Purification of Viruses of Citrus Mosaic and Navel Orange Infectious Mottling

H. Tanaka and J. Imada

Citrus mosaic (CiMV) was found in the 1940s in Wakayama Prefecture and was first described as a virus disease by Ishigai (1958). In addition to symptoms similar to Satsuma dwarf such as leaf malformation, multiple flushes, and dwarfing, affected trees developed ringspots and mosaic on rinds of fruit at the coloring stage. Navel orange infectious mottling (NIMV) has been known in Wakayama Prefecture since the 1930s. Symptoms of the disease consist of large diffused chlorotic blotches and mottling in the leaves, with brown spots on the under surfaces (Tanaka *et al.*, 1971). The casual viruses of these two diseases (CiMV and NIMV) are easily transmitted from affected trees to citrus seedlings by graft inoculation (Tanaka and Yamada, 1972) and to herbaceous plants by mechanical means (Tanaka and Imada, 1974*a*). The host range of these two viruses is similar to that of Satsuma dwarf virus (SDV).

This paper reports purification of CiMV and NIMV, and contrasts the morphology of these two viruses with SDV.

MATERIALS AND METHODS

Virus sources. The sources of CiMV and NIMV were trees maintained at the Laboratory of Plant Pathology, Akitsu Branch, Fruit Tree Research Station, and were propagations of diseased field trees in Wakayama Prefecture.

Test Plants. Plants of *Physalis floridana* served as production hosts for these two viruses. White sesame plants were used for assaying preparations of CiMV and NIMV, and *Nicotiana rustica* plants for the assay of NIMV.

Purification procedure. Purification of CiMV followed the same procedure described for SDV (Tanaka and Imada, 1974*b*). For purification of NIMV, a modified procedure from that for SDV and CiMV was employed. After grinding NIMV-affected *P. floridana* tissue with cold 0.1 M citrate buffer solution containing 0.1 per cent thioglycolic acid (1:3 ratio), the extract was mixed with one-fifth volume of Mg-treated bentonite solution, thoroughly shaken for 15 minutes with a magnetic stirrer, and centrifuged for 15 minutes at 13,500 *g*. The supernatant fluid was mixed with one-fifth volume of cold CCl_4, shaken for 15 minutes by hand, and centrifuged for 15 minutes at 13,500 *g*. Ammonium sulfate was added to the supernatant until it reached a concentration of one-third saturation. After stirring 30 minutes at 4°C, a yellowish flocculent precipitate was produced, which was concentrated by centrifugation for 15 minutes at 13,500 *g*., and then resuspended in 5×10^{-3} M borate buffer solution. The suspension was then dialized against 5×10^{-3} M borate buffer solution for 24 hours. The dialyzate was given two cycles of low speed (5,000 *g*, 10 minutes) and high speed (140,000 *g*, 90 minutes) centrifugation, and then fractionated by density-gradient centrifugation. The material showing UV absorbance at 260 nm was collected.

Electron microscopy. Specimens for electron microscopy were prepared from density-gradient samples. Preparations were negatively strained with 1.0 per cent PTA and examined with an Hitachi, Model HS-9, electron microscope.

RESULTS AND CONCLUSIONS

Rate zonal sedimentation of each partially purified preparation of CiMV and NIMV from sucrose density-gradients produced a faint opalescent band about 20 mm below the meniscus. Each band gave a typical ultraviolet absorption spectrum for nucleoprotein with a maximum at 260 nm, and minimum at 240 nm. The UV-absorbing material of CiMV infected both sesame and *N. rustica* seedlings. Electron microscopy of CiMV and NIMV preparation revealed uniform spherical particles about 27 and 23 nm in diameter, respectively (fig. 1). Particles of CiMV were of the same dimension as those of

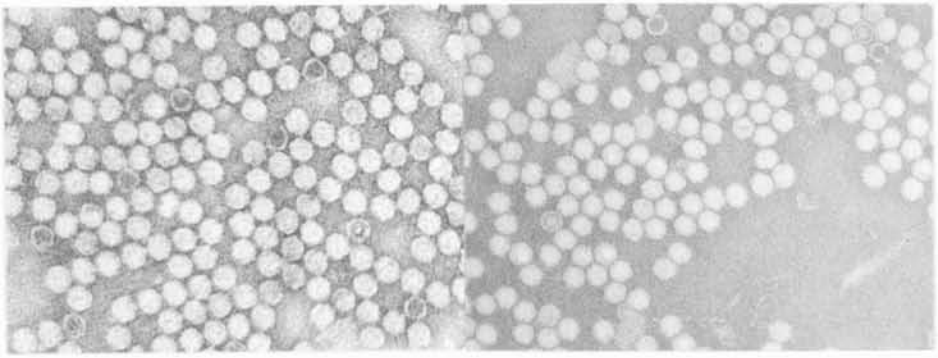

Fig. 1. Electron micrograph of virus particles of citrus mosaic (left) and navel orange infectious mottling (right) (x120,000).

SDV. Particles of NIMV however, were smaller than those of SDV and CiMV.

Symptoms induced in plants of sesame and *N. rustica* inoculated with purified preparations of CiMV and NIMV are identical to symptoms in plants inoculated with viruses extracted from affected citrus leaves. It would seem then that these two purified viruses may be the causal agents of citrus mosaic and navel orange infectious mottling. Back inoculations of these viruses to citrus seedlings are now being conducted. It is also of interest to us to know if CiMV and NIMV are strains of SDV. Further experiments are being planned.

ACKNOWLEDGEMENT

Grateful acknowledgement is made to Dr. L. G. Weathers, University of California, Riverside for reviewing the manuscript.

LITERATURE CITED

ISHIGAI, T.
1958. A virus-like disease of citrus. Kajitsu Nippon, 13:25-26.

TANAKA, H., AND J. IMADA
1974*a*. Mechanical transmission of viruses of satsuma dwarf, citrus mosaic, navel orange infectious mottling and Natsudaidai dwarf to herbaceous plants, p. 141-45. *In* L. G. Weathers and M. Cohen, (eds.), Proc. 6th Conf. Intern. Organization Citrus Virol. Univ. California Div. Agr. Sci., Berkeley.

TANAKA, H., AND J. IMADA
1974*b*. *Physalis floridana*, a good production host for satsuma dwarf virus. Plant Dis. Rep. 58:603-05.

TANAKA, H., AND S. YAMADA
1972. Evidence for a relationship among the viruses of Satsuma dwarf, citrus mosaic, navel infectious mottling, Natsudaidai dwarf, citrus variegation, and citrus crinkly leaf, p. 71-76. *In* W. C. Price, (ed.), Proc. 5th Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.

TANAKA, H., S. YAMADA, AND K. KISHI
1971. Symptoms and occurrence of navel orange infectious mottling and Natsudaidai dwarf. Bul. Hort. Res. Station, Series B, (Hiratsuka) 11:141-47.