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Partial Purification of Tristeza Virus

ATTEMPTS to purify tristeza virus were begun in 1958. Several methods, principally that of Fulton (2, 3) which is based on adsorption on hydrated calcium phosphate, were tried without apparent success. Other methods, including preclarification treatment of the juice by treating it with n-butanol or a mixture of ether and carbon tetrachloride (7), were tried subsequently with better results. This paper presents the results of some of these subsequent attempts to purify tristeza virus from the juice of three species of citrus.

Methods and Materials

Leaves of Galego lime [*Citrus aurantifolia* (Christm.) Swing.] and *Aeglopsis chevalieri* Swing. and, in few cases, Baianinha sweet orange [*Citrus sinensis* (L.) Osbeck] grown in a greenhouse or under field conditions were used as starting material for the experiments. The leaves were generally left overnight in a freezer before starting the work. The Galego lime plants were growing in the field on sweet orange rootstock; seedlings of *A. chevalieri* were used. All greenhouse-grown plants were seedlings that had been infected by means of viruliferous insects.

The leaves were dipped in 0.001 M neutral phosphate buffer containing 0.01 M sodium sulfite. They were then ground in a food chopper and their sap expressed. The liquid thus obtained was subjected to the ether-carbon tetrachloride method (7) or to treatment with n-butanol at 8 per cent of the total volume of the juice. This last treatment was a modification of Steere's method (6). The green liquid so obtained was

centrifuged at 3,000 rpm for 15 minutes. The supernatant fluid was filtered through hydrophilic cotton and was centrifuged alternately at low speed (3,000-4,000 rpm, 15 min.) and high speed (30,000 rpm, 75 min.) one or more times. In some cases, the green liquid after filtration through cotton was layered on top of a sucrose solution increasing in density from top to bottom and centrifuged at 23,000 rpm for two hours, following the method of Brakke (1).

The pellets obtained were usually clear, and sometimes slightly brownish. In general, they were dissolved in phosphate buffer 0.1 M or 0.01 M at pH 7.0 and kept for some days in a refrigerator. The preparation will be referred to as tristeza virus preparation (TVP) when obtained from diseased plants and healthy preparation (HP) when obtained from healthy ones.

To prepare antiserum, rabbits were first injected intravenously with TVP and, after a period of rest, intramuscularly with TVP containing Freund's adjuvant. Precipitin tests were carried out in tubes. The precipitates were centrifuged several times in distilled water and then observed in the electron microscope, using the negative staining technique.

Experimental Results

Partially purified preparations of tristeza virus were used to inoculate Galego lime, cowpea (*Vigna sinensis* Savi), and *Chenopodium amaranticolor* Coste et Reyn. Groups of five plants of each species were inoculated by rubbing their roots and leaves with a brush made of glass cotton after dipping it in the TVP. Sometimes the leaves were first injected with TVP by means of a hypodermic syringe and then pricked with glass cotton bound to the tip of a wooden rod. None of the inoculated plants became infected.

Examination of partially purified preparations from diseased plants revealed two kinds of particles (4, 5). One of them, considered to be tristeza virus, was more flexible, longer, and thicker than the other (Fig. 1). Preparations from healthy plants grown under the same conditions contained only the thinner particles.

The partially purified TVP gave positive biuret and xanthoproteic tests for protein and orcinol tests for nucleic acid. They also gave a typical nucleoprotein absorption curve with a maximum at 260 mμ and a minimum at 240 mμ.

A serological precipitate obtained with material from diseased and

healthy plants in test tubes, when examined in the electron microscope, appeared in preliminary observations to be aggregates of thin and thick particles. The aggregates had a tendency to form separate groups. The diameter of the particles in TVP was about three times the normal.

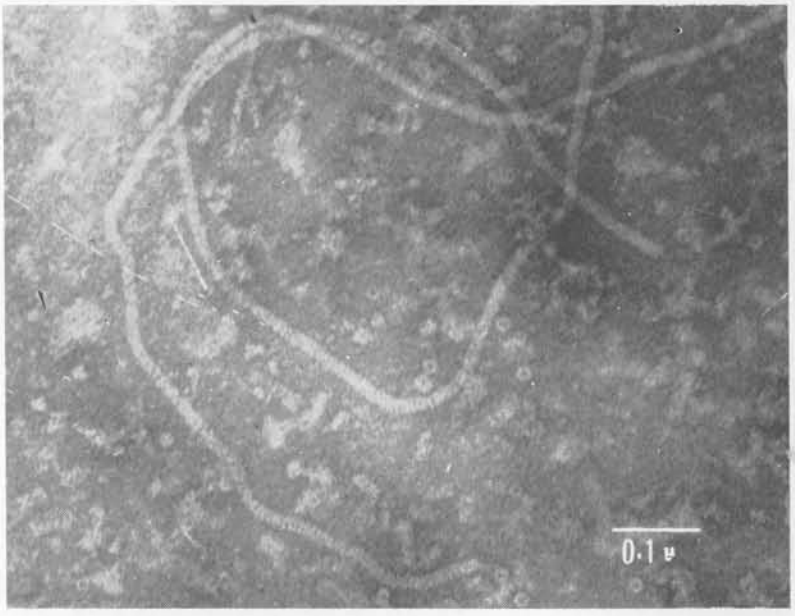

FIGURE 1. *Electron micrograph of a partially purified preparation of tristeza virus negatively stained with phosphotungstic acid.*

Electron microscopical observation revealed that the control mixture of TVP and normal serum did not show a specific precipitation. A mixture of HP and normal serum sometimes exhibited a precipitation like an artifact; this precipitation generally occurred 10-15 minutes later than that between TVP and its specific antiserum.

The long, thick particles were always present in preparations from diseased plants of the three citrus species, independently of the growing conditions and the age of the plants.

Attempts were made to find virus particles in extracts of viruliferous insects. The procedures generally used were the same as those for partial purification of the virus from sap of diseased plants. None of these attempts was successful.

Discussion

Considering the fact that normal plant constituents were not completely eliminated from the partially purified preparations of tristeza virus, there will remain doubt about the chemical nature of the particles present in these preparations. Nevertheless, these preliminary attempts to determine the chemical composition of our materials indicate that they are mainly nucleoprotein in nature.

Since we were unable to transmit tristeza virus by mechanical means, even when partially purified preparations were used as inoculum, the evidence for identity of the virus and the long, thick particles depends upon the fact that the latter were never found in preparations from healthy plants but only in those from plants having symptoms of tristeza. The differences between the HP and TVP were so conspicuous that we believe we have evidence that the long, thick particles are indeed tristeza virus. The constant presence of these particles in preparations obtained from diseased plants by treatment either with n-butanol or a mixture of ether and carbon tetrachloride would seem to show that the particles are intimately associated with the tristeza disease.

The fact that the long, thick particles were always present in preparations obtained from diseased plants of three different species of citrus is good evidence that they represent tristeza virus rather than a secondary product of the diseased plant.

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