# Host Range, Purification, Serology, and Properties of a Carlavirus from Eggplant

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## ABSTRACT

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Mottling of leaves of eggplant (*Solanum melongena*) was found to be related to virus infection. The disease agent was placed in the carlavirus group on the basis of particle length (693 nm) and the lack of cytoplasmic inclusions in crude preparations. The virus is readily transmitted mechanically; it induces leaf mottle and mosaic in various eggplant cultivars and mottle, mosaic, or leaf necrosis in tobacco species and cultivars. Susceptible hosts were found in five of the 26 plant families tested and the largest number of suscepts were in the Solanaceae. The virus was purified by clarification of root extracts in butanol followed by differential centrifugation. The virus was neither transmitted by seeds obtained from diseased eggplants nor by *Myzus persicae*. It was inactivated after 10 min at 70 C, 7 days at 25 C, and in dilutions ranging from $10^{-3}$ to $10^{-4}$. Purified preparations of virus were immunogenic with antisera titers of 1:32 obtained as determined by immunoelectron microscopy and microprecipitin tests.

Eggplant (*Solanum melongena* L.) is grown in many parts of the world as a vegetable crop. A number of virus and mycoplasmalike diseases occur on eggplant in nature (6). Observations of eggplants grown near Daid, Al-Sharja (a United Arab Emirate) in 1975 revealed that about 15% of the plants in the field showed a leaf mottle symptom. This symptom was shown to be associated with a virus and a brief description of its properties has been reported (7). This report presents more details of the biological and physical properties, purification, and serology of the virus.

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## MATERIALS AND METHODS

The virus was originally transferred from eggplant to *Nicotiana tabacum* L. 'Havana 425' and 'Xanthi-nc.' These tobacco cultivars served as a virus source throughout this study, while *Chenopodium*

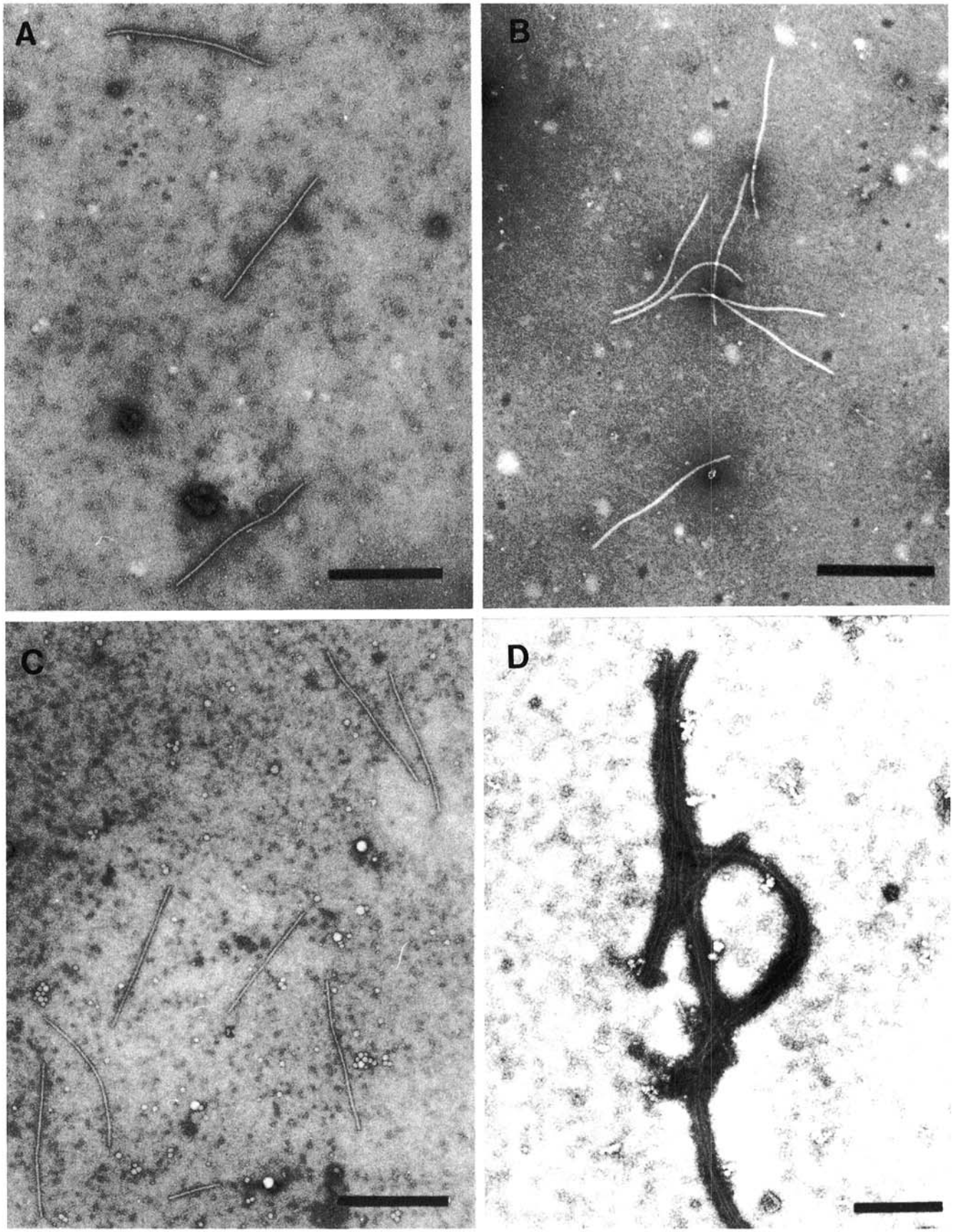


**Fig. 1.** Electron micrographs of eggplant carlavirus showing virus particles from tobacco leaves and serological reactions; negatively stained with 2% phosphotungstic acid (pH 7.0) (Scale bar = 500 nm). **A and B,** virus in crude sap and purified preparations, respectively; **C,** virus mixed with normal serum (1:5 dilution); **D,** virus mixed with homologous antiserum (1:5 dilution).

*amaranticolor* Coste and Reyn. served as a local-lesion host.

**Host range.** Five to 20 plants of 137 species and cultivars from 26 families were mechanically inoculated by using extracts from systemically infected leaves of Havana 425 or Xanthi-nc tobacco plants in two volumes of 0.1 M phosphate buffer (pH 7.0). Test plants were rubbed with this extract after addition of carborundum to the inoculum and evaluated on the basis of symptom development, detection of characteristic virus particles in tissue extracts by electron microscopy, and the appearance of local lesions when assayed on *C. amaranticolor.*

**Stability in sap.** Virus was extracted from leaves of Xanthi-nc tobacco 15–20 days after inoculation by trituration of tissue in 0.1 M phosphate buffer (pH 7.0; 2 ml/g of tissue) with a mortar and pestle. The extract was filtered through double layers of cheesecloth before tests were conducted.

Crude extracts were diluted in 0.1 M phosphate buffer (pH 7.0) for infectivity dilution end point studies. Ten leaves of *C. amaranticolor* were inoculated with samples from each dilution in each of two trials.

Thermal inactivation studies were conducted by heating separate 2.0-ml samples of crude extract for 10 min at temperatures from 50–80 C. Subsequently, each sample was cooled in an ice bath and then used to inoculate 10 leaves of *C. amaranticolor* in each of two trials.

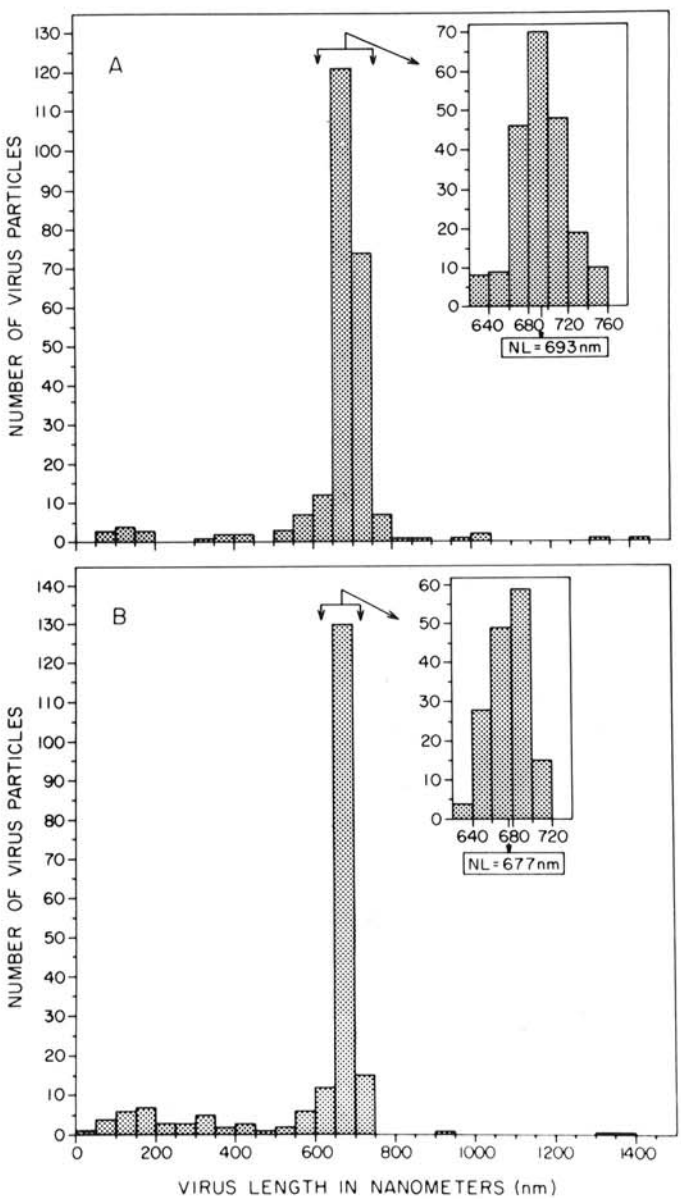


**Fig. 2.** Particle length distribution of eggplant mild carlavirus from tobacco; **A,** virus from crude leaf sap and **B,** purified from leaves.

**Seed transmission.** Seeds were harvested from diseased plants of *N. glutinosa* L., *N. tabacum* L. cultivars Havana 425 and Xanthi-nc, *Physalis floridana* Rydb., and *S. melongena* L. 'Blacknite Hybrid,' 'Burpee Hybrid,' 'Mission Bell Hybrid,' 'Pompano Pride,' and 'Royal Knight Hybrid,' planted in sterilized soil and the resulting seedlings were observed for symptoms over a 2-mo period. Extracts of suspicious seedlings were checked for virus particles in the electron microscope. Otherwise, the striking (but mild) virus symptoms were considered adequate for detection of infected progeny.

**Insect transmission.** *Myzus persicae* Sulz. was reared on healthy eggplant Blacknite Hybrid, healthy *N. tabacum* Xanthi-nc, or on diseased plants of eggplant cultivar Tenderette. Aphids reared on Xanthi-nc tobacco were starved for 3.0–3.5 hr and then allowed to feed for 5, 10, 15, 20, or 25 min on leaf disks from diseased Xanthi-nc tobacco. Subsequently, five aphids from each plant were placed for 24 hr on separate healthy plants of Xanthi-nc tobacco and on *N. glutinosa.* Three replicates were used for each treatment. Aphids reared on Blacknite Hybrid eggplant were starved for 3 hr and placed on leaves of cultivar Tenderette plants for 0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, or 60 min and then transferred to Blacknite Hybrid plants for 24 hr. Six replicates were used for each treatment. Three replicates received five aphids per plant, and three replicates received one aphid per plant. Aphids reared on diseased plants of eggplant cultivar Tenderette were also used for mass transfer. In one trial with 10 replicates, diseased leaves, carrying an unknown number of aphids, were cut and placed on Blacknite Hybrid plants for 48 hr. In another trial with 10 replicates, 15 aphids were transferred with a camel's hair brush to Blacknite Hybrid plants for 48 hr of feeding.

**Purification.** Various clarification techniques were used in attempts to extract the virus from diseased Havana 425 and Xanthi-nc tobacco. Unless otherwise indicated, the virus was extracted from leaves or roots by homogenization of tissue in 0.1 M phosphate buffer (pH 7.0; 2 ml/g of tissue) in a Waring Blendor. Results of preliminary experiments with a variety of denaturants and procedures (*n*-butanol, chloroform, freezing, charcoal, or low pH) indicated that clarification was best accomplished by stirring with *n*-butanol (8% v/v, 30 min). Virus was purified by two cycles of differential centrifugation (10,000 *g,* 10 min; 100,000 *g,* 2 hr).

Virus yield was estimated from the absorbance at 260 nm ($A_{260nm}$). This estimate was checked by electron microscopy of dilutions of the preparation to ensure that changes in particle number were proportional to changes in $A_{260nm}$.

**Electron microscopy.** Electron microscopic examinations were conducted with either Hitachi HS-7S or H-500 electron microscopes. They were calibrated by the use of a carbon grating replica with 2,160 lines per millimeter (Fullam No. 1002, Ernest F. Fullam, Inc., Schenectady, NY 12301); the resulting magnifications were frequently checked against purified preparations of tobacco mosaic virus (TMV) as an internal standard.

Four drops of crude sap from infected plants or purified virus from tobacco roots and leaves, were mixed on a glass slide with four drops of 4% phosphotungstic acid (PTA) (pH 7.0). Carbon-coated grids were placed on the surface of the stained virus preparation for a few seconds. Grids were removed, touched gently with a piece of filter paper to remove excess liquid, and examined with the electron microscope.

**Serology.** Antiserum against virus was produced in rabbits by intramuscular injection of 1 ml of partially purified virus (1.4 mg of virus per milliliter) mixed with 1 ml of Freund's incomplete adjuvant at weekly intervals for 4 wk. Serum was collected 7 days after the last injection.

Serological reactions between antigen and antiserum were examined in the electron microscope (1), by double diffusion in agar gels containing sodium dodecyl sulfate (SDS), by microprecipitin, and by ring-interface precipitin tests. The immunoelectron microscopic decoration technique was used to determine the serological relationships between the virus and three viruses in the carlavirus group, and with four viruses in the potyvirus group. Antisera to tobacco veinal mosaic virus (SC 148)

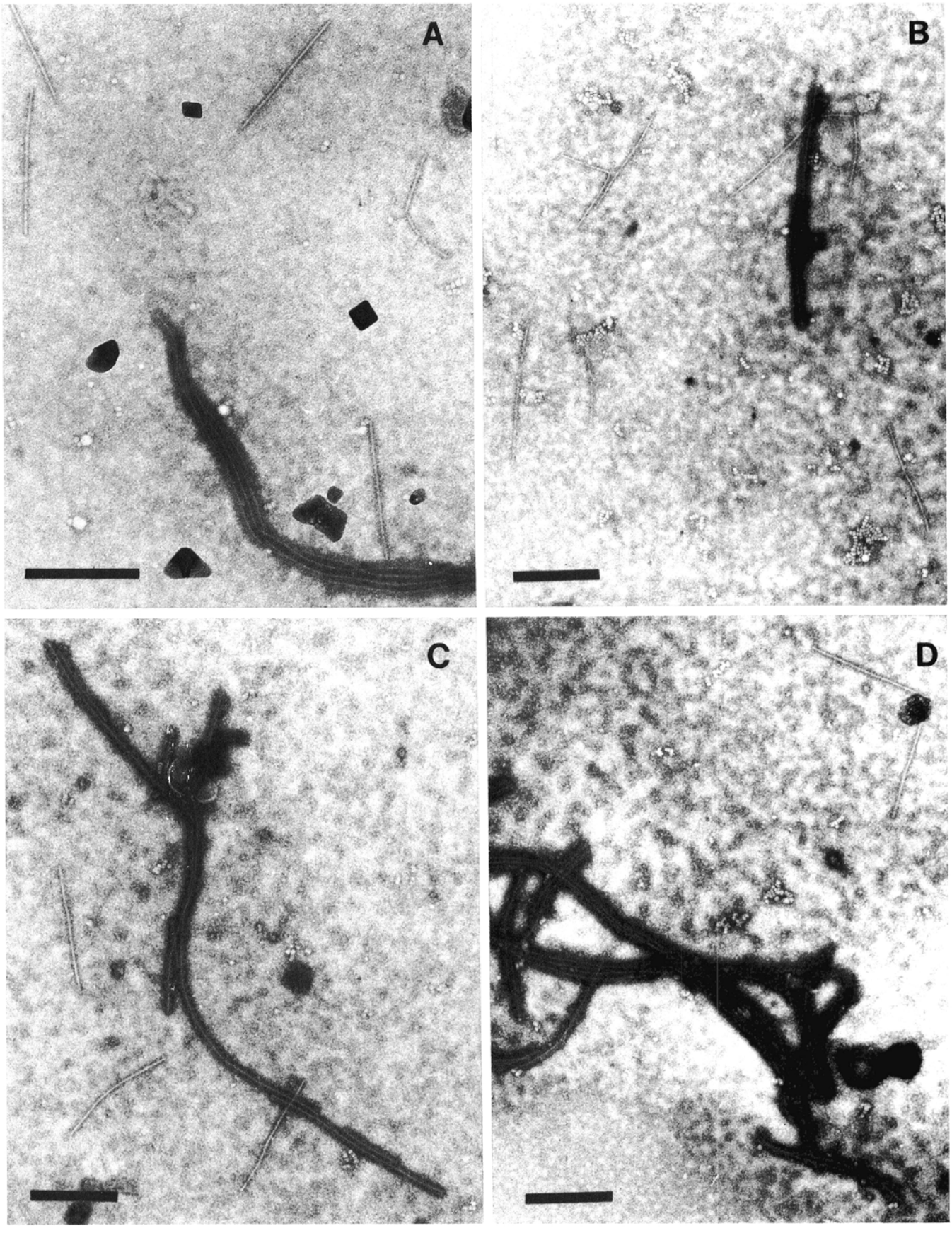


**Fig. 3.** Electron micrographs of eggplant carlavirus mixed with homologous antiserum (1:5 dilution), phosphotungstic acid (pH 7.0) and **A,** pea streak virus; **B,** red clover vein mosaic virus; **C,** pepper mottle virus; and **D,** tobacco etch virus (Scale bar = 500 nm).

and potato virus S (ATCC PV As 103) were tested in one-way tests against the antigen. Reciprocal serological tests were run with tobacco etch virus (ATCC PV69), potato virus Y (NC 57), pea streak virus (ATCC PV87), red clover vein mosaic virus (ATCC PV 110), and pepper mottle virus (Arizona and Florida strains).

## RESULTS

**Host range.** Fifty-four plant species and cultivars in five families were susceptible to the virus. A majority of the susceptible plants were in the genus *Nicotiana.* Such solanaceous hosts as pepper, potato, and tomato were not susceptible based on the absence of symptoms and failure to detect virus particles by electron microscopic examination of sap. Details of the host range studies have been reported elsewhere (6).

**Symptoms.** The largest group of susceptible plants was found in the genus *Nicotiana,* in which all susceptible species reacted to infection with an extreme shock reaction from which they failed to recover. This was manifested as chronic necrotic etch type symptoms, stunting and, with some species, death. Reaction of all eggplant cultivars to the virus was a "mild mottle."

**Stability in sap.** Infectivity was lost at a dilution of $10^{-4}$, by heating for 10 min at 70 C, or at room temperature for 6 days.

**Seed transmission.** No symptoms were noted on any of the following plants grown from seeds collected from infected plants: *N. glutinosa* (50 plants), *N. tabacum* L. Havana 425 (50 plants) and Xanthi-nc (100 plants), *P. floridana* Rydb. (140 plants), and *S. melongena* L. Blacknite Hybrid (276 plants), Burpee Hybrid (296 plants), Mission Bell (319 plants), Pompano Pride (117 plants), and Royal Knight (120 plants).

**Insect transmission.** *Myzus persicae* failed to transmit virus from infected Xanthi-nc tobacco to either healthy Xanthi-nc tobacco or *N. glutinosa.* It also failed to transmit the virus from infected plants of eggplant cultivar Tenderette to healthy plants of eggplant cultivar Blacknite Hybrid.

**Virus yield.** Roots of Havana 425 tobacco plants yielded up to 40 mg of virus per kilogram of virus-infected tissue, whereas leaves yielded a maximum of 7 mg of virus per kilogram of tissue.

**Electron microscopy.** Electron microscopic examinations of crude sap and purified virus preparations from diseased leaves and roots of tobacco showed that the virus particles were rod-shaped and slightly flexuous (Fig. 1A and B). The normal length of 246 virus particles from tobacco leaf crude sap was 693 nm, whereas the normal length of 76.4% of 203 virus particles from purified preparations was 677 nm, with a width of 12–13 nm (Fig. 2A and B) in each case. The preparations contained no inclusions as are commonly found in potyvirus preparations.

**Serology.** Immunoelectron microscopic serology (IEMS) illustrated lack of serological relationships with other viruses (Fig. 3A–D). Microprecipitin tests and ring-interface precipitin tests were also successfully used to detect serological reactions between virus and its antiserum, but the agar (with SDS) double-diffusion tests were not. Eggplant carlavirus antiserum titers were 1:32 or 1:16 as determined by IMS and microprecipitin tests, respectively. At these titers, reactions were very strong. At 1:64 and 1:32 respectively, noticeably weaker reactions occurred. Eggplant carlavirus was not serologically related to the following viruses: carlavirus group—pea streak (ATCC PV 87 and Arizona alfalfa strains), potato S (ATCC PV As 103), and red clover vein mosaic (ATCC PV 110); potyvirus group—pepper mottle (Arizona AzD and Florida strains), potato Y (NC 57), tobacco etch (ATCC PV 69), and tobacco veinal mosaic (NC 148). Criteria for determining relatedness was the decoration or lack of same on the virus particles. Contrast between these two conditions can be clearly seen in Fig. 3 A–D where reacting particles are mixed with nonreacting particles.

## DISCUSSION

The virus is provisionally placed in the carlavirus group because of its particle length (2,4,5) and lack of detectable cytoplasmic inclusions in crude preparations (3). Based on serological reactions, the eggplant carlavirus was not related to other viruses tested that were of similar size; eg, some carlaviruses and potyviruses. The antisera used were low in titer (1:32), but in the immuno-electron microscopic decoration technique the (+) and (−) serological reactions were readily discernible (Figs. 1 and 3) and in the opinion of the authors, provided a clear basis for establishing the presence or absence of serological relationships to the other viruses that were tested.

This carlavirus is different than other viruses described from eggplant such as alfalfa mosaic, cucumber mosaic, eggplant mosaic, tobacco etch, tobacco mosaic, tobacco ringspot, and others (6) on the basis of morphology, host reactions in the genus *Nicotiana* and serology. Full characterization of the virus will require further investigation of its biological and physicochemical properties.

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