Identification of Maize Mosaic Virus in Florida

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ABSTRACT

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Maize mosaic virus was identified in southern Florida on the basis of leaf symptoms in corn (*Zea mays*), rhabdovirus particle morphology and structure, perinuclear virus particle accumulation, immunoelectron microscopy, and persistent transmission by *Peregrinus maidis*. This confirms the identification of maize mosaic virus in the continental United States.

Maize mosaic was recognized as a serious disease of corn (*Zea mays* L.) in Hawaii as early as 1914 (20). Symptoms can be readily recognized as high-contrast chlorotic stripes along leaf veins and dwarfing (shortening of upper internodes) when infected early. The virus is persistently transmitted by the corn delphacid, *Peregrinus maidis* (Ashmead) (6), and the disease is thought to occur with this widely distributed vector throughout humid tropical and subtropical regions (15).

Maize mosaic virus (MMV) was the first rhabdovirus to be described in plants (16). The first rhabdovirus infections of maize in the United States were found in Texas and Hawaii (2,4). Shortly thereafter, rhabdovirus infections of maize were found in Alabama (30), Louisiana (8), and Mississippi (O. E. Bradfute, *unpublished*). These viruses were generally presumed to be MMV on the basis of virus particle structure and vivid leaf symptoms. Vector identification was not reported and serological relationship to MMV was indicated only by serologically specific electron microscopy of the virus found in Louisiana (7,8).

Recently, natural infections of American wheat striate mosaic virus in maize have been identified in South Dakota on the basis of rhabdovirus particle morphology, serology, and transmission by the leafhopper *Endria inimica* (Say) (19).

Previous surveys of maize with viruslike disease symptoms in southern Florida have revealed maize stripe virus, maize rayado fino virus, corn stunt spiroplasma, maize dwarf mosaic virus (strains A and B), maize bushy stunt mycoplasmalike organisms, and long straight tubular viruslike particles (1,5,11,28; O. E. Bradfute and J. H. Tsai, *unpublished*). From continued surveys of maize with viruslike disease symptoms in southern Florida, we report MMV and describe its identification on the basis of leaf symptoms, virus particle morphology and structure, relation to host-cell components, serology, and persistent transmission by *P. maidis*.

MATERIALS AND METHODS

In the spring of 1981 and 1982, leaf samples of dent maize plants with viruslike symptoms were collected by J. H. Tsai in Dade County, FL.

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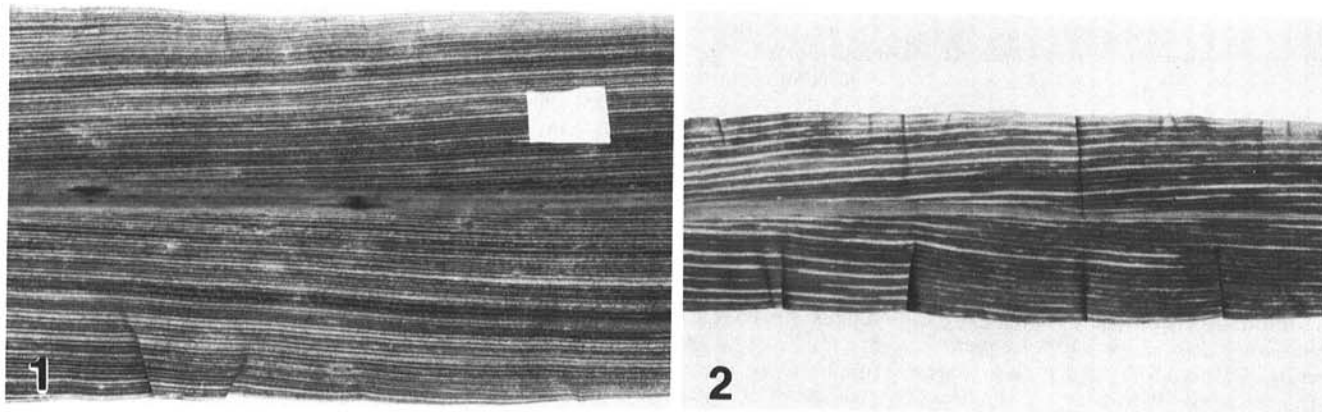
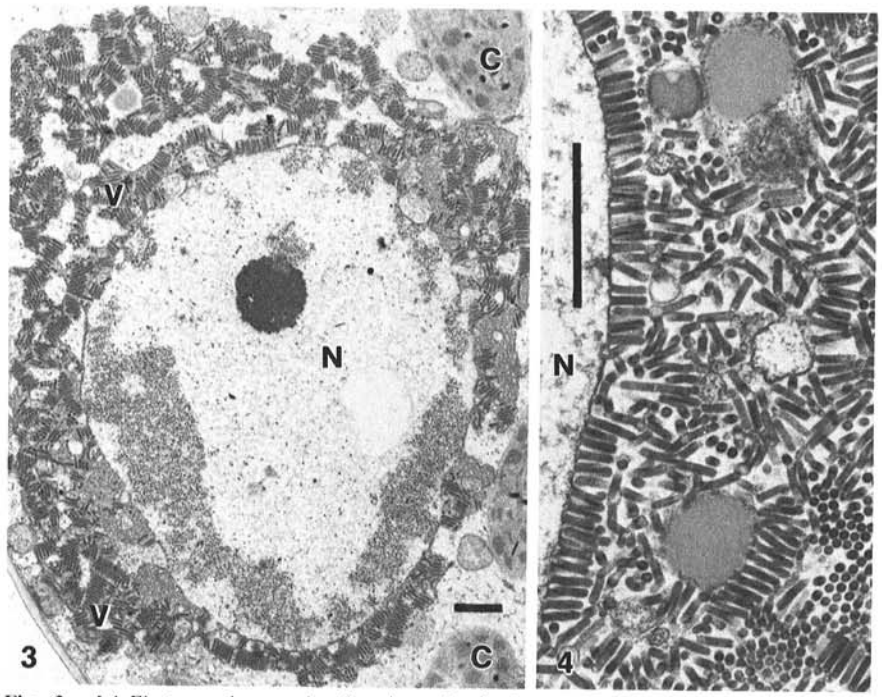


Figs. 1 and 2. Close-up photographs of maize leaves showing symptoms of a Florida isolate of maize mosaic virus. **(1)** Field-collected leaf with natural infection (white space shows location of sample removed for electron microscopic examination). **(2)** Leaf from experimentally infected seedling.



Figs. 3 and 4. Electron micrographs of sections of maize leaves showing rhabdovirus particles (V) attached to the inner nuclear membrane and concentrated in perinuclear spaces. Note chromatin-deficient nuclei (N) and intact chloroplasts (C). **(3)** Low magnification of part of a mesophyll cell from a naturally infected plant shown in Figure 1. **(4)** Higher magnification of a bundle sheath cell from an experimentally infected plant. Scale bars = 1 μm.

Electron microscopy. Isolated virus particles were found by staining expressed leaf sap on formvar-carbon-coated grids with 2% potassium phosphotungstate, pH 6.9. Virus-infected cells were examined in sections cut from selected leaf samples prepared for electron microscopy as described previously (3). Immunoelectron microscopy in the form of the decoration technique (25) was used to test for serological relationship. Antiserum to maize mosaic virus was supplied by Ramón Lastra (Laboratorio de Virus de Plantas, Centro de Microbiología y Biología Celular, Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela) and normal rabbit serum was supplied by D. T. Gordon (Department of Plant Pathology, Ohio State University, Ohio Agricultural Research and Development Center, Wooster). A 2% glutaraldehyde-1% paraformaldehyde-mixture in 0.05 M potassium phosphate, pH 7.2 (3), was used to stabilize virus particles in some of the expressed sap droplets before negative staining, and in some of the leaf samples, before crude virus extraction for the decoration technique. Uniform virus particles, occurring in groups of six to 30 and apparently complete and undistorted, were selected for length and diameter measurements. Electron micrograph magnification was determined with crystalline catalase (29).

Vector transmission. Colonies of *P. maidis* were established from 1974–1979 collections in the Fort Lauderdale area by J. H. Tsai and identified by F. W. Mead (Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville) (5). Colonies of *P. maidis* were reared on maize (*Z. mays* var. *saccharata* 'Guardian') and maintained in an insect-rearing room or growth chamber at 23–25 C with 12 hr of light per day. Guardian sweet corn was used for source and test plants.

RESULTS

Symptoms. Distinguishing leaf symptoms of naturally and experimentally infected plants (Figs. 1 and 2) consist of long, even, distinct, chlorotic stripes on either large or small veins similar to those described previously for maize mosaic (15,20,21) and observed under natural conditions in Hawaii (O. E. Bradfute, *unpublished*).

Ultrastructure of infected maize. Electron micrographs of naturally and experimentally infected cells (Figs. 3 and 4) were similar to those recorded previously for maize mosaic (4,16,23). Rhabdovirus particles appeared to bud through the inner nuclear membrane and accumulate in the perinuclear space and in the dilated cisternae of a connected membrane recticulum extending into the cytoplasm. The perinuclear accumulation of virus particles distinguishes MMV from maize sterile stunt, a rhabdovirus also transmitted by *P. maidis* (13). Virus particle length and diameter were 234–325 × 63 nm compared with previous reports for MMV in thin-sectioned maize cells of 242 × 48 nm (16) and 300 × 75 nm (23). The nuclei were enlarged and showed a loss of electron dense chromatin. In some infected cells, ribosomes were lost, whereas chloroplasts and mitochondria appeared unaltered. There were no other viruslike particles or mycoplasmalike organisms detected in plants infected with rhabdovirus particles or in healthy controls.

Virion morphology and structure. A great variety of altered rhabdovirus particles was observed with or without aldehyde fixation before or after extraction for negative staining. Some bullet-shaped and bacilliform particles

(Figs. 5–8) appeared relatively intact but showed varying stain penetration, plastic deformation, and shrinkage. Surface projections and interior cross-striations were readily seen (Fig. 5) but the distinct hexagonal matrix found in cereal chlorotic mottle virus (CCMV), a rhabdovirus that also infects maize (12), was not evident. The most frequently appearing intact particles were bullet-shaped and measured 220–276 × 71 nm compared to the previous report for MMV negatively stained in phosphotungstate of 255 × 90 nm (17). Generally, longer particles were found in the fixed preparations. These observations are similar to those recorded for many plant rhabdoviruses including MMV (10,17,18).

Serology. The decoration technique of immunoelectron microscopy demonstrated a distinct relationship with MMV. MMV antiserum decorated or coated rhabdovirus particles (Figs. 6 and 7), whereas normal rabbit serum did not (Fig. 8). Although the MMV antiserum used had a relatively low titer (22), the halos of antibodies on intact rhabdovirus particles (Figs. 6 and 7) indicated the presence of antibodies to the virus surface projections or virus membrane.

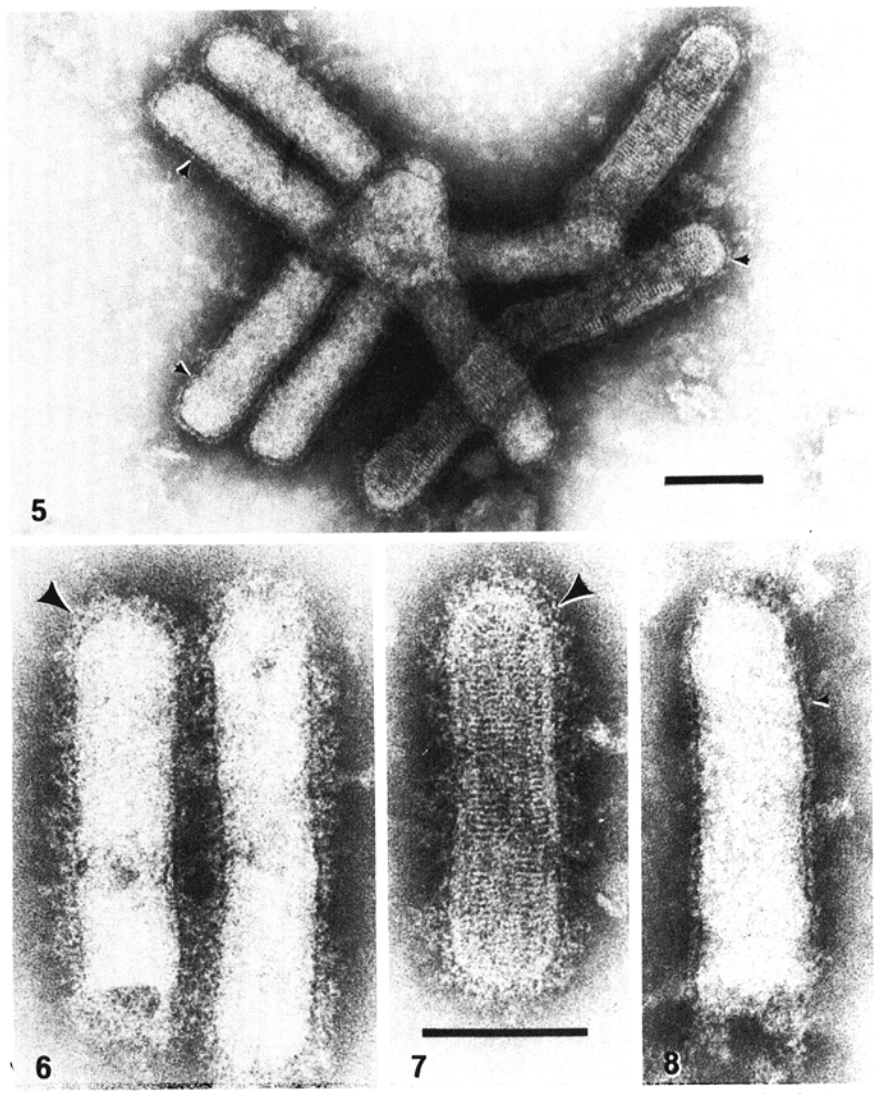
Transmission. Transmission by *P. maidis* was similar to that previously reported for MMV (6,15) and distinguishes MMV from CCMV, a rhabdovirus that infects maize and accumulates in the perinuclear space (14). About 12% of late instar nymphs and about 21% of adults were able to transmit the virus after a 4- to 6-day acquisition access period on experimentally infected plants. The mean latent period of MMV in single-insect tests was 14.5 days, with a minimum of 9 and a maximum of 28 days. The retention period of MMV was highly variable, ranging from 1 to 31 days. Plants exposed to insects taken directly from colonies remained healthy. The virus was not transmitted by the corn leafhopper *Dalbulus maidis* (DeLong & Wolcott). No transmission was obtained in mechanical inoculation tests with sap from infected plants in phosphate buffer-mixed Carborundum powder.

DISCUSSION

Based on leaf symptoms, particle morphology and structure, intracellular relationships, serology, and vector transmission, we conclude that this maize rhabdovirus is MMV or a strain thereof. This is the first definitive identification of MMV in the continental United States. Identification of MMV in Florida together with occurrence of *P. maidis* throughout the Gulf Coast of the United States (27) adds credence to the presumptions that maize rhabdoviruses previously found in Texas (4), Alabama (30), Louisiana (8), and Mississippi (O. E. Bradfute, *unpublished*) were MMV. The occurrence of MMV in southern Florida could be expected from the indigenous population of *P. maidis* and from previous reports of presumptive MMV in the Caribbean Islands (9,15). We have suggested previously that the maize pathogen-inoculative vectors are introduced to southern Florida by weather systems from the Caribbean (5).

The potential loss resulting from MMV in southern Florida is unknown. Leaf-dip electron microscopy of maize samples collected on numerous surveys for maize viruslike diseases in this region have previously failed to find rhabdovirus particles (1,5,28). The prevalence of *P. maidis* and itchgrass (*Rottboellia exaltata* L.), a common host for both virus and vector (27), in addition to summer as well as winter maize production, would be expected to contribute to the incidence and losses resulting from MMV in southern Florida.

The spread of maize mosaic disease beyond the southern coastal regions of the United States is not expected because of the low incidence of *P. maidis* and overwintering plant hosts north of this area (27). Also, incubation periods required in both the vector and the plant hosts would decrease the probability of spread from migrating vectors (18); however, maize-infecting rhabdoviruses of uncertain identity are transmitted by non-*P. maidis* vectors occurring in the United States. A rhabdovirus found in Jamaica and presumed to be MMV is reported to be transmitted to maize by *D. maidis* (9). Sorghum stunt virus, a rhabdovirus found in California, is experimentally transmitted to maize by *Graminella sonora* (Ball) (24). A rhabdovirus found as a greenhouse contaminant in maize was presumably transmitted to maize by *G. nigrifrons* (Forbes) (26; O. E. Bradfute, *unpublished*). Other unidentified rhabdoviruses have been found in maize samples from Iowa, Nebraska, and Ohio (O. E. Bradfute,



Figs. 5–8. Electron micrographs of KPT negatively stained preparations of a Florida isolate of maize mosaic virus (MMV). **(5)** Rhabdovirus particles (RVP) showing surface projections (small arrows) and internal cross-striations of helical nucleoprotein. **(6 and 7)** RVP exposed to MMV antiserum and showing decoration halo or coating of homologous antibodies (large arrows). **(8)** RVP exposed to normal rabbit serum and displaying a negative control reaction. Scale bars = 100 nm.

unpublished). In addition to the identification of these maize-infecting rhabdoviruses in the United States, the relationship among known rhabdoviruses infecting maize and other Gramineae should be determined (18).

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