil particles and to remove organic matter, were carried out so as not to lose infectivity of the samples. The different fractions were suspended in 2 ml. 0.05 M phosphate buffer at pH 8.0 and inoculated on seedlings of cowpea in the usual manner. Soil samples from fourteen healthy trees and fourteen diseased, showing advanced stage of symptoms, were tested. The clay fraction of fourteen and the silt fraction of thirteen diseased trees were infective whereas none of the corresponding samples from the healthy trees were (Table 6).

TABLE 6

*Infectivity of soils in relation to condition of tree*

| | Condition of the tree | | | |
|---|---|---|---|---|
| | Healthy | Early stage of disease | Middle stage of disease | Advanced stage of disease |
| Clay fraction of soil from base of tree | -/10* | 1/14 | 1/14 | 14/14 |
| Silt fraction of soil from base of tree | -/10 | 1/14 | 1/14 | 13/14 |
| Clay fraction of soil 3.7 m. from base of tree | -/10 | 2/14 | 4/14 | 6/6 |
| Silt fraction of soil 3.7 m. from base of tree | -/10 | 2/14 | 2/14 | 6/6 |

* Numerator indicates the number of trees yielding the virus and the denominator the number tested.

Further, it was observed that natural field infection of cowpea seedlings grown at the base of severely diseased trees was 45 to 50% in contrast to 12 to 15% infection observed in the crop grown in a 9 m x 15 m. plot where no coconut was growing but which was surrounded by healthy and diseased ccconuts. As a further check, seedlings of cowpea were raised inside the insect proof house in pots of air-dried soil collected from the base of diseased trees. The plants were watered with sterilised tap water. 35 to 40% seedlings developed typical vein clearing within three weeks of sowing whereas nine grown in sterilised garden soil were diseased.

### *Insect transmission*

Preliminary trials showed that infective adults of *Stephanitis typicus* Dist. when fed forcibly on leaves of cowpea transmitted the virus into this host (Shanta and Menon, 1960). In later experiments, adults of this insect bred on healthy coconut leaves in the laboratory were used for transmission. After a 24 hr. acquisition feeding period on diseased coconut leaves, the insects were confined to the simple leaves of cowpea for a period of 48 hours at the rate of 10 insects per plant. Of the 21 plants so treated, 9 became systemically infected while none of the corresponding 20 plants fed by those which had a 24 hour acquisition feeding on healthy coconut leaves were infected.

## DISCUSSION

The properties of CWV, the virus associated with the root (wilt) disease of coconuts were studied using cowpea as indicator plant. Its thermal inactivation point is fairly high being 76° C. and its stability in extracted leaf sap is about 8 to 9 weeks when frozen. At room temperature (28° to 30° C.) it is active for 3 weeks in extracted leaf sap at a dilution of 1/2.5.

The virus has a wide host range, when artificially transmitted, in the natural orders Leguminosæ, Solanceæ and Palmæ causing systemic symptoms in all. Necrosis and malformation of leaves and stunting of plants are the major symptoms in general. The virus occurs in nature in the roots of many weeds that are found to be common in coconut plantations. It also occurs systemically in coconut, cowpea and *Physalis minima*.

The systemic symptoms exhibited on cowpea are different from those produced on this host by other viruses (McLean, 1941; Snyder, 1942; Yu., 1946; Capoor *et al.* 1947; Dale, 1949, Capoor and Varma, 1956; Chant, 1959) and therefore cowpea can be used as a differential host for CWV.

The mode of transmission of this virus is interesting in that it is highly versatile being soil-borne as well. But unlike the majority of soil-borne viruses (Mckinney, 1923; Smith, 1957, Hewitt *et al.* 1958; Cadman and Harrison, 1959) CWV is also transmitted through insects, a phenomenon met with only in the case of tobacco ringspot virus (Valleau, 1951; Walters, 1952; Smith and Brierley, 1955; Dunleavy, 1957; Hendrix, 1961). The virus is found to be retained in the clay and silt fractions of soil collected from the base of trees in the advanced stage of disease and causes natural infection on cowpea seedlings grown in these soils.


## SUMMARY

Some physical properties and the mode of transmission of Coconut Wilt Virus (CWV), the virus associated with the root (wilt) disease of coconut has been studied using cowpea as indicator plant.

The virus is sap transmissible and its insect vector on coconut is *Stephanitis typicus* Dist. It is also soil-borne, the clay and silt fractions collected from the base of diseased trees being infective. It has a fairly wide host range when mechanically transmitted and occurs naturally in the roots of a number of weeds.


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