Assembly, Morphology, and Accumulation of a Hawaiian Isolate of Maize Mosaic Virus in Maize

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ABSTRACT

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Large accumulations of bullet-shaped and bacilliform particles of a Hawaiian isolate of maize mosaic virus (MMV) were present in the perinuclear space and cytoplasm of epidermal, mesophyll, and vascular parenchyma cells and phloem and xylem elements of infected maize (*Zea mays*). Particles budded through the inner and, less frequently, the outer nuclear membranes and through cytoplasmic membranes. Particles were seen infrequently in the nucleus and only within membrane-bound vesicles that presumably originated from invaginations of the proliferated nuclear membrane. Average particle lengths for bullet-shaped and bacilliform particles in vivo were 244 and 253 nm, respectively; diameters of both, including surface projections, averaged 62 nm. Preparations of glutaraldehyde-fixed, partially purified MMV particles stained with uranyl acetate (UA) (pH 4.0), potassium phosphotungstate (PTA) (pH 7.0), or ammonium uranyl oxalate (AUO) (pH 7.0) contained more bacilliform particles than did preparations of unfixed, similarly stained particles. The virion nucleocapsid was bullet-shaped, as determined by removal of the envelope with Triton X-100. UA caused rupture and collapse of some unfixed particle envelopes, which enfolded upon the nucleocapsid giving an anomalous particle morphology, and PTA caused fragmentation of particles which gave short, stubby rods. AUO did not cause either artifact. Average length and diameter of bullet-shaped particles from partially purified preparations stained with UA were 204 × 67 nm and of the bacilliform particles were 245 × 80 nm. Particles from these preparations were infective.

Maize mosaic was originally described in Hawaii in 1921 (17), but the Hawaiian maize mosaic virus (MMV) and its ultrastructural alterations of infected maize (Zea mays L.) leaf cells have not been reported, except for a brief account of a rhabdovirus presumed to be MMV (4).

Virions of MMV isolates occurring in other states and countries are reported to assemble within maize leaf cells on the inner and outer nuclear membranes and endoplasmic reticulum with nucleocapsid formation possibly occurring within cytoplasmic viroplasmalike structures, and nucleus, and on cytoplasmic strands (5,16,21). Accumulation of MMV virions is reported to occur within membrane-bound vesicles or cisternae (5,21). Massive aggregates of MMV particles are reported to accumulate in the cytoplasm surrounding the nucleus (5,16,21).

MMV virion morphology is reported as bullet-shaped or bacilliform (12). Particle dimensions and structural details may also vary considerably and reflect preparative artifacts (8).

MMV has been classified into subgroup I or A based on the presence of a single virion matrix (M) protein (7,19), whereas sites of assembly and accumulation within infected cells suggest classification in subgroup II or B (5,22).

This paper reports the observed assembly and accumulation sites of a Hawaiian MMV isolate (MMV-HI) in infected maize and the morphology, dimensions, and structural details of the virion. Companion ultrastructural studies of this isolate in its insect vector, *Peregrinus maidis* (Ashmead), are published separately (2). Our MMV-HI is serologically related to the Venezuelan (MMV-VZ) (18) and Florida (MMV-FL) (7) isolates (L. L. McDaniel and D. T. Gordon, *unpublished*).

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MATERIALS AND METHODS

Virus and vector maintenance. MMV-HI and *P. maidis* were provided by R. Namba (Univ. of Hawaii, Honolulu). *Z. mays*, inbred Oh28, or sweet corn cultivar Aristogold Bantam Evergreen was used to propagate MMV-HI and to rear *P. maidis* as described (9). Plants 1–2 wk old were inoculated by feeding of MMV-exposed *P. maidis*. Inoculated plants were maintained in a virus-containment greenhouse with 16 hr light per 24 hr (provided by high-intensity 1,000-W metal halide lamps) and 33 C day and 20 C night temperatures.

Ultrastructure in vivo. Leaf pieces of sweet corn, collected 2–3 wk after inoculation with MMV-HI, were prefixed overnight in cold 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, postfixed in 1% OsO_4 in the phosphate buffer for 3 hr, stained overnight in 1% uranyl acetate (UA), dehydrated in an ethanol-acetone series, and embedded in Spurr's medium (23). Leaf pieces were occasionally prefixed in 2.5% glutaraldehyde containing 6% tannic acid. Sections were stained in 0.5% UA and 0.1% lead citrate and examined in a Philips 201 electron microscope (Philips Electronics Instruments, Inc., Mahwah, NJ).

Virus partial purification. Infected maize leaves, 2–3 wk postinoculation, were homogenized in four volumes of 0.1 M glycine plus 0.5% thioglycollic acid (TGA), pH 8.0. Filtered extract was layered onto linear 200- to 500-mg/ml sucrose density gradients in 0.1 M glycine plus 0.5% TGA, pH 7.0 (suspension buffer). The gradients were centrifuged in a Beckman SW28 rotor (Beckman Instruments, Inc., Palo Alto, CA) at 23,000 rpm for 45 min at 4 C, and the light-scattering virus band was removed. Fractions were diluted and virus was recovered by centrifugation (SW28 rotor at 23,000 rpm for 15 min at 4 C). Pellets were suspended in 0.2–0.4 ml of suspension buffer to concentrate the virus about 10-fold. In attempts to increase virus stability, 0.01 M $MgCl_2$ was added to extraction and suspension buffers. In attempts using glutaraldehyde, the above purification procedure was repeated using 0.1 M sodium citrate plus 0.5% TGA, pH 7.5, as both extraction and suspension buffer. Sucrose density gradients were prepared in the citrate buffer described above, except the respective sucrose solutions (200–500 mg/ml) contained 0, 0.5, 1.0, and 2.0% glutaraldehyde.

Particle morphology in partially purified preparations. MMV-HI, either treated or nontreated with glutaraldehyde, was placed on a carbon-coated grid and stained with 1 or 2% aqueous UA, pH 4.0; 2% ammonium uranyl oxalate (AUO), pH 7.0; or 3% phosphotungstic acid (PTA), pH 7.0. AUO was prepared fresh as described (11). Particles that had not been fixed with glutaraldehyde were treated with Triton X-100 (Rohm & Haas; Philadelphia, PA), as described by Emerson and Wagner (6), to reveal nucleocapsid morphology.

The microscope was calibrated with fragments of a 2,160-line per millimeter, crossed-lines grating replica (E. F. Fullam, Inc.; Schenectady, NY).

Infectivity assay. MMV-HI was purified by filtering the extract, prepared as described above, through a 3- to 4-mm-thick pad of Celite 545 (Fisher Scientific Co.; Pittsburgh, PA). After sucrose density gradient centrifugation, as described above (glycine buffer), virus fractions were layered onto linear sucrose density gradients (250–550 mg/ml) in 0.1 M glycine, pH 7.0. and centrifuged in the SW28 rotor at 23,000 rpm for 2.5 hr at 4 C. The virus was collected and concentrated as above, and the pellet was suspended in 0.4 ml of 0.15 M NaCl + 0.01 M phosphate, pH 7.0. An extract from healthy maize leaves was similarly processed and served as a control.

Partially purified MMV-HI was injected into last instar nymphs of *P. maidis*, and survivors were placed on healthy Oh28 maize plants (five insects per plant) for a 7-day inoculation access period (IAP). Plants were rated for symptoms 16 days later.

RESULTS

Assembly and accumulation sites in MMV-infected maize. Large accumulations of MMV-HI particles frequently were found in cells of mesophyll (Fig. 1) and vascular parenchyma, mature and immature phloem elements (Fig. 2), and less so in epidermal cells and xylem elements. Massive aggregates of these particles, sometimes in crystalline or paracrystalline array, were found in expanded perinuclear space and cisternae within cytoplasm adjacent to the nucleus. Infrequently, small groups of enveloped MMV particles were found within membrane-bound vesicles in the nucleus (Fig. 2); the vesicles presumably resulted from invaginations of perinuclear and/or cytoplasmic membranes.

MMV-HI particles were frequently observed budding through the inner and, to a lesser extent, the outer membranes of the nuclear envelope perpendicular or parallel (a few particles) to the membrane surface (Figs. 2, 3, and 4). Frequently, the viral envelope was continuous with the nuclear membrane. Occasionally, MMV-HI particles were observed budding through vesicular and/or cisternal membranes within the cytoplasm (Fig. 3). The latter membranes were occasionally connected to the rough endoplasmic reticulum (Fig. 5). The vesicles frequently contained MMV-HI particles. Also, an extensive network of membranes, sometimes tubular vesicles, intermingled with ribosomelike particles and budding MMV-HI particles frequently were observed within the cytoplasm surrounding the nucleus (Fig. 3). Groups of vesicles were usually bounded by extensions of the outer nuclear membrane.

No rhabdovirus particles, proliferated nuclear membranes, or vesicles were observed in cells of healthy maize or of maize infected with maize stripe virus (1), which is also vectored by *P. maidis*.

MMV-HI particles were not associated with chloroplasts, tonoplasts, or mitochondria, and no particles with partial or non-enveloped nucleocapsids were observed in the cytoplasm or nucleus.

Particle morphology in thin sections. Most MMV-HI particles were bullet-shaped (one end hemispherical and the other flattened) rather than bacilliform (both ends hemispherical). The lengths of bullet-shaped and bacilliform particles averaged 244 nm (number of particles [N] = 100; 95% confidence interval [CI] = 2.9 nm) and 253 nm (N = 10, CI = 8.8 nm), respectively, with median lengths of 243 and 255 nm. For both, particle diameter (including the surface projections [spikes]) averaged 62 nm.

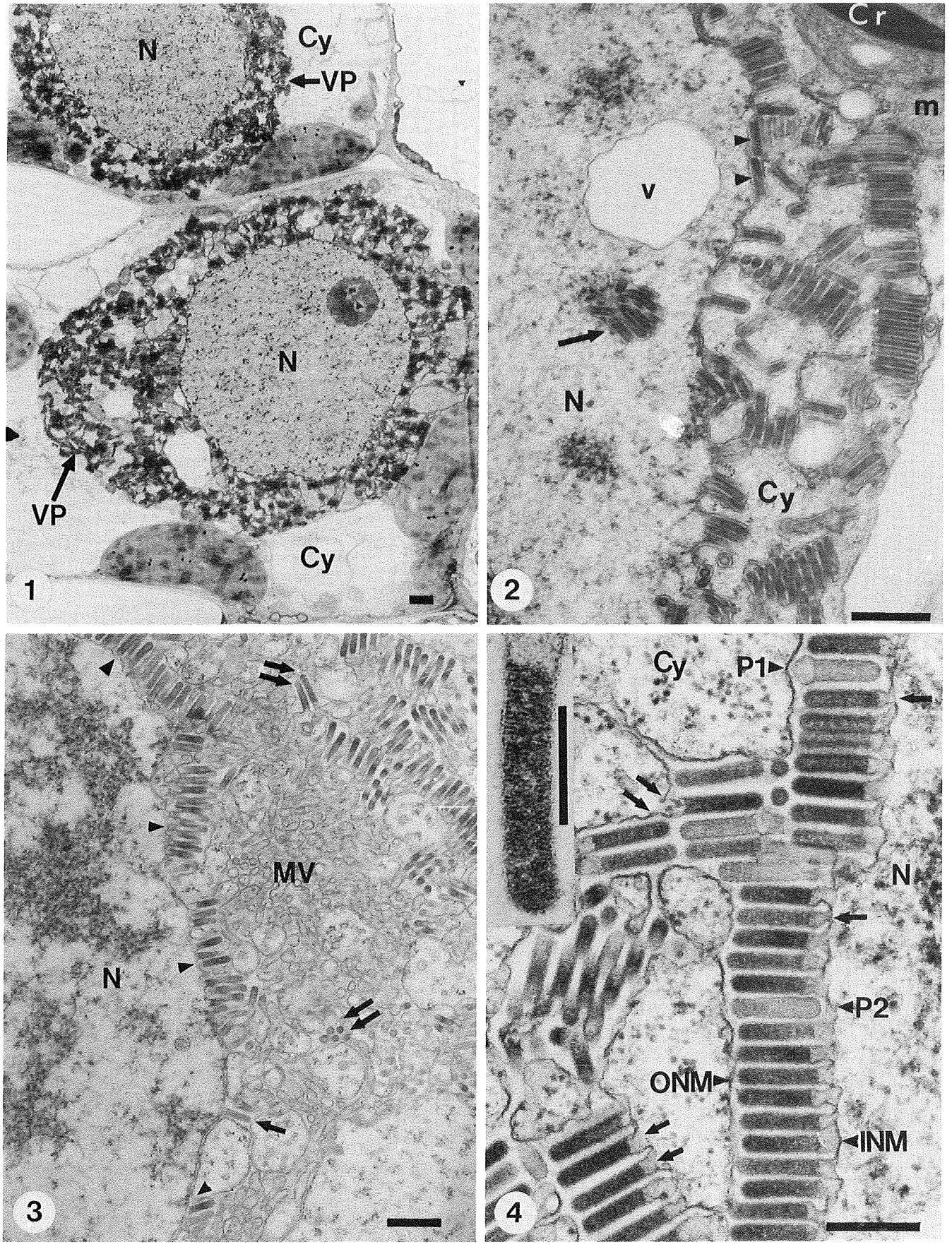
In cross section (Fig. 6), MMV particles had a central electron-lucent cylinder averaging 21 nm (N = 100; CI = 0.3 nm) in diameter that usually contained amorphous, partially electron-opaque material. The electron-opaque nucleocapsid surrounding the central cylinder averaged 11 nm thick. The nucleocapsid was encircled by a clearly visible trilamellar membrane (envelope). Spikes were clearly discernable with an average length of 5 nm. An average of 14 spikes per circumference was observed on undisrupted envelopes.

In longitudinal section (Fig. 4), MMV nucleocapsids showed cross-striation of the helically constructed nucleocapsid. Striations had a periodicity of ~4.7 nm. The nucleocapsid of bacilliform particles was bullet-shaped with an electron-lucent area between its flat end and the envelope (P1 in Fig. 4).

Morphology of particles in partially purified preparations. Although both bullet-shaped and bacilliform particles were

Figs. 1–4. Maize mosaic virus particles in various types of infected maize leaf cells: **1,** Accumulation of virus particles (VP) in the cytoplasmic areas (Cy) around nuclei (N) of two parenchyma cells. Scale bar = 1.0 μm. **2,** Virus particles in the cytoplasm (Cy) of a phloem cell. Arrow indicates membrane-encircled particles in nucleus (N). Arrowheads indicate particles aligned parallel to nuclear membrane. Cr = crystal, m = mitochondrion, and v = vacuole. Scale bar = 0.5 μm. **3,** Virus particles budding on the inner nuclear membrane (arrowheads). Note extensive membranous vesicles (MV) mixed with ribosomelike particles and budding particles (double arrows) and surrounded by extensions of the outer nuclear membrane (single arrows). N = nucleus. Scale bar = 0.5 μm. **4,** Virus particles attached to the inner nuclear membrane (INM) and to intracytoplasmic extensions (double arrows) of the outer nuclear membrane (ONM) which forms dilated cisternae; single arrows indicate constriction of INM. Cy = cytoplasm, N = nucleus, Pl = bacilliform particle, P1 and P2 = alignment suggesting budding on ONM. Scale bar = 0.3 μm.

observed in all preparations stained with UA (Fig. 7), AUO (Fig. 8), or PTA (Fig. 9), the bullet-shaped morphology was predominant. For example, MMV-HI particles in unfixed, UA-stained preparations were 95% bullet-shaped ($N = 120$) compared to 74% ($N = 185$) in glutaraldehyde-fixed preparations. The structural integrity of fixed particles was better than that of particles in unfixed preparations in any of the stains (Figs. 7 to 9). This was quite noticeable when particles from fixed and unfixed PTA-



stained preparations were compared (Fig. 9). Envelopes of UA-stained MMV-HI particles frequently appeared ruptured and collapsed (Fig. 10), apparently enfolding the nucleocapsid and thus producing aberrant particles. The numbers of these aberrant particles were greatly reduced in fixed, UA-stained preparations.

The inner structure of stained bacilliform particles was usually not visible, whereas surface details were clearly evident (Fig. 10). For particles with a ruptured end, the helical structure of the nucleocapsid was revealed by stain uptake (Figs. 7-10). The distance between helix turns averaged 4.5 ± 0.8 nm (N = 40). Treatment of particles with Triton X-100 revealed a bullet-shaped nucleocapsid with envelope fragments attached (Fig. 11). On particles stained with UA the spikes appeared knoblike and had average lengths of 6.6 ± 0.2 nm (N = 57).

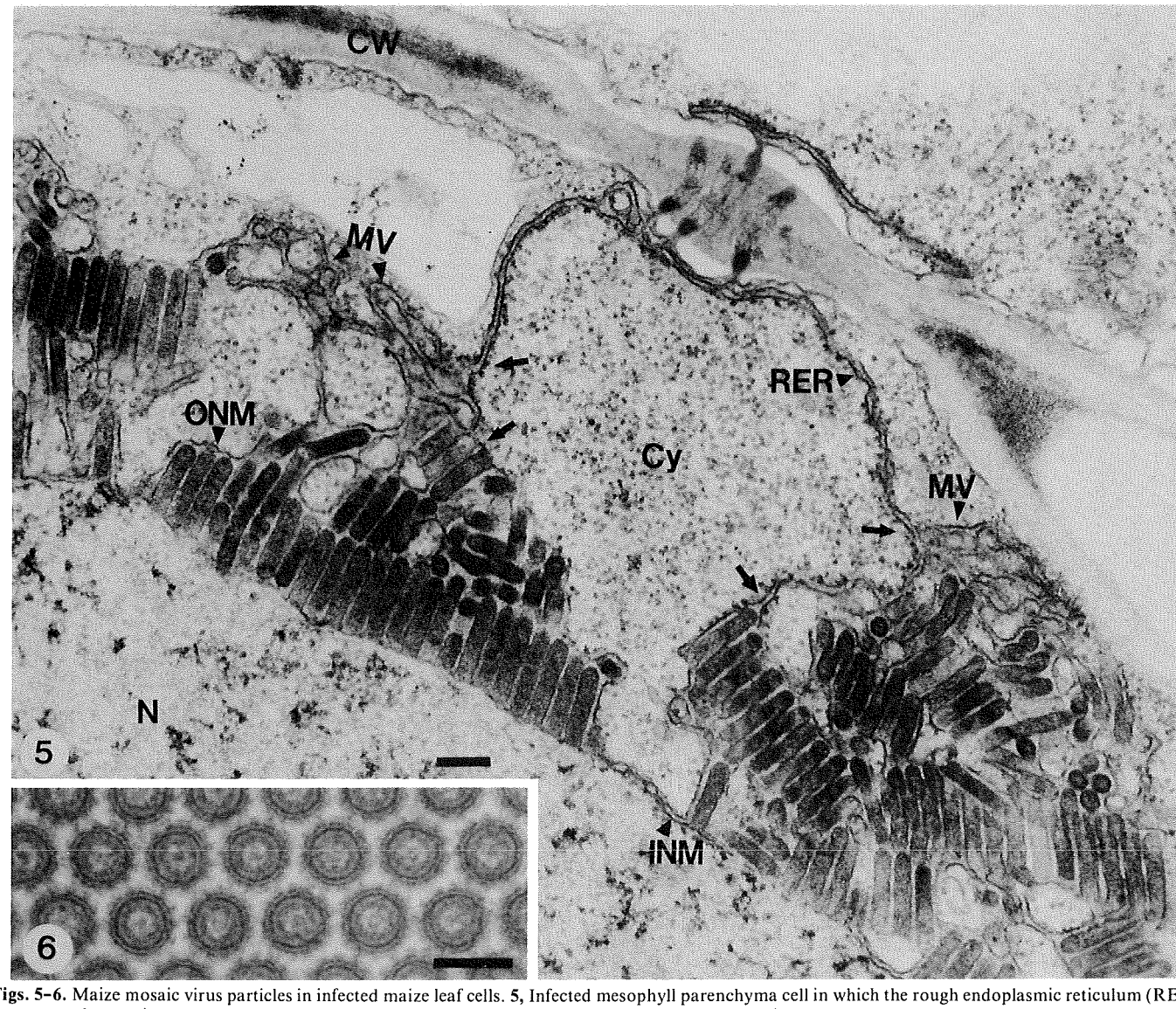
The average length for bullet-shaped particles in unfixed preparations was 204 nm (N = 100, CI = 2.8 nm) with a median length of 204 nm; whereas for bacilliform particles the average length was 245 nm (N = 100; CI = 4.7 nm) with a median length of 245 nm. Diameter of the former was 67.3 nm (N = 100; CI = 0.9 nm) with a median of 67 nm and for the bacilliform 79.9 nm (N = 100; CI = 1.5 nm) with 78 nm for the median. These results indicated that the length of the labile end of the latter was ~40 nm. Mg^{2+} in purification buffers did not increase stability of MMV-HI particles stained with UA, AUO, or PTA.

Infectivity of partially purified particles. All 15 assay plants exposed to MMV-injected *P. maidis* were infected. MMV was serologically detected in extracts from these plants by using MMV-HI antiserum in an agar-gel double-diffusion assay (L. L. McDaniel, *unpublished*), and rhabdovirus particles were observed in leaf dips examined by electron microscopy. Control plants were symptomless, and their extracts neither reacted with MMV-HI antiserum nor contained rhabdovirus particles.

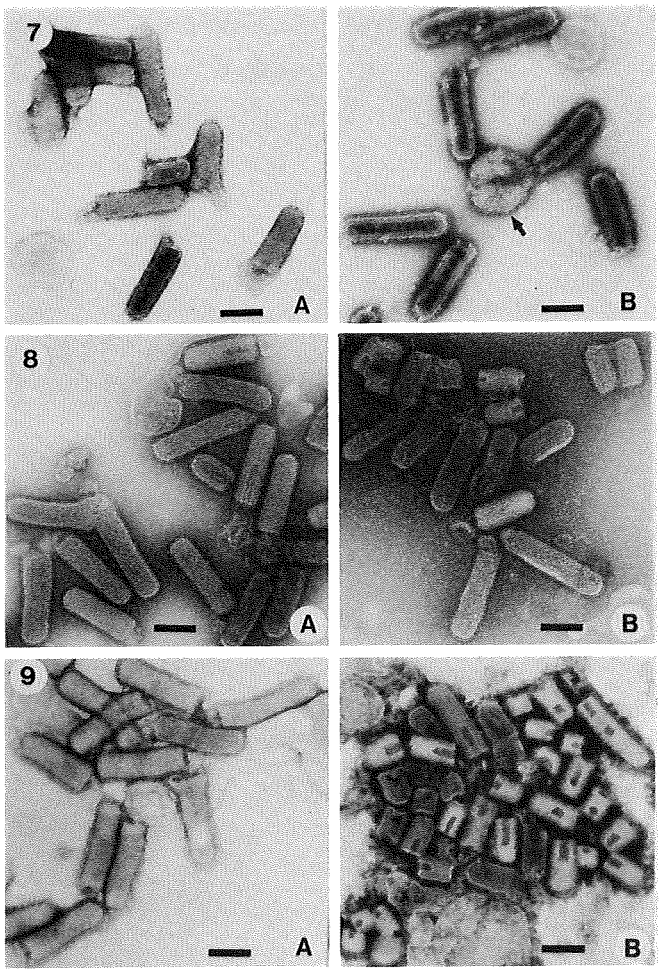
DISCUSSION

Particles of MMV-HI assembled (budded) on the inner and outer nuclear membranes and on membranes of vesicles within the cytoplasm or perinuclear space, matching findings for other MMV isolates (3,5,18,21). In *P. maidis*, MMV-HI particles budded on nuclear, intracytoplasmic, and plasma membranes of salivary gland cells and of nerve axons, indicating a greater diversity of assembly sites in the vector (2).

Fixation with glutaraldehyde increased the frequency of bacilliform particles compared to that for unfixed preparations, suggesting that the morphology of the mature particle was bacilliform and that the bullet-shaped particle either was immature or its labile end had been disrupted. The increased fragmentation (= greater number of short bullet-shaped particles) observed in



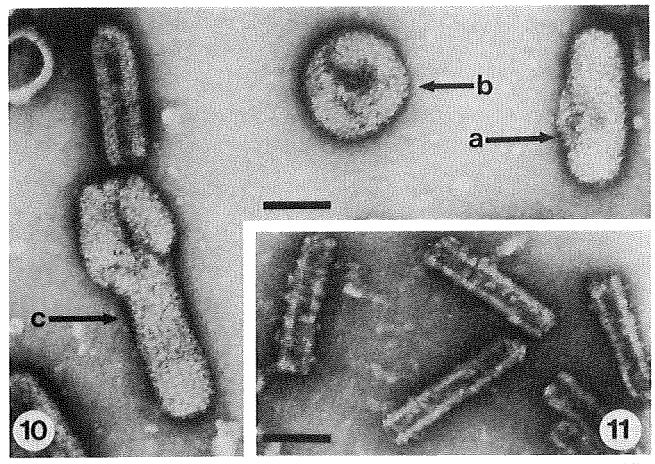
Figs. 5-6. Maize mosaic virus particles in infected maize leaf cells. **5,** Infected mesophyll parenchyma cell in which the rough endoplasmic reticulum (RER) appears to be continuous (arrows) with membranous vesicles (MV) containing rhabdovirus particles and the outer nuclear membrane (ONM). Cy = cytoplasm, CW = cell wall, INM = inner nuclear membrane, and N = nucleus. Scale bar = 0.1 μm. **6,** Cross sections of maize mosaic virus particles showing spikes and interior structure (see text). Tissue was prefixed in glutaraldehyde containing tannic acid. Scale bar = 0.1 μm.



Figs. 7–9. Maize mosaic virus particles partially purified through centrifuged sucrose density gradients. Particles fixed and unfixed with glutaraldehyde and stained with 1 or 2% aqueous uranyl acetate (UA), pH 4.0; 2% ammonium uranyl oxalate (AUO), pH 7.0; or 3% phosphotungstic acid (PTA), pH 7.0. **7, A,** Fixed and **B,** unfixed virus particles stained with 2% UA. Note pleomorphic form (arrow) and stain penetration into particle inner structure in B. Scale bar = 0.2 μm. **8. A,** Fixed and **B,** unfixed virus particles stained with AUO. Same MMV source as for Fig. 7. More of the shorter, bullet-shaped particles were seen in unfixed preparations. Scale bar = 0.2 μm. **9, A,** Fixed and **B,** unfixed (virus particles stained with PTA. Same MMV source as for Fig. 7. Unfixed particles have a distinct flat end and form few of the pleomorphic forms seen in Fig. 7. Structural integrity of fixed particles was good. Scale bar = 0.2 μm.

unfixed PTA- and AUO-stained preparations further supports the artifactual origin of the bullet-shaped particles. In thin-sections of tissue, many MMV-HI particles still had one end attached to cellular membranes, even after reaching their normal length, indicating incomplete particle assembly. This, combined with the lability of the envelope covering the flat end of the nucleocapsid, accounts for the relatively high proportion of bullet-shaped particles relative to bacilliform ones.

Length and diameter measurements of MMV-HI particles in tissue thin sections were similar to those reported for other MMV isolates (202–325 nm) (5,12,13). Average lengths of UA-stained bullet-shaped and bacilliform MMV-HI particles (204 and 245 nm, respectively) in partially purified preparations were shorter than those reported for MMV-VZ (241 and 277 nm, respectively) (14). The average diameter of bullet-shaped particles (67 nm) differed from that of bacilliform particles (80 nm) in UA-stained preparations. Differences in particle diameters may reflect differences in the degree of particle flattening for the two particle morphologies. Although the particle diameter in thin-sections (62 nm) may have been affected by preparative conditions, it is suggestive of the diameter without flattening.



Figs. 10–11. Maize mosaic virus particles from partially purified preparations and nucleocapsids from which the envelopes have been removed. **10,** Aberrant particle types common in preparations fixed in glutaraldehyde and stained with 1 or 2% uranyl acetate UA (arrows): ruptured particle (a), pleomorphic particle (b), and particle ruptured at one end with the envelope folding back onto itself (c). Scale bar = 0.1 μm. **11.** Bullet-shaped nucleocapsids from which most of the envelopes had been removed by treatment with Triton X-100 and stained with 1% UA. Scale bar = 0.1 μm.

The altered morphology of unfixed MMV-HI particles in UA, pH 4.0, was similar to that of Mauritian MMV in PTA, pH 3.5, but not in UA at low pH (3). In UA, the absence of shorter MMV-HI particles, common in unfixed samples in AUO and PTA, suggested that the envelopes of such particles readily ruptured, resulting in aberrant structures. Use of AUO is recommended, since it reduced morphological alterations and resolved envelope surface details better than PTA or UA.

MMV-HI spikes were more contracted or knoblike in UA than in PTA as reported for spikes of MMV-VZ (14). MMV-HI spike length (6.6 nm) was also similar to that of MMV-VZ (7.0 nm) (14). We were not able to resolve a regular surface structure for MMV-HI with any of the stains employed. This lack of clear surface structure is unlike that for cereal chlorotic mottle virus (10), which also naturally infects maize and has a distinct hexameric surface pattern.

Mg^{2+} did not appear to preserve MMV-HI infectivity, since a partially purified virus preparation without Mg^{2+} was equally infectious. Further, MMV-HI particle integrity in partially purified preparations was not improved by Mg^{2+} which suggests that lipase was absent, in low concentration, or nonfunctional in maize leaf extracts or that MMV-HI was not susceptible to its activity.

Sites of assembly and accumulation and particle morphology, size, and stability of MMV-HI resemble those for MMV isolates in Venezuela (18), Florida (5), and Mauritius (3). However, these isolates appear to differ from the isolate from Brazil (16), which is serologically related to MMV-VZ, for these same features. Distinctive features for the Brazilian isolate were the association of naked (unenveloped) rhabdovirus particles with chloroplast and/or tonoplast membranes and the presence of viroplasmalike structures, features not seen in our study. Such a wide array of assembly sites is not typical of plant rhabdoviruses within plant cells, except for wheat striate mosaic virus (WStrMV) (15,20).

Sites of assembly and accumulation suggest that MMV-HI most closely resembles WStrMV and should, therefore, be placed in subgroup I of the plant rhabdoviruses. The report of a single M protein, characteristic of members of subgroup I (22), for isolates from Florida (7) and Venezuela (19) indicates classification in this subgroup. Our preliminary evidence (L. L. McDaniel and D. T. Gordon, *unpublished*) shows that MMV-HI has two M proteins, supporting placement in subgroup II which is not indicated by our electron microscopy findings.

LITERATURE CITED

1. Ammar, E.-D., Gingery, R. E., and Nault, L. R. 1985. Two types of inclu-

sions in maize infected with maize stripe virus. Phytopathology 75: 84-89.
2. Ammar, E.-D., and Nault, L. R. 1985. Assembly and accumulation sites of maize mosaic virus in its planthopper vector. Intervirology (In press).
3. Autrey, L. J. C. 1980. Studies on maize mosaic virus: Its strains and economic importance. Ph.D. Dissertation, University of Exeter, England. 408 pp.
4. Bradfute, O. E., and Robertson, D. C. 1974. Rhabdovirus-like particles in stunted maize from Texas and Hawaii. (Abstr.) Proc. Electron Microsc. Soc. Am. 32:266-267.
5. Bradfute, O. E., and Tsai, J. H. 1983. Identification of maize mosaic virus in Florida. Plant Dis. 67:1339-1342.
6. Emerson, S. U., and Wagner, R. R. 1972. Dissociation and reconstitution of the transcriptase and template activities of vesicular stomatitis B and T virions. J. Gen. Virol. 10:297-309.
7. Falk, B. W., and Tsai, J. H. 1983. Physicochemical characterization of maize mosaic virus. Phytopathology 73:1536-1539.
8. Francki, R. I. B., and Randles, J. W. 1980. Rhabdoviruses infecting plants. Pages 135-165 in: D. H. L. Bishop, ed. Rhabdoviruses. Chemical Rubber Company Press, Inc., Boca Raton, FL. 272 pp.
9. Gingery, R. E., Nault, L. R., Tsai, J. H., and Lastra, R. J. 1979. Occurrence of maize stripe virus in the United States and Venezuela. Plant Dis. Rep. 63:341-343.
10. Greber, R. S., and Gowanlock, D. H. 1979. Cereal chlorotic mottle virus-purification, serology and electron microscopy in plant and insect tissues. Aust. J. Biol. Sci. 32:399-408.
11. Harris, J. R., and Agutter, P. 1970. A negative staining study of human erythrocyte ghosts and rat liver nuclear membranes. J. Ultrastruct. Res. 33:219-232.
12. Herold, F. 1972. Maize mosaic virus. No. 94 in: Descriptions of Plant Viruses. Commow. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 4 pp.
13. Herold, F., Bergold, G. H., and Weibel, J. 1960. Isolation and electron microscopic demonstration of a virus infecting corn (*Zea mays* L.). Virology 12:335-347.
14. Herold, F., and Munz, K. 1967. Morphological studies of maize mosaic virus I. J. Gen. Virol. 1:227-234.
15. Jackson, A. O., Milbrath, G. M., and Jedlinski, H. 1981. Rhabdovirus diseases of the Gramineae. Pages 51-76 in: Virus and Viruslike Diseases of Maize in the United States. D. T. Gordon, J. K. Knoke, and G. E. Scott, eds. South. Coop. Ser. Bull. 247. 218 pp.
16. Kitajima, E. W., and Costa, A. S. 1982. The ultrastructure of the corn chlorotic vein banding (Brazilian maize mosaic) virus-infected corn leaf tissues and viruliferous vector. Fitopatol. Bras. 7:247-259.
17. Kunkel, L. O. 1921. A possible causative agent for the mosaic disease of corn. Hawaii. Sugar Plant. Assoc. Exp. Stn. Bull. Bot. Ser. 3:44-58.
18. Lastra, R. J. 1977. Maize mosaic and other maize virus and virus-like diseases in Venezuela. Pages 30-39 in: Proc. Int. Maize Virus Dis. Colloq. Workshop. L. E. Williams, D. T. Gordon, and L. R. Nault, eds. 16–19 August 1976, Ohio State Univ., Ohio Agric. Res. Devel. Cent., Wooster. 145 pp.
19. Lastra, R., and Carballo, O. 1983. Maize virus disease problems in Venezuela. Pages 83-86 in: Proc. Int. Maize Virus Dis. Colloq. Workshop. D. T. Gordon, J. K. Knoke, L. R. Nault, and R. M. Ritter, eds. 2–6 August 1982. Ohio State Univ., Ohio Agric. Res. Devel. Cent., Wooster. 266 pp.
20. Lee, P. E. 1970. Developmental stages of wheat striate mosaic virus. J. Ultrastruct. Res. 31:282-290.
21. Martelli, G. P., Russo, M., and Malaguti, G. 1975. Ultrastructural aspects of maize mosaic virus in the host cells. Phytopathol. Mediterr. 14:140-142.
22. Peters, D. 1981. Plant rhabdovirus group. No. 244 in: Descriptions of Plant Viruses. Commow. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 6 pp.
23. Spurr, A. R. 1969. A low-viscosity epoxy embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.