Properties and Cytopathology of a Tymovirus Isolated from Eggplant

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ABSTRACT

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An isometric virus measuring 28 nm was isolated from eggplant (*Solanum melongena*) and mechanically transmitted to several species of the families Amaranthaceae, Chenopodiaceae, Labiatae, and Solanaceae. The virus was vectored by the flea beetle, *Epitrix fuscula*. Ultrastructural studies showed that chloroplasts of infected plants were clustered and formed tymovirus-characteristic double membrane-bound vesicles. In *Nicotiana rustica*, immunogold labeling studies showed that the largest numbers of virus particles were in the cytoplasm and vacuole of infected cells. Purified virus formed two bands in CsCl and sucrose gradients. The capsid contained a single polypeptide of about 24 kDa;

RNase digested the nucleic acid, which had a molecular weight of 2.0 $\times 10^6$ Da. The virus particles had one positively charged electrophoretic form, as demonstrated by migration toward the cathode during agarose electrophoresis. The virus is serologically related to tymoviruses of the subgroup of eggplant mosaic virus (EMV) and could not be distinguished from Abelia latent strain (EMV-AL). Several differences, however, were found in the studies of host range and symptomatology of these two strains. These differences and some other properties of the Arkansas isolate of EMV are reported in this paper.

In late summer of 1987, eggplants (*Solanum melongena* L.) in a home garden in Fayetteville, AR, were observed showing viruslike symptoms. These symptoms were severe mosaic, leaf crumpling, mild stunting, and fruit abnormalities including malformation, reduced size, reduced number, bitter taste, and hardening of the pulp. Plants showing similar symptoms were found during 1988 in the same garden. Preliminary electron microscopic studies showed that eggplants were doubly infected with a tymovirus and a possible rhabdovirus.

In 1988, tymovirus-infected eggplants occurred in four out of five gardens surveyed in Fayetteville. Unlike the original doubly infected plants, these new plants showed only a mild stunting, and electron microscopy showed them to be infected with the tymovirus alone.

In the United States, only one eggplant mosaic virus (EMV), the Abelia latent strain of eggplant mosaic virus (EMV-AL), has been demonstrated to infect eggplant (15,30). Recently, three other strains of eggplant mosaic virus (EMV) have been reported from South America, two of which were shown to infect eggplant (1,22); eggplant was not mentioned in connection with the third (8).

The purpose of our work was to characterize the tymovirus responsible for a latent infection of eggplant in Fayetteville, AR. The virus found in eggplant from Fayetteville is different from the new South American strains. Although it was shown to be serologically indistinguishable from EMV-AL, it is different from EMV-AL in a number of biological properties and will be referred to as eggplant mosaic virus-Arkansas strain (EMV-AR). Preliminary results have been published (4).

MATERIALS AND METHODS

Virus sources and maintenance. Leaves collected from a field-grown eggplant showing severe mosaic symptoms caused by a double infection with a tymovirus and a rhabdovirus were the source of the virus in preliminary tests. Mechanical inoculation of sap to Carborundum-dusted leaves of *Nicotiana rustica* L. resulted in transmission of only the tymovirus. After seven successive local lesion transfers to *N. rustica*, the virus was maintained in this host and in eggplant. The tobacco strain of eggplant mosaic virus (EMV-T) and tomato white necrosis virus (TWNV), two South American strains of EMV, were obtained from E. W. Kitajima (Instituto de Biologia, Universidade de Brasilia, Brazil) and maintained in *Nicotiana tabacum* L. and *Datura stramonium* L., respectively.

Host range and transmission tests. Carborundum-dusted leaves of test plants were mechanically inoculated with inoculum prepared by macerating leaves of infected eggplant or *N. rustica* in deionized water. To determine the host range of EMV-AR, at least five plants of each species or cultivar were inoculated. All plants were evaluated for symptom expression for 8 wk and tested serologically with antiserum to EMV-AR by Ouchterlony double-diffusion tests. Back-inoculations to *N. rustica* followed by serological tests were done when the results of serology were not clear.

Seed transmission tests were done with seeds collected from systemically EMV-AR-infected eggplant (Black Beauty and Long Black), *Chenopodium quinoa* Willd., *D. stramonium*, *Lycopersicon esculentum* Mill. (VF Hybrid and Long Keeper), *N. clevelandii* Gray, *N. glutinosa* L., and *N. rustica*. Seeds were sown in sterile potting medium. Seedlings (50–100) of each species were picked at random after 1 and 2 mo and individually assayed for virus by Ouchterlony double-diffusion tests. Eggplant was more extensively investigated. From infected plants of both cultivars mentioned, 699 seeds were collected from four different fruits. Out of 423 Long Black and 276 Black Beauty, respectively, 367 and 130 seeds germinated, 50% of which were randomly tested for the presence of the virus.

Insect transmission tests were carried out with flea beetles (*Epitrix fuscula* Cr.) collected from eggplants in the field from May through July of 1988. The beetle was identified by Drs. R. T. Allen and C. E. Carlton, University of Arkansas, Fayette-

ville. After a period (3–7 days) on healthy eggplant seedlings, individual beetles were given an acquisition access period of 24–48 hr on detached leaves of EMV-AR infected eggplant (cultivars Black Beauty or Long Black) and then transferred to healthy, 2-wk-old seedlings of the same cultivar. The insects were individually transferred to new test hosts every 24 hr for a total of 4 days. In two separate experiments, 160 seedlings and 72 beetles were used, but only the 61 beetles that fed on the infected leaves during the acquisition period were used until the end of the experiment. All plants were assayed individually for the presence of virus, 1 and 2 mo later, by Ouchterlony double-diffusion tests.

Physical properties. Thermal inactivation point (TIP), longevity in vitro (LIV), and dilution end point (DEP) were determined with sap extracted from systemically infected *N. rustica* and treated as described by Walkey (29). Temperatures between 50 and 100 C, at 5 C intervals, were tested for determining the TIP. LIV was determined at room temperature. Phosphate buffer (0.01 M, pH 7.0) was used only for determining the DEP. The sap aliquots were inoculated onto *N. rustica*, the local lesion assay host. Infection was assessed visually, and the plants were individually tested by Ouchterlony double-diffusion tests 2–4 wk after inoculation.

Purification. EMV-AR, EMV-T, and TWNV were purified from *N. rustica*, *N. tabacum*, and *D. stramonium*, respectively, by the low pH method described for desmodium yellow mottle virus, another tymovirus (23,24). The high-speed centrifugation times were increased, however, to 2.5 hr to sediment the top component. The viruses were further purified by centrifugation in sucrose density gradients (10–40%) for 4 hr at 76,000 g or in CsCl isopycnic gradients for 18–22 hr at 140,000 g in 0.1 M phosphate buffer, pH 7.2. Virus concentrations were calculated based on an extinction coefficient of 9.0 previously determined for the bottom component of EMV-AL (30). The buoyant density of EMV-AR was determined for both top and bottom components collected separately after equilibrium centrifugation in CsCl. The refractive index was read in an ABBE Mark II digital refractometer (A.O. Reichert, Buffalo, NY) and converted into buoyant density (9). Percentage of RNA was estimated with the formula developed by Sehgal et al (25).

Serology. Antiserum against EMV-AR was obtained by weekly subcutaneous injections of a rabbit with 2.6 mg/ml purified virus. The first injection was with virus emulsified in Freund's complete adjuvant (Difco, Detroit, MI), whereas the two subsequent injections contained virus emulsified with incomplete adjuvant. The antiserum was collected by bleeding the rabbit at weekly intervals for 7 wk starting 1 wk after the last injection. Antiserum titer was determined by reacting twofold serial dilutions of the antiserum with undiluted sap of EMV-AR-infected *N. rustica* in Ouchterlony gel double-diffusion tests. The gels consisted of 1% agarose in water with 0.02% NaN₃. Serological relationships were determined in Fayetteville, AR, and in Braunschweig, West Germany. In Fayetteville, Ouchterlony double-diffusion tests were used after intra-gel cross-absorption of antisera with healthy plant sap. Undiluted expressed sap from plants infected with various tymoviruses was reacted with dilutions of homologous and heterologous antisera to these viruses. In addition, antisera to strains of APLV (Andean potato latent virus) and to BMV-LANE (belladonna mottle virus, LANE strain) were tested in Braunschweig by Dr. R. Koenig as previously described (13).

Chemical properties. Capsid proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (17) using a minislab system (5) with 12 or 15% resolving and 5% stacking gels (30/0.8 acrylamide/bisacrylamide). Capsid protein was prepared from purified EMV-AR and stained with Coomassie Brilliant Blue (28) or labeled with MDPF (2-methoxy-2, 4-diphenyl-2[2H]-furanone) for observation under ultraviolet (UV) light (5,18). Capsid proteins from EMV-T and TWNV were prepared simultaneously with EMV-AR protein and included in the gels for comparison. Molecular weight standards were purchased from Bio-Rad (Richmond, CA).

Nucleic acid was extracted by the phenol method described by Larsen et al (19), but bentonite was omitted, and the sample was incubated for 2–3 min in the extraction buffer, which consisted of 0.1 M Tris-HCl, 1.5% SDS, 1.5% N-lauroylsarcosine, and 0.01 M EDTA, pH 8.0. Nucleic acid was pelleted by centrifugation (12,000 g, 10 min), washed twice with 70% ethanol, dried, and stored dry or resuspended in sterile, double-distilled water at −20 C.

The type of nucleic acid was determined by subjecting the extracted viral nucleic acid to DNase and RNase-A digestion. For DNase digestion, the nucleic acid (in water) was incubated for 30 min at room temperature with 22 Kunitz units per milliliter (16) of RNase-free DNase (Boehringer Mannheim GmbH, West Germany) in 0.2 M NaCl, 0.1 M Tris-HCl, 0.01 M MgCl₂, pH 7.5. For RNase digestion, the nucleic acid preparation was incubated with 10 μg/ml RNase-A (Sigma) in water at room temperature for 30 min. Both nuclease reactions were stopped by adding 50 μg/ml Proteinase K (E. Merck, Darmstadt, Germany) in 0.01 M Tris-HCl (pH 7.8), 0.005 M EDTA, and 0.5% SDS and incubating at room temperature for 30 min (21), or for 2 hr at 4 C (all concentrations given are final concentrations in the reaction mixture). Samples were electrophoresed at 100 V for 70 min at room temperature in a 3-mm horizontal agarose gel (1.2% agarose in 0.02 M HEPES, 1 mM EDTA, pH 7.0). Activity of the enzyme preparations was confirmed by a microtiter nuclease assay (MNA) as described by Burke and Slinker (2).

The molecular weight of the nucleic acid was determined by electrophoresis as described by Ribeiro (22) with glyoxal as the denaturing agent, and acridine orange as the stain (3). RNA from tobacco mosaic virus (TMV) both denatured and nondenatured, as well as denatured RNA from EMV-T, were used as standards.

To determine the surface charge of EMV-AR, purified whole virus particles were electrophoresed in 1% agarose gels for 18–20 hr, stained with ethidium bromide, and visualized on a UV transilluminator as described by Serwer et al (26).

Electron microscopy. The morphology of EMV-AR was determined by leaf dips of infected plants or purified virus preparations that were negatively stained with 2% aqueous uranyl acetate or 2% neutral phosphotungstate for 2–4 min. Purified TMV particles were added to purified virus preparations to serve as internal standards for size determinations; 100 particles of each virus were measured from micrographs magnified to 100,000×.

For in situ observations of EMV-AR and its cytopathology, small leaf pieces from healthy (controls) and infected *C. amaranticolor*, eggplant, *N. glutinosa*, and *N. rustica* were prepared for thin sectioning as described by Kim and Fulton (11). Leaf pieces of the original, doubly infected eggplant were

TABLE 1. The response of host plants to the Arkansas strain of eggplant mosaic virus

Host plants	Symptoms[a]	
	Local	Systemic
Capsicum frutescens L. 'California Wonder'	RS	M,S
Chenopodium amaranticolor Coste & Reyn.	CL,RS	M
C. quinoa Willd.	CL,RS	M
Datura stramonium L.	PPL,CL	M
Gomphrena globosa L.	PPL,CL	M,N
Lycopersicon esculentum Mill. 'VF Hybrid' and 'Long Keeper'	RS	SI,S
Molucella laevis L.	SI	S
Nicotiana benthamiana Domin.	SI	M,LC,RS
N. clevelandii Gray	SI	M,Y,S
N. debneyi Domin.	PPL,RS	M,Y
N. glutinosa L.	SI	M,Y
N. megalosiphon Heurck & Muell.	PPL,RS	M,Y
N. multivalvis Lindl.	PPL,RS	M,Y
N. rustica L.	PPL,RS	M,N,RS,S
Petunia hybrida Vilm.	PPL,RS	M,Y
Solanum melongena L. 'Black Beauty' and 'Long Black'	SI	SI,S
Vinca rosea L. 'Carousel' and 'Standard'	SI	M

[a]CL = chlorotic lesions; LC = leaf crumpling; M = mosaic; N = net pattern; PPL = pinpoint lesions; RS = ringspots; SI = symptomless infection; Y = yellowing; S = stunting.

similarly treated. To verify whether the particles observed in infected tissue were EMV-AR particles, tissue samples from healthy (control) and EMV-AR-infected *N. rustica* were subjected to immunogold labeling. Samples were processed as described above, but postfixation in osmium tetroxide and the en bloc staining with uranyl acetate were omitted. Spurr's medium (27) was the embedding resin. The sections collected on uncoated nickel grids were placed inside a drop of PBS (0.14 M NaCl, 1.5 mM KH_2PO_4, 1.5 mM Na_2HPO_4, pH 7.0) on Parafilm, transferred to a drop of PBS containing 0.1% bovine serum albumin (BSA) for 30 min, then to a drop of 1% BSA solution for 10 min and washed three times (1 min each) in PBST-BSA (PBS, 0.05% Tween 20, 0.1% BSA). Grids containing sections of infected tissue were then incubated for 30 min with either EMV-AR antiserum (1:100 dilution), or PBS alone (control 1), or rabbit preimmune serum (control 2); healthy tissue sections incubated with EMV-AR antiserum was the third control. After being washed with 10 drops of PBST-BSA (1 min in each drop), the grids were incubated with Protein A-gold (Sigma) (1:50 in PBS) for 30 min, washed again with 10 drops of PBST-BSA (1 min each), and rinsed under a stream of double-distilled water. All steps were done in a moist chamber. The grids were double-stained with uranyl acetate for 45 min and lead citrate for 10 min. Sections were examined with a JEOL 100 CX electron microscope.

RESULTS

Host range, symptomatology, and transmissibility. The hosts of EMV-AR, as determined by mechanical inoculation, as well as the symptoms induced in these hosts, are listed in Table 1. The susceptible plants were in the families Amaranthaceae, Chenopodiaceae, Labiatae, and Solanaceae.

As opposed to the doubly infected source eggplant, EMV-AR-infected eggplant was symptomless except for a slight stunting. EMV-AR-infected *N. rustica* developed very distinctive symptoms starting with pinpoint lesions on inoculated leaves 4–10 days after inoculation. Some of these enlarged to form white concentric ringspot lesions after 2–4 wk (Fig. 1B and C). Systemic symptoms were fully developed after a month and appeared as a netted mosaic or mottle with some reticulated yellowing on portions of the leaves (Fig. 1D). The tips of some leaves became distorted. Large ringspots were also seen occasionally on systemically infected leaves, and plants were slightly stunted when compared to controls.

Plants not infected by EMV-AR in mechanical inoculation tests were: *Cassia occidentalis* L.; *Cucumis melo* L.; *C. sativus* L. 'Model' and 'Early Pride'; *Cucurbita pepo* L. 'Early Prolific Straightneck'; *Diodia virginiana* L.; *Glycine max* (L.) Merr. 'Lee 74'; *Lunaria annua* L.; *Malva parviflora* L.; *N. sylvestris* Speg. & Comes; *N. tabacum* L. 'Havana 38', 'Kentucky 16', 'NC-95', 'Xanthi NN', 'X-73', 'Judy's Pride', and 'Turkish'; *Phaseolus vulgaris* L. 'Black Valentine', 'Pinto', and 'Cherokee Wax'; *Pisum sativum* L. 'Early Alaska'; *Poinsettia* sp.; *Sida carpinifolia* L.; *S. cordifolia* L.; *S. rhombifolia* L.; *S. santaremnensis* H. Mont.; *Solanum tuberosum* L.; *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'Monarch' and 'Georgia 21'; and *Zinnia elegans* Jacq.

Seedlings grown from seeds harvested from the systemically infected plants developed no symptoms, and EMV-AR could not be detected in these seedlings by serology.

The flea beetle, *E. fuscula*, transmitted EMV-AR. Typically a beetle transmitted EMV-AR once and did not retain infectivity for more than 24 hr. In a total of two experiments, 14/61 beetles (23%) transmitted the virus within the first 24 hr after acquisition. On days 2, 3, and 4 following acquisition, only 1/61, 2/61, and 2/61 beetles, respectively, transmitted EMV-AR.

Physical properties. The results of the TIP, LIV, and DEP experiments showed that EMV-AR was infective after 10 min at 75 C but not at 80 C, after standing at room temperature for 12 days but not 16 days, and after dilution of infectious sap to 10^{-6} but not to 10^{-7}.

Purification. Purification of EMV-AR from infected *N. rustica* yielded about 40 mg/100 g of leaf tissue. Two components were observed after rate zonal and equilibrium centrifugation. Electron microscopic examination of negatively stained preparations revealed that the top component (T) consisted mainly of particles penetrated by the stain (empty shells), while the bottom component (B) contained mostly unpenetrated particles. Both components were infective, indicating an incomplete separation of T and B components. Purified preparations showed a typical UV-absorption spectrum for nucleoproteins with a maximum at 260 nm and an $A_{260/280nm}$ of 1.71. Buoyant densities of T and B components were 1.26 and 1.44 g/cm³ respectively. RNA percentage, based on buoyant density data, was 34.82% for the B component.

Serology. The antiserum against EMV-AR had a titer of 1:320 in Ouchterlony gel diffusion tests. A reaction was observed with the undiluted antiserum and sap of healthy *N. rustica*; this reaction was not seen with antiserum dilutions of 1:10 or higher, or after intra-gel absorption with healthy sap from *N. rustica*. In Ouchterlony double-diffusion tests, no reactions occurred between EMV-AR antigen and antisera to the four tymoviruses of the TYMV-subgroup tested (Table 2). EMV-AR reacted positively with all the members of the EMV-subgroup tested in Fayetteville (Table 2) and in Braunschweig, except dulcamara mottle virus (DMV). Reciprocal serological tests with EMV-T, TWNV (Fig.
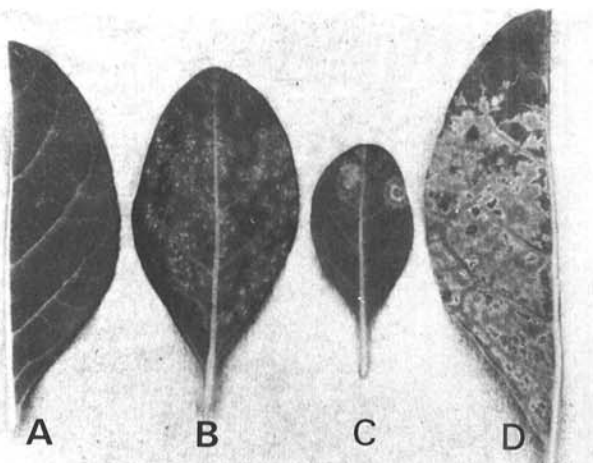

Fig. 1. Symptoms induced by eggplant mosaic virus-Arkansas in *Nicotiana rustica*. **A,** Healthy control. **B,** Inoculated leaf, 15 days after inoculation (DAI). **C,** Inoculated leaf, 22 DAI. **D,** leaf showing systemic mosaic, 30 DAI.

TABLE 2. Serological relationships of the Arkansas strain of eggplant mosaic virus (EMV-AR) with other tymoviruses as shown by Ouchterlony gel diffusion tests

Tymovirus antisera used		Source[a]	Serological reaction of EMV-AR[b]
EMV-subgroup of tymoviruses			
BMV-I	Belladona mottle virus (Iowa strain)	1	+
BMV-K	Belladona mottle virus (Kansas strain)	1	+
DMV	Dulcamara mottle virus	2	−
EMV-AL	Eggplant mosaic virus (Abelia latent strain)	3	+
EMV-T	Eggplant mosaic virus (tobacco strain)	5	+
EMV-type	Eggplant mosaic virus (type strain)	3	+
MRMV	Melon rugose mosaic virus	2	+
TWNV	Tomato white necrosis virus	5	+
TYMV-subgroup of tymoviruses			
CYMV	Cocoa yellow mosaic virus	2	−
DesYMV	Desmodium yellow mottle virus	4	−
PfYMV	Passionfruit yellow mosaic virus	5	−
TYMV	Turnip yellow mosaic virus	4	−

[a] The antisera were kindly supplied by: 1, Dr. R. F. Lee (Inst. of Food and Agric. Sci., Univ. of Florida); 2, Dr. P. Jones (Dept. Plant Pathol., Rothamsted Exp. Station, Harpenden, England); 3, Dr. R. Koenig (Inst. fur Viruskrankheit der Pflanzen, Biologische Bundesanstalt fur Land- und Fortwirtschaft, Braunschweig, West Germany); 4, Dr. H. A. Scott (University of Arkansas); 5, Dr. E. W. Kitajima (Instituto de Biologia, Universidade de Brasilia, Brazil).
[b] + = visible precipitin line, − = no visible precipitin line.

2), and BMV (Iowa and Kansas strains) showed positive reactions with formation of spurs. Serological tests run in Fayetteville and in Braunschweig indicated serological identity between EMV-AR and EMV-AL.

Chemical properties. Results of nine 12% and two 15% gels showed an average molecular weight of 23.9 kDa for the coat protein of EMV-AR when labeled with MDPF before the run and 23.2 kDa when stained with Coomassie blue. The estimates for the molecular weight of EMV-T (23.7 with MDPF and 23.97 with Coomassie blue) and TWNV (23.94 with MDPF and 24.27 with Coomassie blue) coat proteins were very close to EMV-AR values, and the bands formed by the proteins of all three viruses were always closely aligned in the gels independent of the staining procedure used. In most cases, the coat protein of EMV-AR was observed as a single band in the gels (Fig. 3), but in two instances, two bands were detected. These had molecular weights of 23.5 and 24.7 kDa, as estimated from the two gels. The occurrence of two closely migrating bands in electrophoresis of other tymoviruses is thought to represent a degradation of the coat protein upon storage (1), but in the case of EMV-AR coat protein it happened once with material electrophoresed shortly after purification of freshly harvested leaves, suggesting a possible degradation of the particles in vivo.

Nucleic acid from EMV-AR as well as from TMV (control) was digested by RNase but not by DNase, thus demonstrating that the nucleic acid of EMV-AR is RNA. The MNA confirmed that both the RNase and the DNase preparations were active and highly specific. The molecular weight of EMV-AR RNA was found to be 2.0×10^6 (average of four gels, under denaturing conditions), and the bands were always aligned with the bands of EMV-T RNA, whose molecular weight has been reported to be 2.0×10^6 (22). These values agree with the value reported for the type strain of EMV (7).

When whole virus particles were electrophoresed, only one electrophoretic form that migrated toward the cathode was observed.

Electron microscopy. In negatively stained preparations, EMV-AR particles appeared isometric (Fig. 4A) and had an average diameter of 27.7 nm (± 4.2) with two modal classes at 26 and 29 nm corresponding to the T and B components, respectively.

In thin sections of leaf tissues, particles were observed in varying amounts in the lumen of xylem vessels and the cytoplasm and vacuoles of all cell types except the mature sieve tube members. In highly disorganized cells, densely packed virus particles were seen and were occasionally in crystalline arrays. Double-membrane bound vesicles containing fibrils at the periphery of chloroplasts, a characteristic cytopathic feature of tymovirus infection, were invariably observed in infected cells of all plant hosts studied (Fig. 4B). Vacuoles of various sizes also occurred in some chloroplasts that were seen at various stages of disintegration. In *C. amaranticolor*, the particles present in cells of local lesions apparently reached much higher concentrations than in cells of systemically infected tissue, and many mesophyll nuclei contained large numbers of empty protein shells (Fig. 4C). Some empty shells were also observed in nuclei of leaf tissue from systemically infected *N. glutinosa*, but they were not found in the cytoplasm or in other cell organelles. In all hosts tested, some chloroplasts showed a typical alteration in the form of fingerlike projections (Fig. 5A and B) and/or myelinlike structures. Often chloroplasts were surrounded by large numbers of viruslike particles. In some instances, mitochondria were swollen or presented inflated crystae or a dense matrix. Clusters of rounded chloroplasts and mitochondria were observed in the cytoplasm of some host cells. Elongated crystals, such as those described for other strains of EMV (15,20,22) were only observed in the chloroplasts of *N. rustica* (Fig. 5A). None of these changes was observed in the healthy samples.

Most of the gold label in the immunogold-treated material was found on the cytoplasm and vacuoles of parenchyma cells in the infected tissues treated with the antiserum to EMV-AR (Fig. 5A). This confirmed the distribution of particles described above and provided direct evidence that the particles are EMV-AR virus particles. Fewer gold particles were observed over the chloroplasts, the nucleus, and the cell wall. No label was found in tissues treated with PBS alone, but some label was randomly scattered over tissues from EMV-AR-infected plants treated with preimmune serum (Fig. 5B, control 2) and healthy plants treated with EMV-AR antiserum (Fig. 5C, control 3), which indicated that there
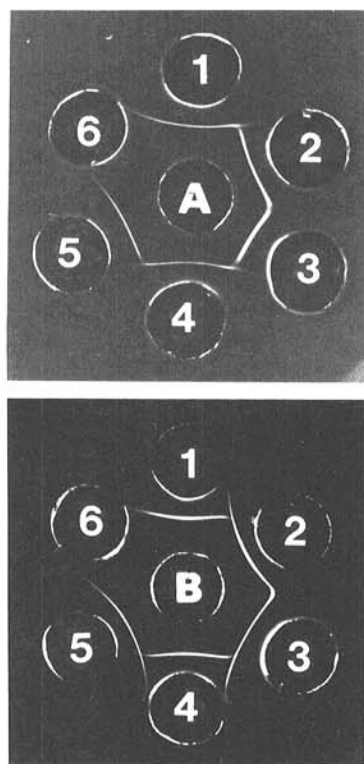

Fig. 2. Ouchterlony double-diffusion tests in 1% agarose gels containing 0.02% NaN₃ after cross absorption with sap of healthy *Nicotiana rustica* (**A**) and *N. tabacum* 'Turkish' (**B**). The center wells contain antisera against the Arkansas strain of eggplant mosaic virus (EMV-AR) (**A**) and tobacco strain of eggplant mosaic virus (EMV-T) (**B**). The external wells contain crude sap of: 1 and 4, EMV-AR-infected *N. rustica*; 2 and 5, EMV-T-infected *N. tabacum* 'Turkish'; 3, tomato white necrosis virus-infected *Datura stramonium*; 6, healthy *N. rustica* in A, and healthy *N. tabacum* 'Turkish' in B.
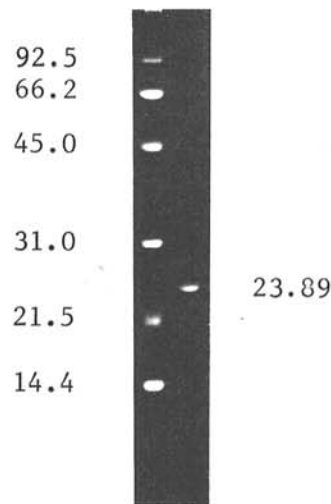

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the Arkansas strain of eggplant mosaic virus coat protein (right); molecular weight standards (left) in increasing molecular weights (kDa) are: lysozyme, soybean trypsin inhibitor, carbonic anhydrase, ovalbumin, bovine serum albumin, and phosphorylase B (Bio-Rad).

was some nonspecific binding of the Protein A-gold conjugate. This and the healthy reaction detected with the antiserum explain the presence of the few gold particles over chloroplasts and cell walls in the infected material treated with EMV-AR antiserum. The gold particles over the nucleus could represent a nonspecific reaction as well, since empty shells were not observed in nontreated *N. rustica*.

DISCUSSION

The results of this study show that EMV-AR is a member of the tymovirus group (6,14,15). Cytopathic effects such as the presence of double-membrane bound vesicles at the periphery of the chloroplasts, rounding and clumping of chloroplasts, and accumulation of empty shells in nuclei are diagnostic for these viruses (14,15,20). The electrophoretic behavior of EMV-AR particles, which migrate toward the cathode, and the serological tests confirmed that EMV-AR is a tymovirus related to other

viruses in the EMV-subgroup of tymoviruses (12,15). However, serological tests have showed that EMV-AR is not identical to BMV-I, BMV-K, BMV-LANE, EMV-T, TWNV, and several isolates of APLV, which are all members of the EMV-subgroup (12,15). In reciprocal serological tests with EMV-AR and EMV-AL antisera, EMV-AR could not be differentiated from EMV-AL, the only strain of EMV known to occur in North America. However, extracts from EMV-AL-infected plants were reported to react with antiserum to DMV (30), whereas those of EMV-AR-infected plants did not react with the DMV antiserum used in this study. As discussed by Koenig (12), however, a large number of antisera from different rabbits and several bleedings should be used to determine the serological relatedness of tymoviruses. Since only limited antisera were available, a thorough comparison of EMV-AR and EMV-AL was not possible.

Although the cytopathic effects of EMV-AR were similar to those described by Lesemann (20) for EMV-AL, there were some differences. For example, contrary to what happens with EMV-
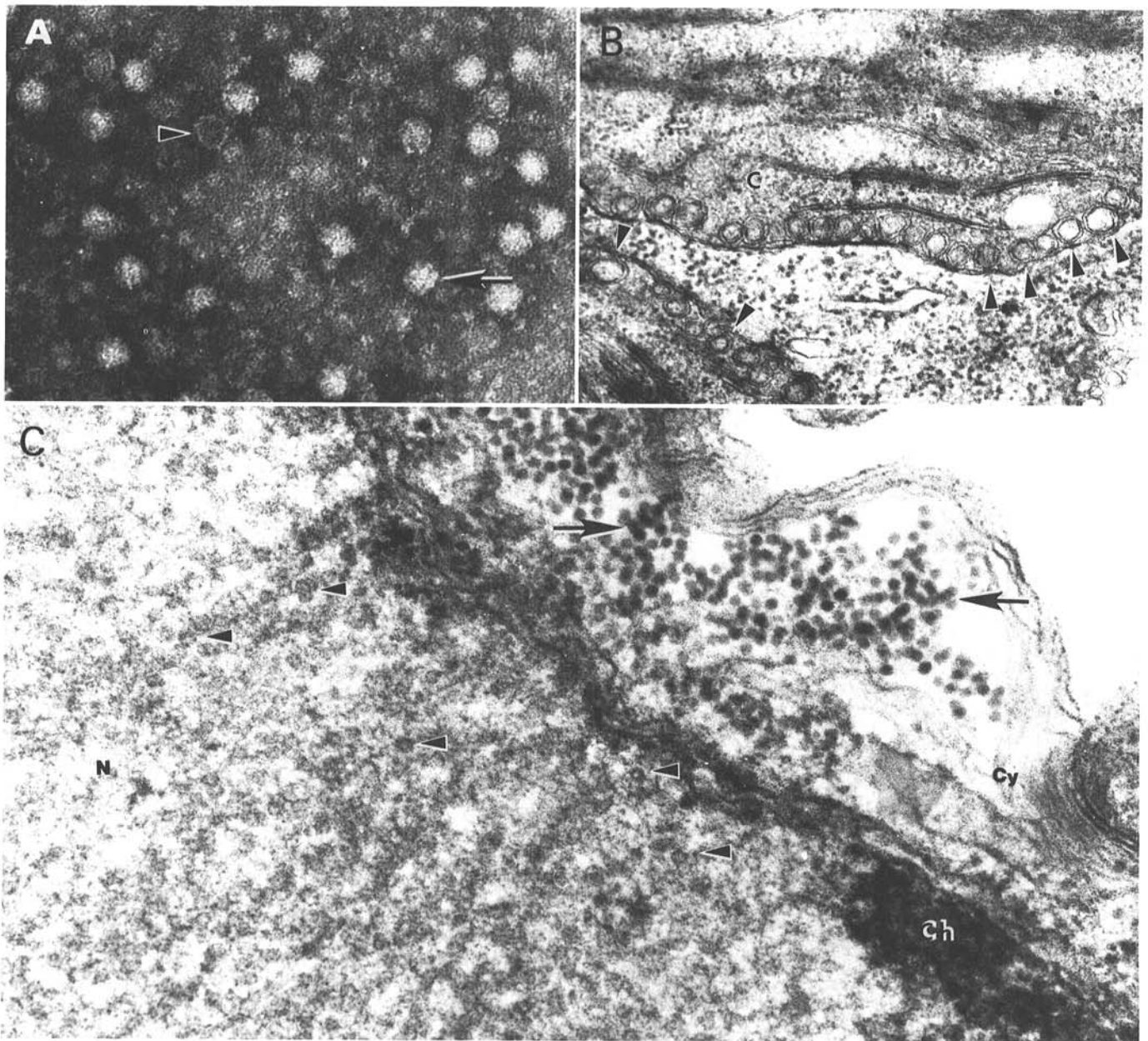

Fig. 4. Ultrastructure of the Arkansas strain of eggplant mosaic virus (EMV-AR). **A,** Leaf dip preparation from infected eggplant, showing complete particles (arrow) and empty shells (arrowhead) ×200,000. **B,** Chloroplasts (C) in vascular parenchyma cells of systemically infected *N. rustica*, showing peripheral double membrane-bound vesicles (arrowheads) characteristic of a tymovirus infection. ×52,000. **C,** Local lesion tissue from *Chenopodium amaranticolor*, showing part of a mesophyll cell infected by EMV-AR. Complete particles (arrows) appear in the cytoplasm (Cy) while empty shells (arrowheads) are seen in the nucleus (N). Ch = chromatin. ×100,000.
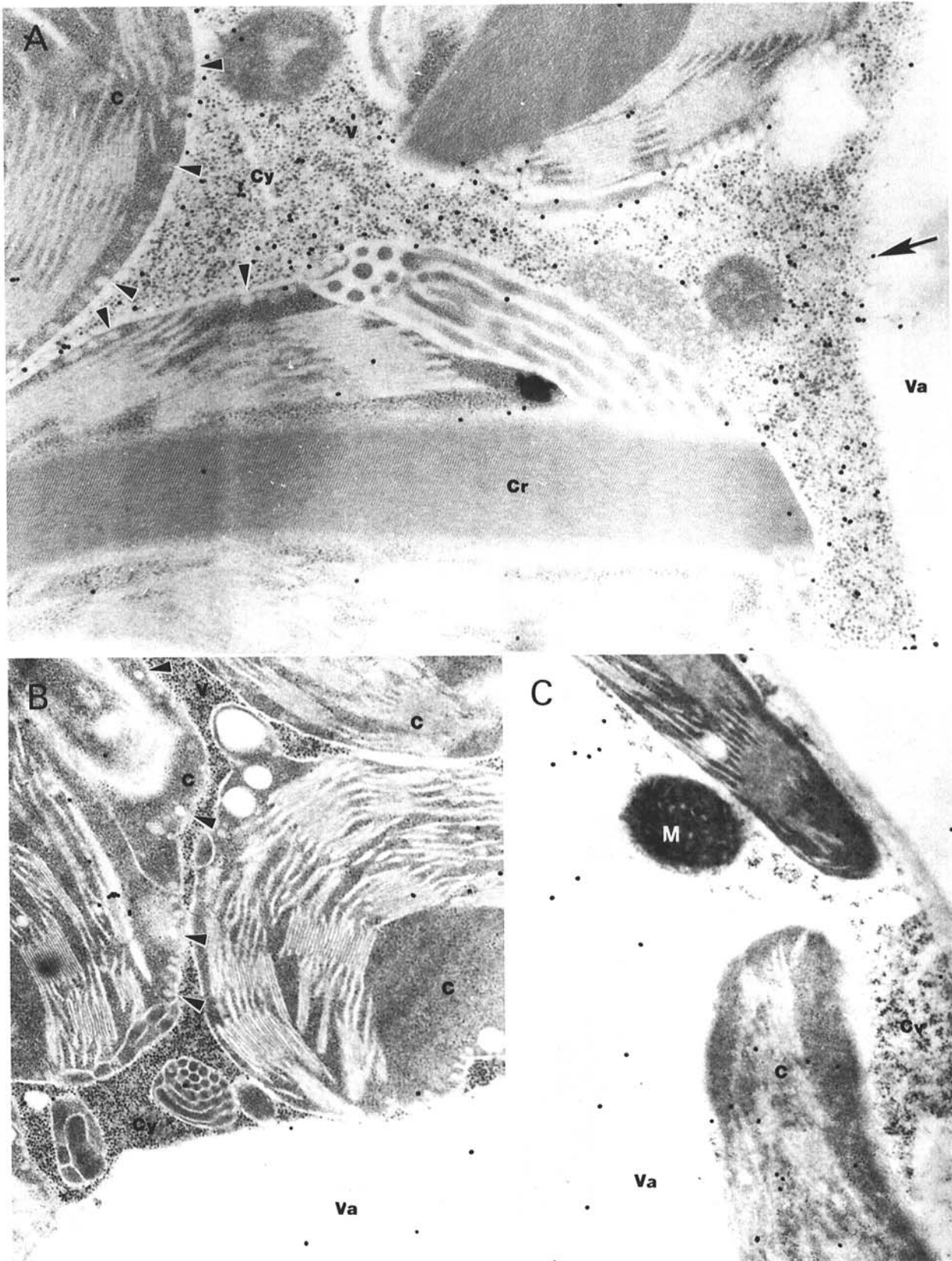
Fig. 5. Immunogold labeling (using 30 nm gold particles) of leaf tissue from infected and healthy *Nicotiana rustica*. **A,** 24 days after inoculation with the Arkansas strain of eggplant mosaic virus (EMV-AR), incubated with antiserum to EMV-AR before incubation with Protein A-gold. The higher concentrations of gold particles are seen over the cytoplasm, where high numbers of virus particles are discernible. Some nonspecific binding of the gold label occurred to the chloroplasts or chloroplast crystals. Arrowheads indicate chloroplast peripheral vesicles. The arrow points to one gold particle, which can be distinguished from the virus particles by its larger diameter and more electron-dense aspect. ×40,000. **B,** 24 days after infection with EMV-AR, incubated with rabbit preimmune serum before incubation with Protein A-gold (control 2). A few gold particles are seen scattered without any preferential binding site. Arrowheads indicate chloroplast peripheral vesicles that characteristically accompany the infection by tymoviruses. ×24,000. **C,** Healthy tissue incubated with antiserum to EMV-AR before incubation with Protein A-gold (control 3). A few gold particles are scattered over the section and no specificity of binding is observed. ×24,000. C = chloroplast; Cr = crystal; Cy = cytoplasm; M = mitochondrion; V = virus particles; Va = vacuole.

AL, empty shells of EMV-AR were always intranuclear and were never observed in mitochondria of any host. On the other hand, densely packed virus crystals were occasionally seen in EMV-AR-infected tissues, and these were not found in EMV-AL-infected tissues (15,20). Absence from sieve tubes of particles of an otherwise ubiquitous virus has also been described for EMV-T, based on studies using immunogold labeling (22).

The molecular weight of EMV-AR coat protein was greater than has been reported as typical of tymoviruses but was similar to the molecular weight of the coat protein of the other two tymoviruses analyzed in the same gels. It is believed that the molecular weight of tymovirus coat proteins is overestimated by electrophoresis, as suggested by Jones et al (10). They also found molecular weights for the coat protein of other tymoviruses to be higher than had been estimated from sequencing data.

Information on coat protein and RNA molecular weights, vector transmission, and buoyant densities are lacking for EMV-AL and thus cannot be compared with the results presented in this paper.

Under the conditions described here, the host range and symptomatology of EMV-AR are different from those of EMV-AL as described by Waterworth et al (30). EMV-AR does not induce formation of local symptoms in *N. clevelandii* or *N. glutinosa*, while EMV-AL induces formation of chlorotic local lesions in both hosts. On the other hand, EMV-AR induces formation of very characteristic concentric ringspots on inoculated leaves of *N. rustica*, but EMV-AL induced no local symptoms on inoculated leaves of the same host (30). Furthermore, EMV-AR does not infect *Glycine max*, *Sida rhombifolia*, or *Solanum tuberosum* (which are infected by EMV-AL) but does infect *C. frutescens* (not infected by EMV-AL). *L. esculentum* reacts to EMV-AR with a symptomless systemic infection and a few local lesions on inoculated leaves. In contrast, EMV-AL causes systemic mosaic without local lesions in this host. *C. amaranticolor*, *C. quinoa*, and *P. hybrida* react to EMV-AR with a few local ringspot lesions (not reported in the infection with EMV-AL). Chlorotic lesions were seen in *D. stramonium*, which shows no local symptoms when inoculated with EMV-AL.

Based on differences in host range, symptomatology, and cytopathology, EMV-AR could be distinguished from EMV-AL. It appears, therefore, that EMV-AR is the second strain of EMV found in the United States.

Because EMV-AR-infected eggplant does not show symptoms other than a slight stunting, this virus may go undetected and yet affect the growth of the plant. The fact that EMV-AR was isolated in two consecutive years from eggplant that had been grown from seed in different gardens in Fayetteville indicates that the virus may be endemic and can overwinter in this area. Because eggplants are planted annually and the virus was not seed transmitted in our tests, it is believed that EMV-AR is being disseminated by flea beetles from a biennial or perennial host, which is also fed upon by the beetle. Two potential hosts, which are solanaceous weeds commonly found in this area, were tested for EMV-AR infection with negative results. Therefore, wild reservoirs of EMV-AR have not been identified.

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