8

Purification and Electron Microscopy

Citrus, a Local Lesion Host of Tobacco Necrosis Virus

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FIELD SOURCES of cristacortis virus are generally contaminated with concave gum psorosis virus (2). In the course of work undertaken to obtain a culture of cristacortis virus free from that of concave gum, we encountered another virus, one that produces necrotic local lesions in citrus. This virus was shown, as reported herein, to be a strain of tobacco necrosis virus (TNV); it is referred to for convenience as TNV-CT.

Source Material

Young citrus leaves used for purification studies were showing strong symptoms of psorosis. They came from the following sources. 1. Sour orange seedlings inoculated in 1967 with a source of cristacortis and concave-gum viruses; the source was Tarocco orange tree C4-7. 2. Eureka lemon seedlings infected with a strain of psorosis virus (Code 340) obtained from Dr. L. G. Weathers. 3. Eight-year-old, field-grown Orlando tangelo seedlings infected with the same source of cristacortis virus as in 1.; they were provided by R. Vogel.

Leaves from healthy seedlings or trees were used for controls.

Experimental Work

No transmission was obtained when crude extracts of citrus leaves were used for mechanical inoculation of various herbaceous plants. Partially purified preparations were then tried. Leaves were homogenized in buffer containing 0.02 M sodium phosphate, pH 8, and 0.02 M mercaptoethanol. Two low-speed centrifugations, 5,000 *g* and 10,000 *g*, eliminated most of the green material. Polyethylene glycol (PEG, Carbowax 6,000) was added to the 10,000 *g* supernatant, in the presence of 0.02 M NaCl, to obtain a final concentration of 6 per cent. The sediment that formed when the preparation stood for 1 hour at 5°C was collected by centrifugation and resuspended in 0.02 M phosphate buffer, pH 8. The suspension was

centrifuged at 11,000 *g*, and then at 100,000 *g*. The final pellet was suspended in 0.02 M sodium phosphate buffer, pH 8.

Preparations from the sour orange, Orlando tangelo, and Eureka lemon seedlings produced a few red necrotic local lesions in inoculated leaves of Blackeye No. 5 cowpea leaves and in Scotia bean leaves. Extracts from the local lesions were highly infectious, producing many local lesions in cowpea leaves. When examined in an electron microscope by the dip method, such lesions were seen to contain numerous pseudospherical particles.

TNV-CT was purified from infected cowpea leaves by employing a butanol clarification step, precipitation of the virions with PEG (8 per cent), centrifugation at low speed and high speed, and sucrose density centrifugation. Electron microscopy of the purified preparations revealed pseudospherical particles approximately 30 nm in diameter (Fig. 1,A).

Band centrifugation in CsCl in the analytical centrifuge revealed only a single component with physical properties as follows: an optical density (OD) spectrum with a maximum at 259 nm and a minimum at 243 nm; a ratio OD 280/OD 260 of 0.6; a ratio OD 260/OD 240 of 1.29; a sedimentation coefficient $S_{20}°$ of 115S; and a buoyant density in CsCl at 20° C of 1.373.

The purified preparations were highly infectious, producing lesions in practically all susceptible hosts inoculated. Herbaceous hosts in addition to cowpea and bean are National Pickling Cucumber and Xanthi n.c. tobacco. Red necrotic lesions were also produced in Eureka and Lisbon lemon, Madam Vinous sweet orange, sour orange, Orlando tangelo, West Indian lime, and Etrog citron No. 60-13 (Fig. 2). Lesions appeared in cowpea, tobacco, and citrus, 2, 5, and 10 days after inoculation, respectively.

The pseudospherical particles were demonstrated, by the dip method, in local lesions of the various host plants, particularly citrus (Fig. 1,B).

TNV-CT became systemic in cowpea when the inoculated primary leaves were not too heavily infected. Citrus seedlings infected by mechanical inoculation of their leaves sometimes developed necrosis and gumming of the shoots where the leaves were attached. Young-leaf symptoms of psorosis have not, however, been observed in the infected seedlings as long as 4 months after inoculation.

The type of symptom produced, host range, and physical properties of the virions suggested that they were related to TNV. A strain of TNV, free of the satellite virus, was obtained from Dr. M. K. Corbett for comparative purposes. Its physical properties were determined after purification from inoculated primary leaves of cowpea. In common with TNV-CT, it had the following properties: sedimentation coefficient, 115S; pseudospherical particles about 30 nm in diameter; infectious for Xanthi n.c. tobacco, Blackeye No. 5 cowpea, Scotia bean, lemon,

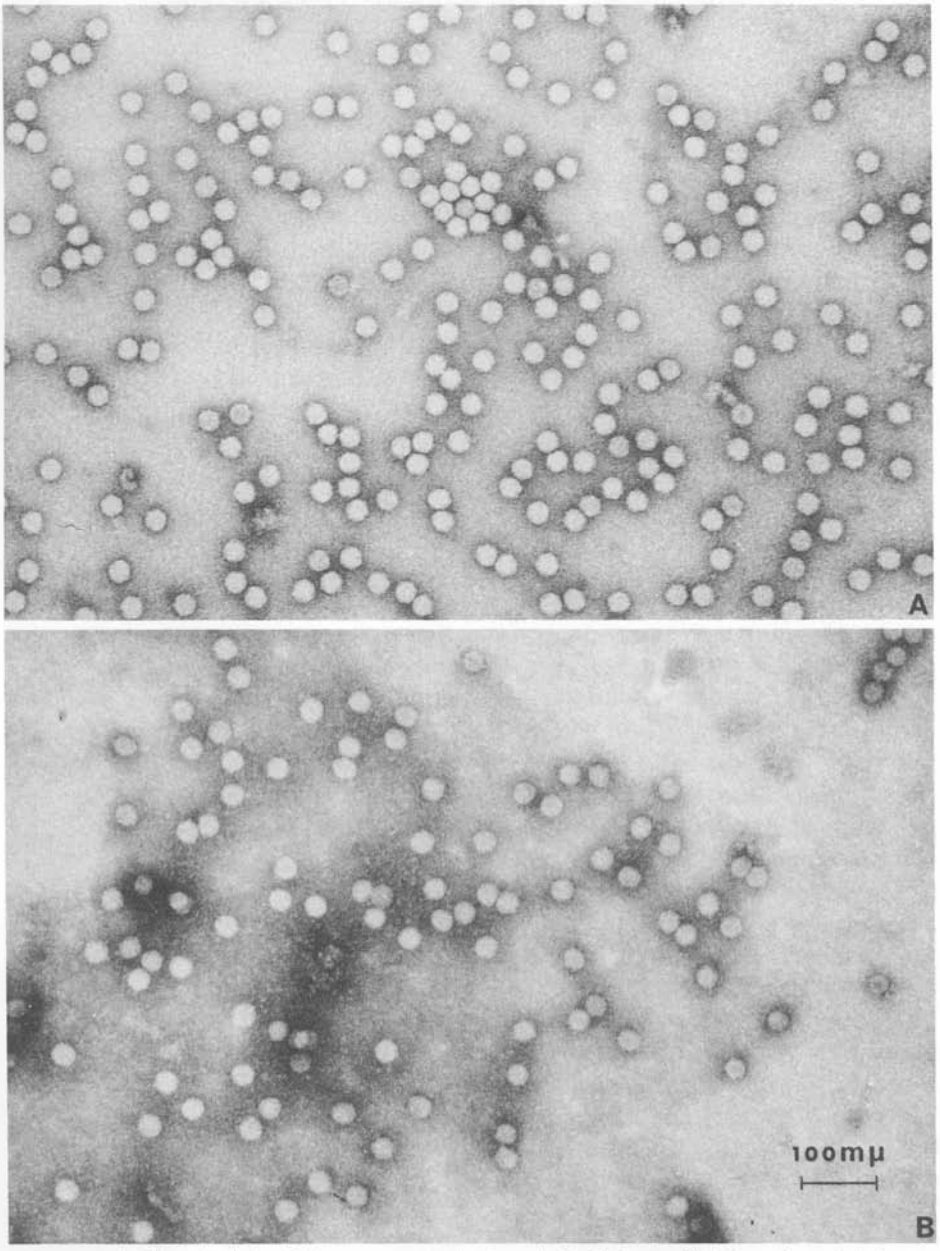

FIGURE 1. *Citrus isolate of tobacco necrosis virus. A. Particles purified from inoculated cowpea leaves. B. Particles obtained with the "dip" method from a local lesion on a Eureka lemon leaf.*

and sour orange—in all of which it produced necrotic local lesions. The 2 viruses were not separated from a mixture by band centrifugation in CsCl (Fig. 3).

No heterologous reaction was obtained in the Oucterlony test between TNV-CT and TNV antiserum; under similar conditions, the homologous reaction between TNV and its antiserum was strongly positive. Several serotypes of TNV are, however, known. Recent—February 1970—serological tests carried out in collaboration with Dr. B. Kassanis, show that TNV-CT belongs to the D group. It produces angular necrotic lesions similar to those produced by strain TNV-E in leaves of French bean. TNV-CT activates the Rothamsted strains of satellite virus SV1 and SV2 but not the American strain SVC.

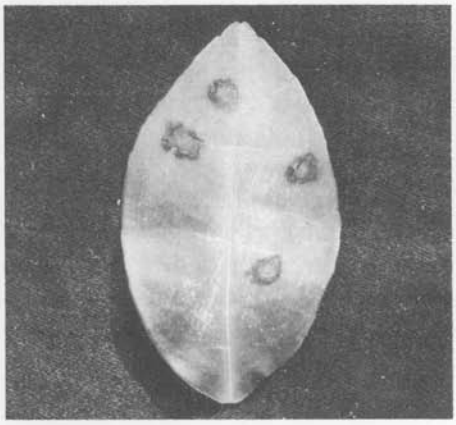

FIGURE 2. *Necrotic local lesions in an Orlando tangelo leaf inoculated with a purified preparation of the citrus isolate of TNV.*

Discussion

TNV is the second virus naturally occurring in herbaceous plants to be transmitted experimentally to citrus; the first is potato mottle virus, which was demonstrated by Holmes (1) to induce chlorotic lesions in inoculated leaves of sweet orange, Orlando tangelo, and Meyer lemon

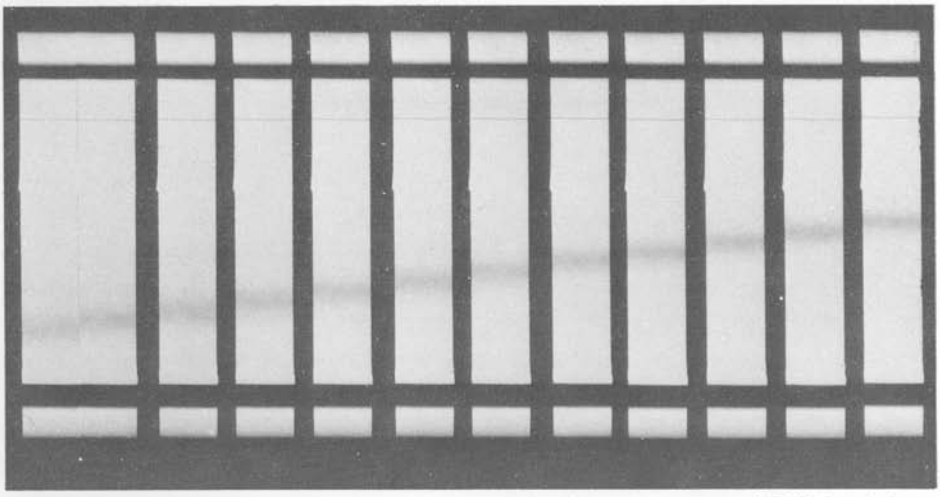

FIGURE 3. *Band sedimentation pattern of a mixture of equal amounts of TNV (strain from Dr. M. K. Corbett) and of the citrus isolate of TNV. Sedimentation velocity, 20,410 rpm.*

—from which it was recovered by subinoculation to tobacco.

Even though the exact identity of TNV-CT has not been definitely established, its properties, infectivity, and serology thus far studied make it extremely likely that TNV-CT is a strain of TNV. Two questions then arise: was TNV-CT present in the citrus leaves used to obtain the partially purified extract and, if so, what is the relationship of TNV-CT to psorosis virus?

TNV-CT was obtained when leaves of the following plants were used as sources: Orlando tangelo seedlings growing in Corsica and infected with cristacortis and concave gum psorosis viruses; sour orange seedlings infected with the same inoculum as the tangelo seedlings, but growing in the greenhouse in Versailles; and Eureka lemon seedlings infected with psorosis virus (Code 340), and also growing in the greenhouse in Versailles.

When leaves of healthy sour orange and lemon seedlings from Corsica and Versailles were used under the same conditions, TNV-CT was not encountered. It was encountered only when young leaves with psorosis symptoms were used as a source of purified material; this evidence is, of course, only circumstantial and may not be of significance. Even though our results establish that citrus is a local lesion host of TNV, additional experiments need be performed to ascertain the origin of TNV-CT and its possible relationship to psorosis virus.

Literature Cited

1. HOLMES, F. O. 1959. Transmission of potato mottle virus to, and from, citrus plants by mechanical inoculation. Phytopathology 49: 729–31.

2. VOGEL, R., and BOVÉ, J. Relation of cristacortis virus to other citrus viruses. In this volume.