Mosaic Disease of *Rhoeo discolor* Caused by a Strain of Tobacco Mosaic Virus

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ABSTRACT

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Rhoeo discolor plants showing mosaic symptoms were systemically infected with a rigid rod virus of the tobamovirus group. The virus was mechanically transmissible but had a limited host range. It did not infect *Nicotiana tabacum* 'Turkish,' *N. glutinosa, Phaseolus vulgaris* 'Pinto,' or *Datura stramonium*, all of which are susceptible to the type strain of tobacco mosaic virus (TMV). Properties of the virus in crude *R. discolor* sap were thermal inactivation between 92 and 94 C for 10 min, aging in vitro over 8 mo, and dilution end point between 10^{-5} and 10^{-6}. Healthy plants of *R. discolor* inoculated with partially purified preparations of the virus developed a systemic mosaic 1–2 mo after inoculation. The virus gave precipitin zones of serological relatedness in reciprocal gel double-diffusion tests with type TMV.

Rhoeo discolor (L. Her.) Hance, an herbaceous perennial monocotyledon in the family Commelinaceae, is commonly cultivated as an ornamental. It is often used in cytogenetical teaching and research because it has fairly large chromosomes that frequently produce abnormal ring configurations during meiosis (11).

During the past 10 yr, *Rhoeo* plants with mosaic symptoms have been frequently received from growers, and infected plants are often offered for sale through retail outlets. Electron microscopy of leaf-dip preparations from such plants showed the presence of rigid rod viruslike particles similar to those of the tobamovirus group.

This paper characterizes the causal agent of the mosaic disease of *R. discolor* as a strain (RD) of tobacco mosaic virus (TMV). Preliminary results have been published (14).

MATERIALS AND METHODS

Inoculation and host range. Crude sap from young leaves of greenhouse-grown plants of *R. discolor* showing irregular pale greenish yellow streaks and patches on the upper dark green leaf lamina (Fig. 1) and a corresponding "bleaching" of the anthocyanin pigments on the lower leaf surface was used for mechanical inoculation by the Carborundum gauze-pad method. Plants of 35 species either susceptible and diagnostic to type TMV or susceptible to many other viruses were inoculated. In preliminary tests, *Chenopodium quinoa* Willd. reacted with local lesions; thus it was used for all subsequent bioassays.

Virus properties. Sap from infected *R. discolor* plants was used to determine dilution end point, thermal inactivation, and aging in vitro by the methods described by Ross (13).

Purification. Infected leaves of *R. discolor* ground in a food chopper yielded about 0.6 ml of crude sap per gram of tissue. Phosphate buffer (0.1 M, pH 7.3) was added at 0.5 ml/ml of leaf extract, the mixture was expressed through cheesecloth, and activated charcoal, Merck NF 18351, was added at a rate of 0.1 g/10 ml of slurry (4). The charcoal was removed by centrifugation (6) for 10 min at 12,000 *g* and the supernatant filtered through Whatman No. 1 filter paper. The clarified sap was given three cycles of differential centrifugation of 90 min at 75,000 *g* and 10 min at 12,000 *g*. Total virus protein was determined by dry weight and spectrophotometry. Ultraviolet (UV) absorption spectra of purified preparations of TMV-RD and type TMV were made in a Zeiss PMQ-II spectrophotometer.

Final purification of TMV-RD was obtained by centrifugation in 10–40% sucrose density gradients for 90 min at 52,000 *g* in an SW 25.1 rotor. Sedimentation coefficients were obtained by centrifuging 2 mg/ml of TMV-RD, compared with 2.5 mg/ml of type TMV, at 23,150 rpm for 25 min in a Beckman Model E analytical centrifuge equipped with schlieren and UV optics.

Electron microscopy. Leaf-dip preparations, partially purified preparations, and samples from light-scattering zones after density-gradient centrifugation were shadowed with chromium or treated with 2% phosphotungstic acid, pH 7.0, and examined in a Hitachi HU-11C electron microscope. Comparative preparations of type TMV were used for particle size determination.

Infected *R. discolor* tissue was fixed in 6% buffered glutaraldehyde, postfixed in 1% buffered osmium tetroxide, dehydrated in a graded series of ethyl alcohol, and embedded in a mixture of Maraglas-Cardolite. Ultrathin sections, cut with glass knives on a Porter-Blum MT-1 microtome, were stained with lead citrate and uranyl acetate and examined in the electron microscope.

Serology. Over a period of 30 days, a New Zealand rabit was given seven intravenous and two intramuscular injections of TMV-RD. Intravenous and intramuscular injections were 2 mg of virus in sterile physiological saline and Freund's incomplete adjuvant, respectively. Ten days after the last injection, the titer of the antiserum was determined by microprecipitin tests.

In gel double-diffusion tests, TMV-RD was reciprocally tested against type TMV and six strains of TMV. Antisera to TMV-RD and type TMV were cross-absorbed by the tube absorption test described by Matthews (10) and by the intragel absorption technique of Van Regenmortel (16).

RESULTS AND DISCUSSION

Symptomatology and host range. TMV-RD is easily differentiated from type TMV and most of the natural strains of TMV on the basis of symptomatology and host range. Of the 35 species in 10 monocot and dicot families, only *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa, Capsicum annuum* L. 'Yolo Wonder' and 'California Wonder,' *C. frutescens* L. 'Tabasco,' and *Nicotiana tabacum* L. 'Kentucky 35' developed symptoms after inoculation with TMV-RD. Chlorotic local lesions appeared about 6 days after inoculation on leaves of *C. quinoa*, 8–10 days on *C. amaranticolor*, and 4–10 days on the inoculated leaves of tobacco cultivar Kentucky 35. *C. quinoa* gave the most reliable and consistent lesion number. *Emilia sagittata* (Vahl.) DC., *Torenia fournieri* Lind., and *Nicotiana clevelandii* Gray showed no symptoms, but TMV-

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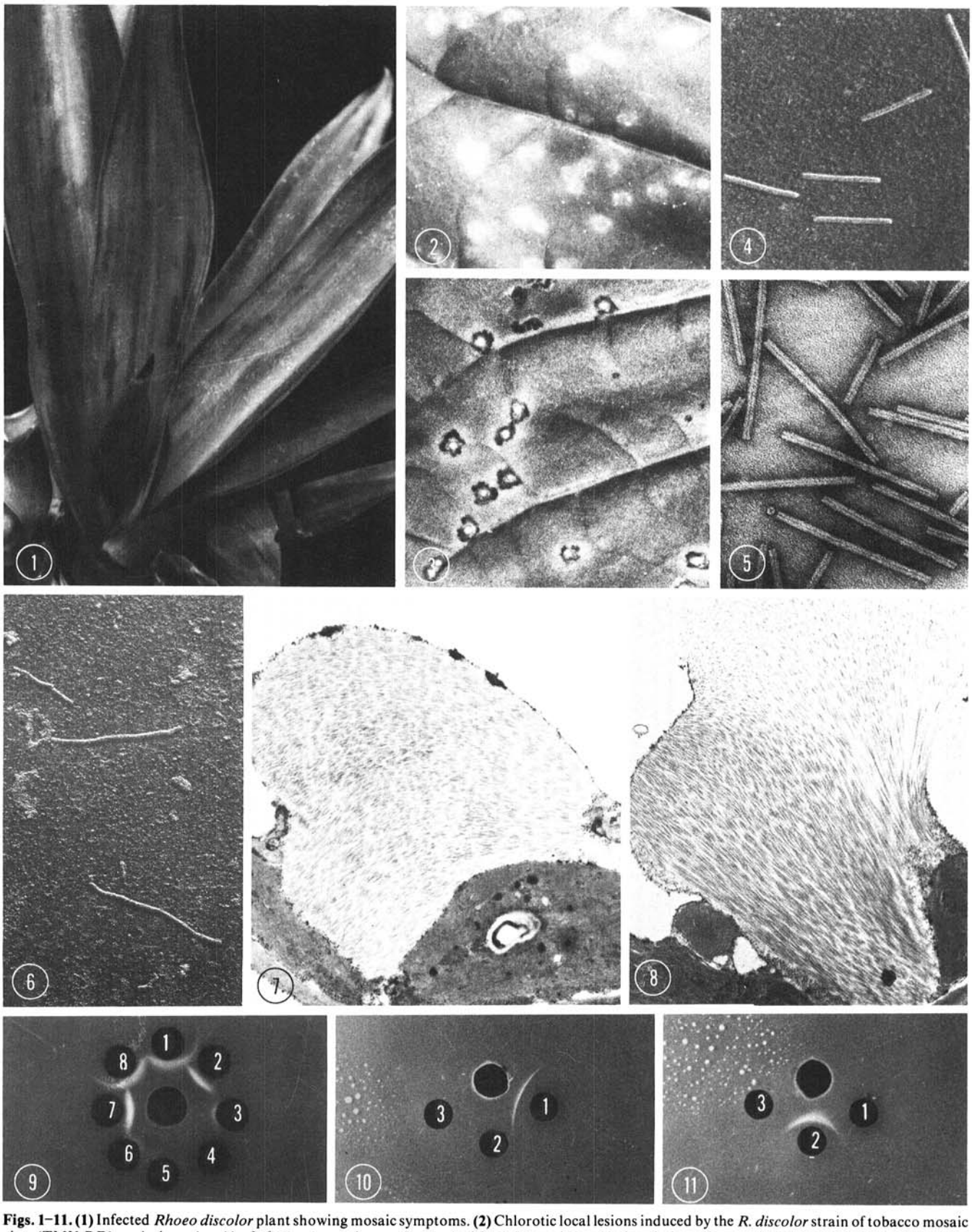

Figs. 1–11. (1) Infected *Rhoeo discolor* plant showing mosaic symptoms. **(2)** Chlorotic local lesions induced by the *R. discolor* strain of tobacco mosaic virus (TMV-RD) on the inoculated leaf of *Nicotiana tabacum* 'Kentucky 35.' **(3)** Necrotic local lesions induced by type TMV on the inoculated leaf of *N. tabacum* 'Kentucky 35.' **(4)** Electron micrograph of a partially purified preparation of TMV-RD shadowed with chromium (×50,000). **(5)** Electron micrograph of a purified preparation of TMV-RD treated with 2% phosphotungstic acid (×100,000). **(6)** Electron micrograph of flexuous rod viruslike particles in a chromium-shadowed leaf-dip preparation from *R. discolor* (×50,000). **(7 and 8)** Electron micrographs of ultrathin sections of infected *R. discolor* leaf tissue (×2,500). **(9)** Gel double-diffusion plate: center well received TMV-RD antiserum; peripheral wells 1 and 8, TMV-RD; wells 2 and 7, type TMV; well 3, TMV aucuba strain; well 4, cucumber virus 3; well 5, TMV ribgrass strain; and well 6, yellow tomato atypical mosaic virus. **(10)** Gel double-diffusion plate: center well received type TMV antiserum cross-absorbed three times with purified TMV-RD; well 1, type TMV; and wells 2 and 3, TMV-RD. **(11)** Gel double-diffusion plate: center well received TMV-RD antiserum cross-absorbed three times with purified type TMV; wells 1 and 3, purified type TMV; and well 2, TMV-RD.

RD was recovered from the uninoculated leaves. Unlike type TMV, TMV-RD has a limited host range and did not induce necrotic local lesions on Kentucky 35 (Figs. 2 and 3), *N. glutinosa* L., and the tobacco varieties carrying the NN gene (9). The inability of TMV-RD to infect some members of the Solanaceae is similar to that reported for rigid rod viruses of the tobamovirus group isolated from orchids, cactus, and cucumber. Bald and Tinsley (2) noted that if a virus is well adapted to a single genetically stable host, it may be restricted in its ability to infect widely different species. This may be true of TMV-RD.

The following plants did not show symptoms and TMV-RD was not recovered from the uninoculated leaves: *Gomphrena globosa* L.; *Vinca rosea* L.; *Cassia occidentalis* L.; *Zinnia elegans* Jacq.; *Cucumis sativus* L. 'National Pickling,' 'Long Marketer,' 'Ohio MR17,' and 'Chicago Pickling'; *Cucurbita maxima* Duch.; 'Early Prolific Straightneck'; *Citrullus vulgaris* Schrad. 'Charleston Grey'; *Phaseolus vulgaris* L. 'Bountiful' and 'Pinto'; *Pisum sativum* L. 'Alaska'; *Glycine max* (L.) Merr. 'Harosoy'; *Vicia faba* L. 'Long Pod'; *Hordeum vulgare* L. 'Wong'; *Oryza sativa* L. 'Nato'; *Secale cereale* L. 'Abruzzi'; *Setaria italica* L. 'German'; *Sorghum vulgare* L.; *Triticum aestivum* L. 'Redcoat'; *Zea mays* L. 'Golden Cross Bantam'; *Datura stramonium* L.; *Lycopersicon esculentum* Mill. 'Marglobe'; *Nicotiana glutinosa*; *N. rustica* L.; *N. sylvestris* Speg. & Comes.; *N. tabacum* 'Samsun,' 'Samsun NN,' and 'Turkish'; and *Solanum tuberosum* L. 'USDA 41956.'

Virus was not transmitted from 13 apparently healthy *R. discolor* plants and one *R. discolor* 'Concolor' (leaves green on both surfaces) plant, and viruslike particles were not observed in leaf-dip preparations from 12 of the plants. These plants were mechanically inoculated with crude sap from symptomatic *R. discolor* plants. Inoculated leaves did not develop symptoms, but 1–2 mo after inoculation, the young uninoculated leaves showed a greenish yellow mosaic with corresponding "bleaching" of the lower leaf surfaces; uninoculated leaves of *R. discolor* 'Concolor' showed darkening of the green pigmentation of the upper leaf surface. Virus was recovered from all inoculated plants as indicated by chlorotic local lesions on *C. quinoa*. Electron microscopy of leaf-dip preparations revealed rigid rod viruslike particles. The recovered virus did not infect plants of *N. glutinosa*, indicating that the plants were not infected with type TMV.

TMV-RD in crude *R. discolor* sap had a dilution end point between 10^{-5} and 10^{-6}, thermal inactivation point between 92 and 94 C, and was still infectious after 253 days at 25–31 C. These properties of TMV-RD are similar to those reported for tobamoviruses (18).

Purification. Partially purified preparations contained, on the average, 0.9 mg of virus per milliliter of crude sap. Partially purified preparations of TMV-RD gave two light-scattering zones after rate-zonal density-gradient centrifugation. Comparable preparations of type TMV also gave two zones when centrifuged under similar conditions. Samples from the top and bottom zones of TMV-RD produced an average of 180 and 155 lesions, respectively, per inoculated leaf. Zone separation has been attributed to aggregation (3), but electron microscopy of samples from the two zones did not show any differences in the amount of aggregation. Both zones contained fragments and unit length particles; this may have resulted from preparation of the samples for electron microscopy. Regardless of the purification method, virus concentration (0.2–2 mg/ml), sucrose concentration (10–40% to 30–60% saturation), or centrifugation time (40 min to 15 hr), two zones always occurred. Sedimentation coefficients were 182 S and 196 S for type TMV and 170 S and 204 S for the top and bottom zones, respectively, of TMV-RD.

The UV absorption spectra of TMV-RD and type TMV at a concentration of 0.2 mg/ml were similar, except TMV-RD did not show increased absorption at 291 nm. Ginoza and Atkinson (7) attributed the 291-nm absorption to the tryptophan/tyrosine ratio in the viral protein and separated TMV strains into three groups based on the extent of that absorption. In this respect, TMV-RD appeared similar to Holmes' (9) ribgrass strain of TMV.

Electron microscopy. Chromium-shadowed leaf-dip preparations, partially purified preparations, and samples from the zone in density gradients contained rigid rod particles about 312 ± 2 nm long (Fig. 4). Preparations examined in 2% phosphotungstic acid, pH 7.0, showed particle substructure similar to that reported for TMV (Fig. 5). Leaf-dip preparations from one apparently healthy *R. discolor* plant showed the presence of flexuous rod viruslike particles about 574 nm long (Fig. 6). Rigid rod viruslike particles were not detected in the preparation, and no additional studies were made.

Electron microscopy of ultrathin sections of infected *R. discolor* tissue showed the presence of packets of viruslike particles in the cytoplasm (Figs. 7 and 8). The particles were not in any orderly arrangement as shown by Warmke and Edwardson (17) for type TMV or the aucuba strain of TMV but appeared as masses that greatly extended the tonoplast, which was often ruptured into the vacuole.

Serology. TMV-RD antiserum had a homologous dilution end point of 1:256 in microprecipitin tests. In gel double-diffusion tests, the virus reacted with precipitin zones against antisera to type TMV, aucuba strain (AV), Holmes' ribgrass virus (HRV), yellow tomato atypical mosaic virus (YTAMV), and Sammons' Opuntia virus (SOV). TMV-RD gave no precipitin zones against antisera to cucumber virus 3 (CV3) or the orchid strain of TMV (TMV-O). Antiserum to TMV-RD gave precipitin zones when reacted with type TMV, YTAMV, and AV but not with HRV, CV3, or SOV. Preimmune serum did not react in gel diffusion tests with any viruses used. Precipitin zones of identity occurred between TMV-RD preparations, and spur formation occurred with type TMV (Fig. 9). The spurs were long and the point of intersection was angular rather than rounded, indicating a distant serological relationship (5,8,16). In reactions with cross-absorbed antisera by either the tube absorption procedure (10) or by intragel absorption (16), type TMV antiserum absorbed with TMV-RD formed a precipitin zone with type TMV but not with TMV-RD (Fig. 10). TMV-RD antiserum absorbed with type TMV formed a precipitin zone with TMV-RD but not with type TMV (Fig. 11), indicating that TMV-RD and type TMV have some different antigenic sites or that slightly different antibodies were produced to similar types of reactive antigenic sites (1,12,15).

TMV-RD differs from type TMV in host range and symptomatology but is serologically related to type TMV. The degree of this serological relationship has not yet been determined, but at this time, TMV-RD can be placed in the TMV group of virus strains. It appears to be a unique strain of TMV that may offer interesting possibilities for molecular studies.

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