LONG FLEXUOUS THREADS ASSOCIATED WITH HASSAKU DWARF DISEASE OF CITRUS TREES

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INTRODUCTION

A dwarf disease of Hassaku orange trees (*Citrus hassaku* HORT. et Y. TANAKA), first described by TANAKA *et al.* (1960) in Japan, was assumed to be caused by the citrus tristeza virus (SASAKI 1963, 64, 67, TANAKA and YAMADA 1964) on the basis of host range and symptomatology studies. CHEN, MIYAKAWA and MATSUI (1967) briefly reported that long threads of 10 to 13 mμ in diameter of variable length were found in the dip preparations and thus the causal virus of Hassaku dwarf may be identical with tristeza.

As for the tristeza virus, electron microscopic studies by KITAJIMA *et al.* (1964, 1965), SILVA *et al.* (1965), and PRICE *et al.* (1966) established its average length of 2000 mμ and the intracellular accumulation in phloem cells of diseased lime plants.

To confirm the causal virus of Hassaku dwarf, attempts were made to observe the virus morphology in ultrathin sections of diseased plants under an electron microscope. A part of this work was presented at the International Citrus Symposium, Riverside, California, March, 1968, and at the annual meeting of the Japan Phytopathological Society, Sapporo, Japan, June, 1968. (TANAKA *et al.* in press, SASAKI *et al.* 1968). This paper deals with the finding of long flexuous threads, detected either in exudate preparations from Mexican lime plants infected with severe and mild strains of Hassaku dwarf, or in ultrathin sections of phloem cells from leaves of Hassaku trees and Mexican lime plants infected with the severe strain of Hassaku dwarf.

MATERIALS AND METHODS

Two strains of Hassaku dwarf, selected by the junior author at the Hiroshima Agriculture Experiment Station from diseased Hassaku trees, were used in the study. The HM–50 strain caused very mild symptoms on the Hassaku trees and Mexican lime plants (*Citrus aurantifolia* (CHRISTMANN) SWINGLE), sometimes causing no apparent symptoms or resulting in slight stunting of the infected plants. Strain HS–34 was very severe, showing typical vein clearing on young leaves and later corky veins on the mature leaves, but remaining nearly symptomless on the very young Hassaku plants, except for severe stem pitting and the very slight swelling on the vein.

The exudate method was employed to prepare negatively stained preparations from the Mexican lime plants infected with HM–50 and HS–34 strains. The exuded drops from the stem were placed on the specimen grids and then an equal volume of 2% PTA (phosphotungstic acid) was added to the drops. After the preparations dried, the grids were examined by an electron microscope.

Small pieces of the vein portion of the diseased leaves of Mexican lime plants and Hassaku trees infected with HS–34 strain were cut and fixed in 5% glutaraldehyde in phosphate buffer at pH 7.0 for 90 minutes. After post-fixation in 2% osmium tetroxide for 60 minutes, the materials were dehydrated in graded alcohol and then embedded in Epon.

The ultrathin sections were cut with a Porter-Blum MT–1 microtome, equiped with glass knives and stained witn 6% uranyl acetate for 60 minutes and lead acetate for 15 minutes.

RESULTS

Exudate Preparations: Electron microscopy of the negatively stained preparations of the exudates from the Mexican lime plants infected with the severe strain, HS–34 that showed clear vein clearing and corky vein on the leaves, revealed the presence of long flexuous threads. Their size was approximately 2000 mμ in length and 10 to 12 mμ in diameter (Fig. 1, 2). Similar threads of the same size and shape were detected in the exudate preparations of the lime plants infected with a mild strain. HM–50, showing slight stunting of the lime plants, but no apparent symptoms on the leaves. There were no such long threads in the exudate preparations from the healthy lime plants. Their size and shape corresponded with those of tristeza virus particles described by several workers (KITAJIMA *et al.* 1964 a, b, c., SILVA *et al.* 1965, PRICE 1966).

Sometimes, some tubular structures of approximately 15 to 18 mμ in

diameter of variable length were seen in the exudate preparations (Fig. 3), while there were no such fine threads of 6 to 7 mμ in diameter as described by KITAJIMA *et al.* (1965).

Ultrathin Sections: Ultrathin sections of the vein portions of young leaves of lime and Hassaku plants infected with a severe strain, HS-34, showing vein clearing symptom, were examined by an electron microscope. As shown in Fig. 4, a cross section of the vein portion of the diseased lime plants revealed some cytological changes in the phloem cells. There were some necrotic cells and densely packed cells filled with uniformly apppearing substances. Such cells as indicated in Fig., 4, (V) contained large accumulations of thread-like particles. High magnification of these cells clearly demonstrated masses of fine flexuous threads within the cytoplasm (Fig. 5). The size of the threads was approximately 10 to 12 mμ in diameter and the length was variable (Fig. 6). In some of the cells, bundles of tubular structures were seen, which were usually surrounded by fine flexuous threads (Fig. 7). These tubular structures, arranged parallely, were appoximately 15 to 18 mμ in diameter. The size and shape of the tubules corresponded with those of tubular structures appearing in the fresh exudate preparations. As shown in Fig. 8, these tubules seemed to be connected with fine flexuous threads. Similar fine threads and tubules were also detected in the phloem cells of Hassaku trees infected with a severe strain, HS-34. No similar fine flexuous threads were found in the healthy lime and Hassaku trees.

DISCUSSION

The present electron microscopic studies of Hassaku dwarf revealed the presence of fine flexuous threads in the exudate preparations from Mexican lime plants infected with either a severe strain, HS-34 or a mild strain, HM-50. They appeared either in the phloem cells of the Hassaku trees infected with a severe strain, HS-34, or in phloem cells of the Mexican lime plants infected with a severe strain, HS-34. Since no such threads were found in either exudates or thin sections of healthy controls, the results suggest that the fine flexuous threads associated with Hassaku dwarf represent the causal virus of this disease.

The size and shape of these threads correspond with the size of tristeza virus described by KITAJIMA *et al.* (1964) and PRICE (1966). SASAKI *et al.* (1964, 1967) assumed from the studies of host range and symptomatology that the disease was caused by tristeza virus. They reported that tristeza virus in Japan is represented by several strains which cause severe or mild symptoms

on Hassaku orange and Mexican lime plants. The present studies of ultrathin sections and negative staining of the exudates from the severe and mild strains of the Hassaku dwarf strongly support their assumption that the causal agent of the Hassaku dwarf is tristeza virus.

The role of tubular structures found in exudates and in ultrathin sections is unknown. The structures resemble the protein slime of the X-component described by ESAU and CRONSHAW (1968). Small accumulations of similar tubules were also found in the phloem cells of healthy leaves of lime and Hassaku trees, but were not as abundant as those of diseased leaves.

SUMMARY

Fine flexuous thread-like particles of approximately 2000 mμ in length and 10 to 12 mμ in diameter were found in exudate preparations from Mexican lime plants infected with either a severe strain, HS-34, or a mild strain, HM-50 of Hassaku dwarf.

Accumulations of fine flexuous thread-like particles of approximately 10 to 12 mμ in diameter were detected in cells of leaves from either Mexican lime or Hassaku trees infected with a severe strain, HS-34. They appeared in the cytoplasm of phloem cells of the diseased plants.

No such flexuous threads were found in exudate preparations and in cells of leaves from healthy controls.

These results confirm the postulation that Hassaku dwarf is caused by tristeza virus.

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LEGENDS OF FIGURES

Fig. 1, 2. Long flexuous thread-like particles detected in dip preparations from a lime plant infected with a mild strain of Hassaku dwarf, HM-50. × 100,000

Fig. 3. Along flexuous thread-like particle (left) and a tubular structure (right) detected in a dip preparation from a lime plant infected with a severe strain of Hassaku dwarf, HS-34. × 100,000

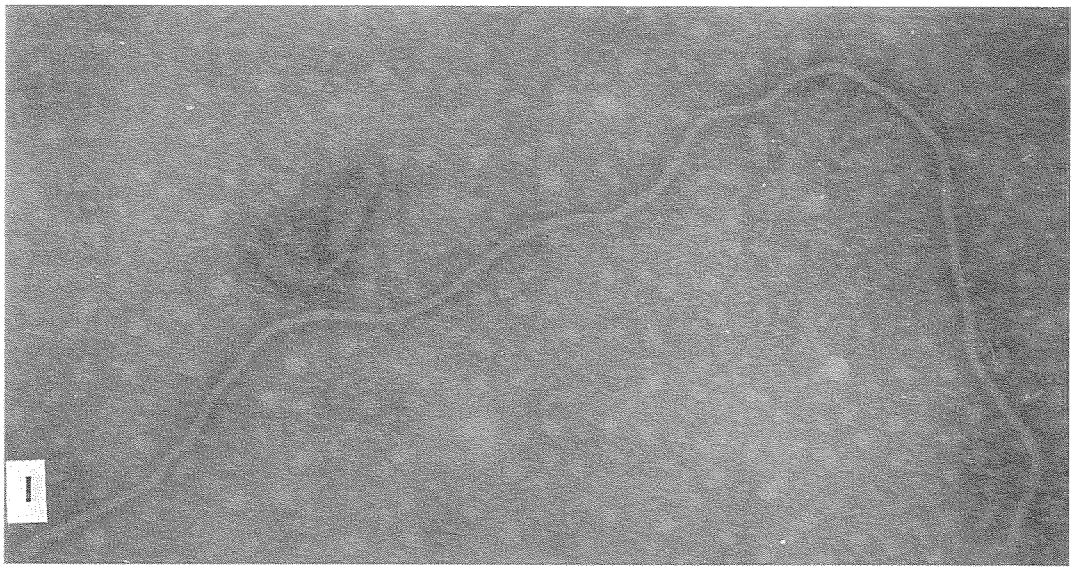
Fig. 4. An ultrathin section of phloem cells in leaves of a lime plant infected with a severe strain of Hassaku dwarf, HS-34. (V) represents cells filled with dense uniform substances. Note that some of the cells exhibited necrotic degenerations. × 5,000

Fig. 5. An ultrathin section of an infected cell of a Hassaku dwarf (HS-34 strain) diseased lime plant showing cross and longitudinal section of the long flexuous threads-like particles. × 66,000

Fig. 6. High magnification of a part of Fig. 5, showing a mass of flexuous thread-like particles within the cell. × 132,000

Fig. 7. An ultrathin section of a cell in a lime plant infected with Hassaku dwarf, HS-34 strain. Note that a bundle of parallely arranged tubular structures (T) is surrounded by fine flexous threads (V). × 34,000

Fig. 8. High magnification of a part of Fig. 7. Note that the tubules seem to be connected with fine flexuous threads. × 91,000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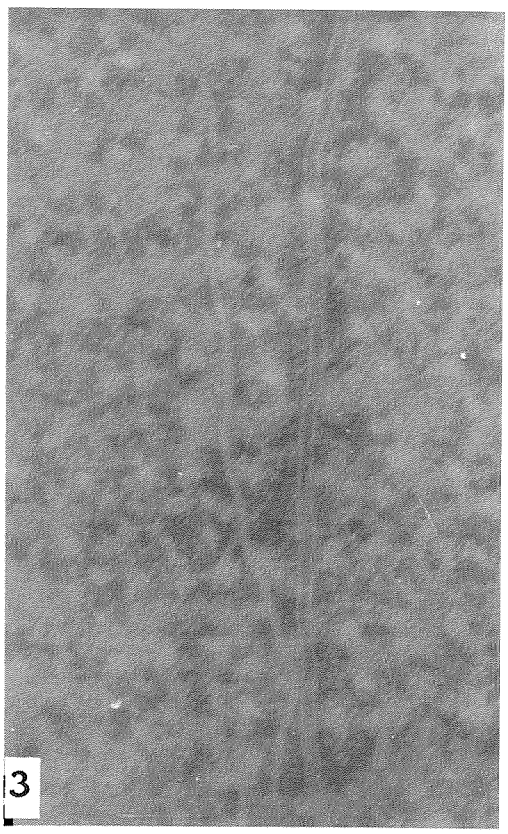
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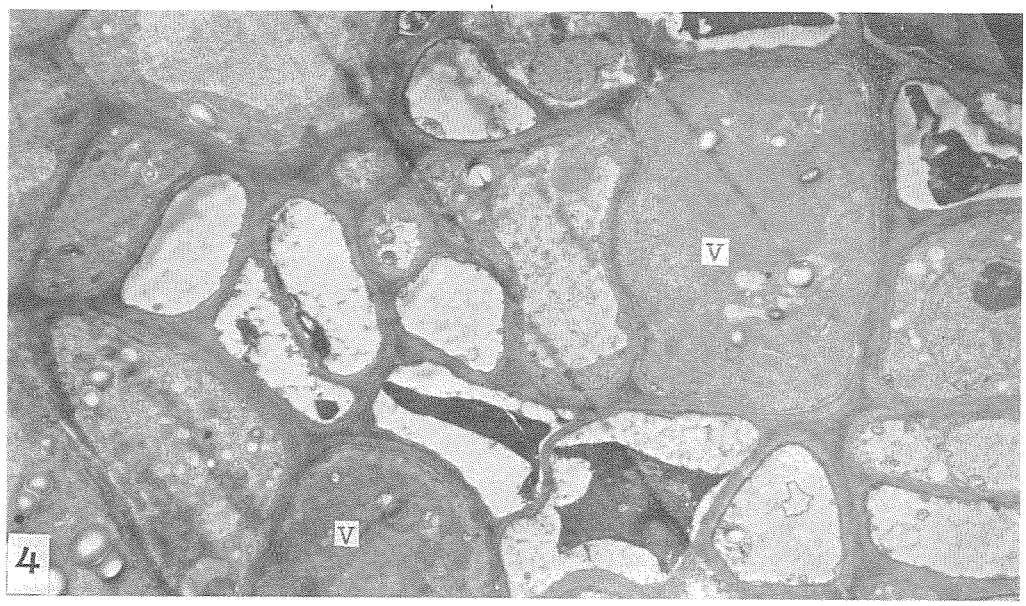
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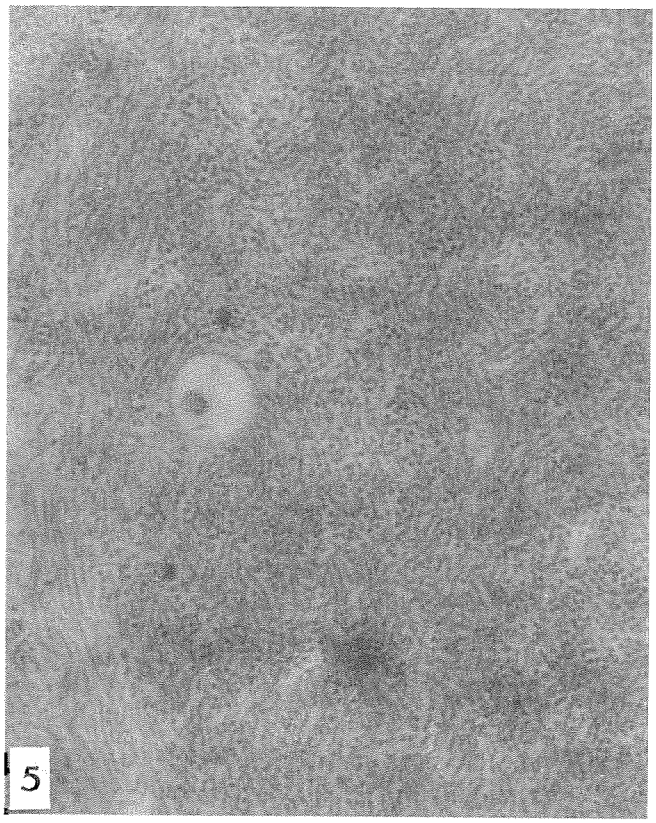
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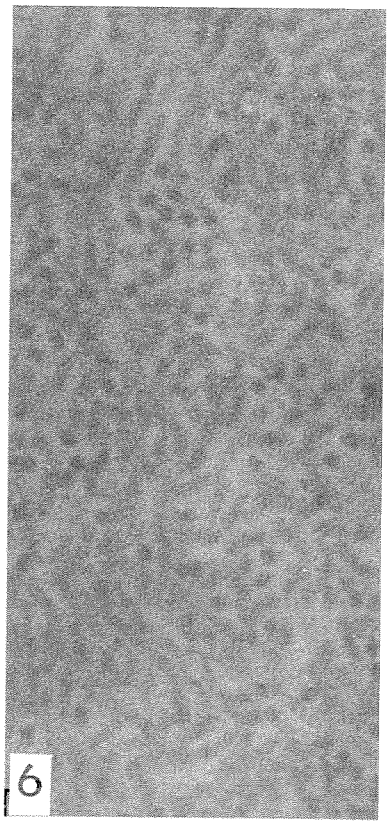
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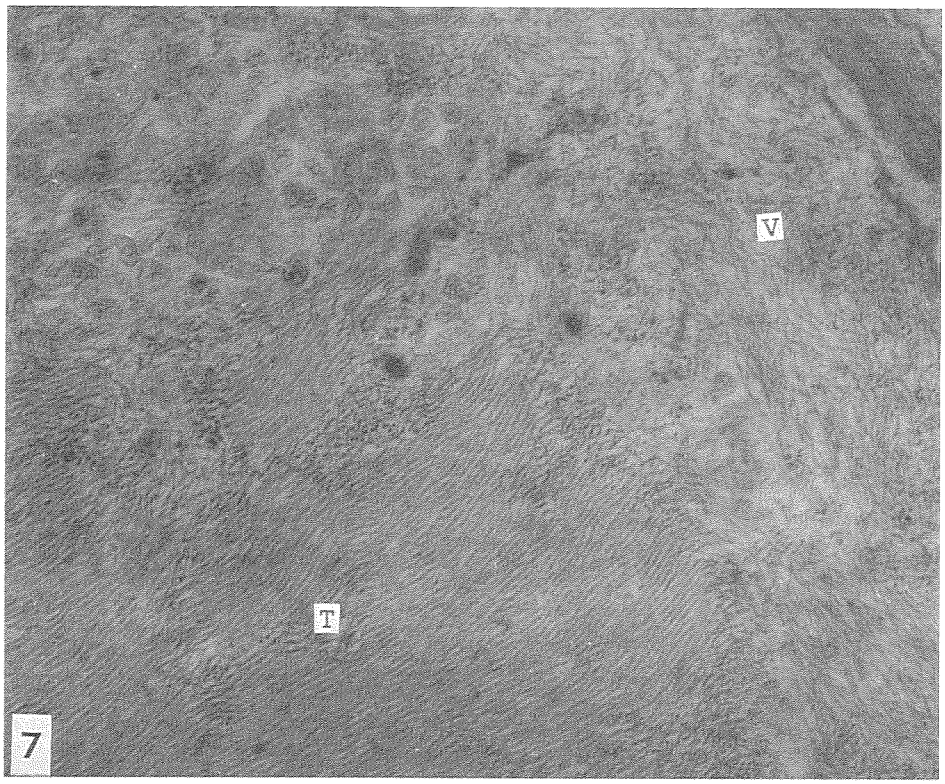
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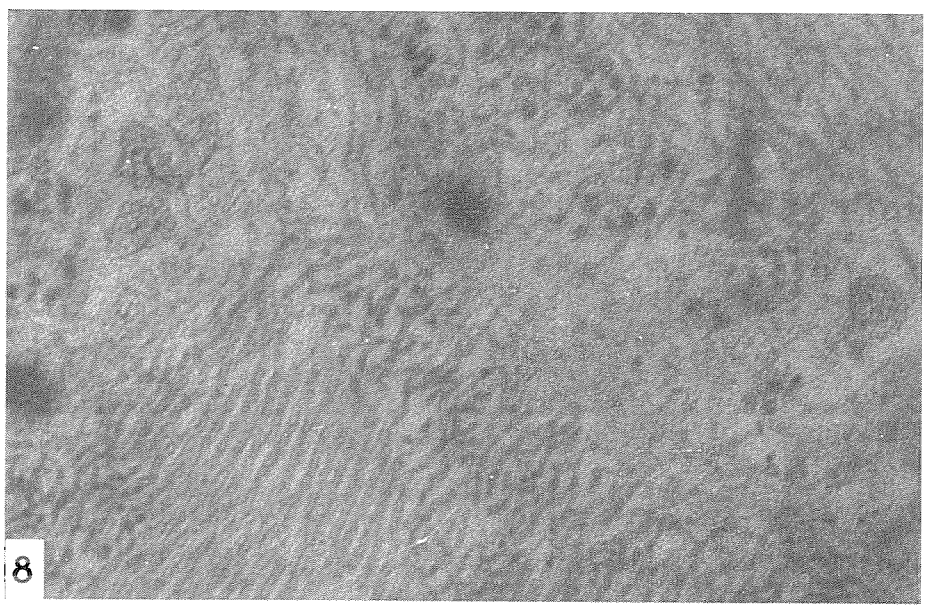
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