# Maize Rayado Fino and Maize Dwarf Mosaic Viruses in Ecuador

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**ABSTRACT**

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Symptoms of rayado fino disease were found in about 3% of open-pollinated maize plants in central Ecuador at seven locations (elevation 2,000–2,500 m). Maize rayado fino virus (MRFV) was identified on the basis of leaf symptoms, virus particle morphology, and immunoelectron microscopy. Maize dwarf mosaic virus strain B (MDMV-B) was identified by the double-antibody sandwich enzyme-linked immunosorbent assay. *Dalbulus maidis*, leafhopper vector of MRFV, and *Rhopalosiphum maidis*, aphid vector of MDMV-B, were collected on maize. This report extends the known distribution of these damaging maize viruses within the neotropics.

Rayado fino (Spanish for fine striping) disease of maize (*Zea mays* L.) was first reported in El Salvador (1). The viral nature of the pathogen and confirmation of its transmission by the leafhopper *Dalbulus maidis* (Delong & Wolcott) were demonstrated by Gamez (5,6) with an isolate he found in Costa Rica. Subsequently, maize rayado fino virus (MRFV), or a serologically related strain thereof, has been identified in Uruguay, Brazil, Colombia, Panama, Nicaragua, Honduras, Guatemala (10), Peru (10,18), Venezuela (14), Mexico (10; O. E. Bradfute, *unpublished*), and the southern United States (2). Maize rayado Colombiano virus (15) and Brazilian maize streak virus (12) are considered strains of MRFV (8).

Yield losses of local maize cultivars in Central America may be up to 40–50% of MRFV-infected plants with field incidences up to 20%. Losses and incidences may reach 100% for newly introduced or developed maize cultivars (7). All maize cultivars screened for resistance in Colombia, Costa Rica, El Salvador, and Mexico were susceptible to the virus (9), and no commercially acceptable level of resistance was found in North American germ plasm tested (21).

Maize dwarf mosaic virus (MDMV), found in many countries throughout the world, has been reported in Venezuela (13), Colombia (15), Peru (18), Brazil (11), and Argentina (20). However, MDMV is considered less important than MRFV in Latin America.

From a survey of maize with viruslike symptoms in Ecuador, we report MRFV and MDMV. Identification of MRFV was on the basis of leaf symptoms, virus particle morphology, and serology. MDMV was found by serological screening for several maize viruses. An insect previously reported as a vector of each virus was also found. This is the first report of these viruses in Ecuador.

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## MATERIALS AND METHODS

**Survey sites and sample collection.** The ecology of Ecuador is affected by the Andes Mountains, which are separated into a Western Cordillera and an Eastern Cordillera with a mountainous valley in between. There are three ecological zones: the coastal lowlands, the sierra (or highland area), and the area east of the Andes known as the jungle lowlands. The survey areas lie in the central intermontane basins of the Ecuadorian sierra, ranging in altitude from 1,800 to 3,000 m. The climate is relatively mild and uniform with an average temperature of 15 C. A total area of 20 ha in the sierra was surveyed, including locations at Hacienda Perafin, 40 km north of Quito; Gonzales Suarez, 35 km north of Quito; Finca experimental, 24 km east of Quito; and Quito vicinity. J. H. Tsai collected leaf samples from maize plants with viruslike symptoms. Leafhoppers and aphids were collected from maize leaves with an aspirator.

**Assays.** Electron microscopy of negatively stained leaf sap on carbon/Formvar-coated grids was used to find virus particles in leaf samples. Immunoelectron microscopy, including immunosorbent trapping of virus particles on antibody-coated grids (4,19) and the antibody coating or decoration of trapped virus particles (16), was used to test for serological relationship. Antiserum to MRFV was supplied by R. Gamez (Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica, Ciudad Universitaria, Costa Rica), and normal rabbit serum was supplied by D. T. Gordon (Department of Plant Pathology, Ohio State University, Ohio Agricultural Research and Development Center, Wooster).

Fifty symptomatic leaf samples were tested by double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) for MDMV strain A (MDMV-A), MDMV strain B (MDMV-B), maize mosaic virus (MMV), maize stripe virus (MStpV), and MRFV. Standard ELISA procedures described by Clark and Adams (3) were used in all assays. Antisera to MDMV-A and MDMV-B were obtained from C. L. Niblett (Department of Plant Pathology, University of Florida, Gainesville). Antisera to MMV, MStpV, and MRFV were produced in Florida (B. W. Falk and J. H. Tsai, *unpublished*). The enzyme label was alkaline phosphatase and *p*-nitrophenyl phosphate in dimethanolamine buffer was used as the substrate. Results were assessed photometrically at 405 nm using a BioTek EIA Reader (Burlington, VT).

## RESULTS

**Field surveys.** Symptoms of rayado fino disease were found in about 3% of the maize plants at seven locations surveyed. Leaf symptoms (Fig. 1), similar to those typically associated with MRFV (2,8), consisted of rows of fine, bleached dots and streaks along second-, third-, and fourth-order veins contrasting vividly with green intervening leaf areas.

Specimens of *D. maidis* were found abundantly on maize plants 2,000–2,500 m above sea level. Their identity was confirmed by D. M. DeLong (Department of Entomology, Ohio State University, Columbus). Aphid vectors of MDMV-B, *Rhopalosiphum maidis* (Fitch), were found on maize and wheat plants. Transmission tests were not performed.

**Assays.** Immunoelectron microscopy revealed small isometric virus particles serologically related to MRFV in three of three samples with symptoms similar to those shown in Figure 1. Abundant virus

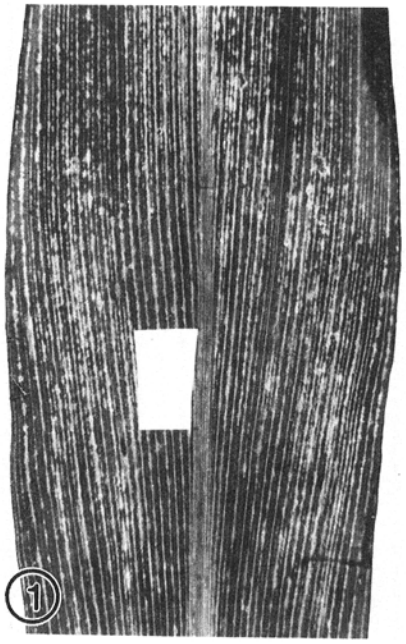
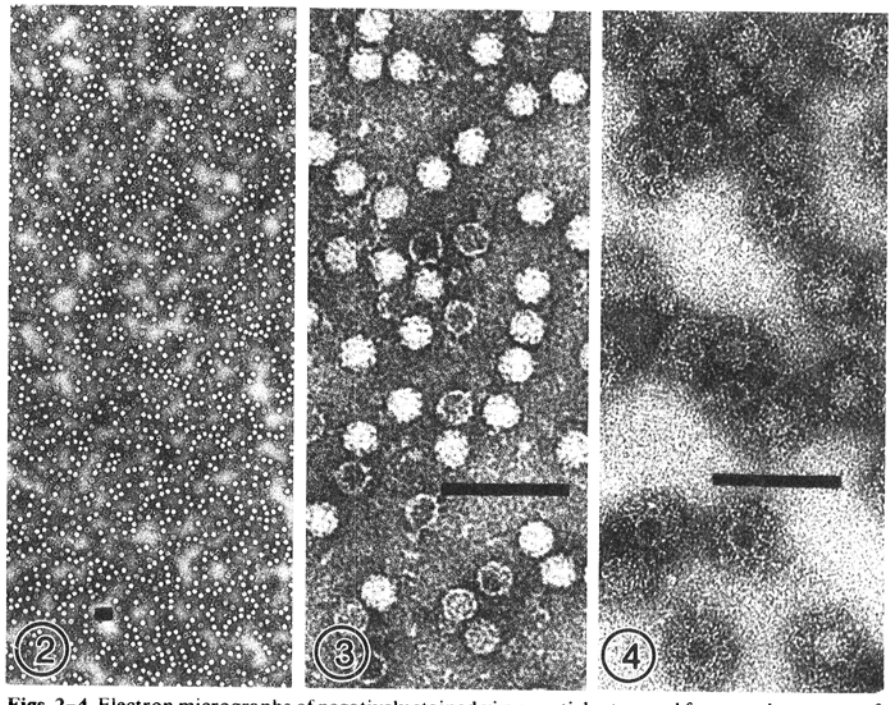


**Fig. 1.** Close-up of a naturally infected maize leaf collected in central Ecuador (2,100 m above sea level) showing symptoms of maize rayado fino virus. White rectangle shows typical location of a sample removed for electron microscopic examination.



**Figs. 2–4.** Electron micrographs of negatively stained virus particles trapped from crude extracts of leaf samples with symptoms of maize rayado fino virus (MRFV) on carbon/Formvar grids coated with MRFV antiserum. **(2)** Low magnification of abundant virus particles on typical grid area. **(3)** High magnification of particles characteristic of MRFV (isometric morphology, about 30 nm in diameter, showing stain penetration of some presumably empty particles and morphological subunits in other particles). **(4)** Virus particles trapped on a grid as in Figures 2 and 3, then exposed to MRFV antiserum. Note coating or decoration halo of homologous antibody molecules around each virus particle. Scale bars = 100 nm.

particles, appearing as a continuous lawn, were trapped on grids coated with MRFV antiserum (Figs. 2–4). In contrast, virus particles were infrequently found on grids not exposed to antiserum or grids coated with normal rabbit serum. In Figure 3, the appearance of the virus particles (isometric morphology, about 30 nm diameter, stain penetration of some presumably empty particles, and morphological subunits in other particles) is characteristic of negatively stained MRFV virions (2,8). Decoration or coating of these particles with MRFV antiserum confirms their serological relationship to MRFV (Fig. 4). Coating of virus particles was not observed when grids with trapped virus particles were exposed to normal rabbit serum. No other viruslike particles were detected.

ELISA showed 7 of 50 leaf samples reacted positively to MRFV antiserum and 4 of 50 leaf samples reacted positively to MDMV-B antiserum. No reaction to MDMV-A, MStpV, or MMV antisera was found in the 50 samples.

## DISCUSSION

On the basis of leaf symptoms, virus particle morphology, serology, and vector presence, we conclude that MRFV, or a strain thereof, occurs in Ecuador. Our results are consistent with the previous findings of MRFV and *D. maidis* occurring together with maize over a wide range of ecological conditions throughout the neotropics (9). The elevation at which we found MRFV in Ecuador suggests its similarity to the serologically related maize rayado Colombiano virus that occurs in Colombia exclusively above 1,000-m elevation (15). A potential for increased losses caused by this damaging maize virus is suggested by 1) its apparent penetration of regions subjected to seasonal low temperature, including the southern United States; 2) its experimental transmission by *Graminella nigrifrons* (Forbes), an oligophagous leafhopper that occurs abundantly throughout the eastern half of the United States; and 3) the lack of resistant maize genotypes (2,17,21).

The occurrence of MDMV-B with its vector, *R. maidis*, in the same ecological zone is consistent with previous reports of this virus in other South American countries (11,13,15,18,20). The low importance given this virus in South America (11,13) may result from the presence of more severe maize virus diseases rather than the actual incidence and losses caused by MDMV.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