Purification and Properties of Foxtail Mosaic Virus

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ABSTRACT

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Foxtail mosaic virus (FMV) infected 56 grass and 35 dicotyledonous species. Development of systemic symptoms first on maturing leaves rather than on emerging leaves was unique and characteristic. Foxtail mosaic virus was seedborne in *Briza maxima* (2%) and Clintland oats (1%). *Myzus persicae* and *Nephotettix impicticeps* were not vectors. Crude sap dilution endpoint was 10^{-6}, thermal inactivitation point 70 C, longevity in vitro was 46 days in undiluted sap and 105 days in buffered extract. Foxtail mosaic virus was purified from Reno barley by chloroform-butanol clarification and differential centrifugation. Igepon-T-73 and sucrose added to the phosphate buffer for extraction and resuspension reduced aggregation. Purified FMV had an $A_{260/280}$ ratio of 1.2 and was very stable at 4 C. Two components sedimenting at 122S and 144S were detected. Extracted nucleic acid sedimented at 32S and its infectivity was eliminated by RNase treatment. Leaf-dip and purified preparations contained rod-shaped particles averaging 430 × 15 nm. Serological tests showed no relationship between FMV and barley stripe (BSMV), brome mosaic (BMV), clover yellow mosaic, panicum mosaic, papaya mosaic, potato X, or tobacco mosaic viruses. Cross-protection tests showed no relationship between FMV and BSMV or BMV.

Additional key words: foxtail, *Setaria viridis*, *S. italica*, grass viruses.

A sap-transmissible virus was isolated in 1967 from the foxtails *Setaria viridis* (L.) Beauv. and *S. italica* (L.) Beauv. with mildly chlorotic striped leaves. The plants were collected in a cornfield in Leavenworth, Kansas. Assays on indicator hosts for several sap-transmissible grass viruses suggested that this virus differed from known grass viruses. We designated this virus foxtail mosaic virus (FMV). Based on earlier data (7, 8) and that reported here, we concluded that FMV is distinct from other known sap-transmissible grass viruses.

MATERIALS AND METHODS

Foxtail mosaic virus was maintained on either Pawnee wheat (*Triticum aestivum* L.) or Reno barley (*Hordeum vulgare* L.). Inoculum routinely was prepared from plants infected 1-4 mo. One part leaves was ground with mortar and pestle in 15-20 parts 0.03 M potassium phosphate buffer, pH 8.0, containing 2% Celite and 1% 2-mercaptoethanol. The extract was filtered through cheesecloth and rubbed on Carborundum-dusted leaves with a cheesecloth pad. In virus characterization experiments, inoculum was prepared only from leaves with distinct mosaic symptoms and dilutions were made with 0.03 M phosphate buffer, pH 8.0. All plants were grown from seed and inoculated while very young. Back-inoculations and other bioassays were made on half-leaves of *Chenopodium amaranticolor* Coste & Reyn. All inoculations were done above 24 C. Plants were grown under supplemental light that provided a 12- to 14-hr daylength during winter.

Seed transmission.—Several species that were systemically infected in the host range trials were allowed to set seed in the greenhouse. Approximately 1,000 seedlings of each species were grown and tested for virus by inoculation to *C. amaranticolor*.

Virus purification.—Foxtail mosaic virus was inoculated to 10- to 12-day-old Reno barley seedlings and both inoculated and systemically-infected leaves were harvested 21-30 days later. Leaves were cut into short strips and ground in small batches in a Waring Blendor with 1.5 ml of cold 0.03 M phosphate buffer, pH 8.0, and 1% 2-mercaptoethanol/g tissue. The liquid was squeezed through cheesecloth and used to grind additional batches. An equal volume of chloroform:butanol (1:1, v/v) mixture was added slowly with gentle stirring. The emulsion was kept at 4 C for 30-60 min then centrifuged 10 min at 5,000 *g*. The aqueous phase was filtered through glass wool and centrifuged 1.5 hr at 147,000 *g*. Pellets were resuspended in 3-4 ml 0.02 M phosphate buffer, pH 7.5, overnight at 4 C, and resuspension was completed the next day by gently stirring with a glass rod. The preparations were pooled and centrifuged 10 min at 12,000 *g*. After two additional cycles of differential centrifugation (60 min at 269,000 *g*, then 10 min at 12,000 *g*), final pellets were resuspended in buffer (2-3 ml/100 g tissue). These were combined, centrifuged 10 min at 12,000 *g*, and the resulting supernatant liquid (purified virus) was stored at 4 C.

An alternative procedure involved grinding tissue in 0.5 M phosphate buffer, pH 7.5, containing 0.1% Igepon T-73, 1% 2-mercaptoethanol and 1% sucrose. Antifoam A (Dow Corning) was sprayed into the Blendor jar before

TABLE 1. Symptoms induced by foxtail mosaic virus on mechanically inoculated hosts

Family and species	Symptoms[a]
Amaranthaceae	
Celosia plumosa Hort.	CL
Gomphrena globosa L.	RL
Apocynaceae	
Vinca rosea L.	ISL
Chenopodiaceae	
Beta vulgaris L.	RL
Chenopodium album L.	CL, NL, SCR, LP
C. amaranticolor Coste & Reyn.	CL, NL, SCR, LP
C. ambrosioides L.	RL, SCR, LP
C. capitatum (L.) Asch.	SL, NL, SCR, LP
C. foetidum Lam.	CL, NL, SM
C. hybridum L.	CL, NL, SM, LP
C. murale L.	NL, SM
C. quinoa Willd.	CL, NL, SM
C. rubrum L.	CL, NL, SCR, LP
C. urbicum L.	CL, CLR, SCR, LP
Spinacea oleracea L.	CL, NL, CLR, ISS
Compositae	
Zinnia elegans Jacq.	ISL
Cruciferae	
Arabis alpina L.	ISL
Brassica juncea (L.) Coss	ISL
Cucurbitaceae	
Cucumis sativus L.	ISL
Gramineae	
Aegilops bicornis Jaub & Spach.	CL, SM
A. cylindrica Host.	CL, SM
A. juvenalis L.	CL, SM
A. triaristata Ref. E Bertol.	CL, SM
A. triuncialis L.	CL, SM
Agropyron elongatum (Host) Beauv.	ISL
Alopercurus pratensis L.	ISL
Andropogon caucasius Trin.	ISL
A. halli Hack	ISL
A. ischaemum L.	ISL
Avena sativa L.	SM
A. sterilis L.	SM
Bouteloua curtipendula (Michx.) Torr.	SM
B. gracilis (HBK) Lag. & Steud.	ISS
Briza maxima L.	SM
Bromus inermis Leyss	ISL
Buchlöe dactyloids (Nutt.) Engelm.	SM
Coix lacryma-jobi L.	ISS
Cynodon dactylon (L.) Pers.	SM
Digitaria sanguinalis (L.) Scop.	ISS
Echinochloa crus-galli (L.) Beauc.	ISS
Hordeum vulgare L.	SM
Lagurus ovatus L.	SM
Oryza sativa L.	ISS
Panicum anceps Michx.	SM
P. antidotale Retz.	SM
P. capillare L.	SM
P. decompositum L.	SM
P. maximum Jacq.	SM
P. miliaceum L.	SM
P. ramosum L.	SM
P. scribnerianum Nash	SM
P. stapfianum Fourc.	SM
P. turgidum Forsck.	SM
P. virgatum L.	SM

TABLE 1. (continued)

Family and species	Symptoms[a]
Paspalum notatum Flugge	ISL
Pennisetum glaucum (R.) Br.	SM
Phalaris arundinacea L.	CL
Phleum pratense L.	ISL
Poa pratensis L.	ISL
Secale cereale L.	SM
Setaria faberii Herm.	SM
S. italica (L.) Beauv.	SM
S. sphacelata (Schramm) Stapg. & Hubb.	SM
S. verticillata (L.) Beauv.	SM
S. viridis (L.) Beauv.	SM
Sorghastrum nutans (L.) Nash	SM
Sorghum bicolor (L.) Moench	SM
S. vulgare var. *sudanense* Hitch.	SM
Tripsacum dactyloides L.	SM
Triticum aestivum L.	CL, SM
T. dicoccum Schrank	CL, SM
T. durum Desf.	CL, SM
T. monococcum L.	CL, SM
T. timopheevi Zhukolv.	CL, SM
Zea mays L.	SM
Leguminosae	
Cassia florida Vahl.	NL
Crotalaria spectabilis Roth.	NL
Cyamopsis tetragonoloba (L.) Taub.	NL
Glycine max (L.) Merr.	ISL
V. sinensis (Torn.) Savi	ISL
Scrophulariaceae	
Torenia fournieri Lindl.	ISS
Solanaceae	
Lycopersicon esculentum Mill.	ISS
Nicandra physaloides Gaertn.	ISL
Nicotiana glauca Graham	ISS, SCR
N. megalosiphon Heurck & Muell.	NL, ISS, SCR
N. multivalvis Lindl.	ISL
N. raimondi L.	ISS
N. rustica L.	NL
N. tabacum L.	ISS
Physalis floridana Rydb.	ISL

[a]Abbreviations for symptoms: CL, RL, or NL - chlorotic, red, or necrotic local lesions; CLR - chlorotic local rings; ISL or ISS - infection symptomless - local (L) or systemic (S); LP - line patterns; SCR - systemic chorotic rings; and SM - systemic mosaic.

use to prevent foaming. Clarification and further purification were as described above and the final pellets were resuspended in 0.02 M phosphate, pH 7.5, containing 0.1% Igepon T-73 and 1% sucrose.

Sedimentation coefficients of foxtail mosaic virus and foxtail mosaic virus nucleic acid.—Sedimentation coefficients were determined using linear-log sucrose gradients as described by Brakke and Van Pelt (2). Virus and RNA standards were prepared, analyzed, and fractionated as previously described (6). All fractions were dialyzed overnight in 0.02 M potassium phosphate buffer, pH 7.0. One-half of each dialyzed nucleic acid fraction was incubated with 2 μg ribonuclease A (RASE, Worthington Biochemical Corp., Freehold, NJ 07728) for 30 min at 37 C. All preparations were inoculated to *C. amaranticolor* with autoclaved cotton swabs.

Electron microscopy.—One part purified FMV(A_{260} = 1) was mixed with nine parts of 1% sodium phosphotungstate (NaPT) and the mixture incubated 20-30 min at 4 C. A drop of the mixture was placed on carbon stabilized, Formvar-coated grids and viewed in a RCA-EMU4 or Hitachi HU-11B electron microscope. Leaf-dip preparations were made by touching a freshly cut, healthy or infected barley leaf to a drop of NaPT on a grid and viewed similarly.

Serology.—One ml of purified FMV (A_{260} = 5.0 was emulsified with an equal volume of Difco Freund's incomplete adjuvant and injected intramuscularly into a rabbit. Seven injections were given at 4-day intervals, and blood was sampled for antiserum at weekly intervals. Antiserum titer was determined by the microprecipitin method (1) with FMV at A_{280} = 0.03. Routine assays for FMV and tests for relationships to some viruses were performed using the Ouchterlony double-diffusion procedure (0.75% Ionagar No. 2 in 0.05 M Tris-HCl, pH

7.5, 0.85% NaCl, .02% NaN_3)(1). Relationships with potex viruses [potato virus X (PVX) clover yellow mosaic virus (CYMV) and papaya mosaic virus (PaMV)] were investigated by three procedures; (i) agar double diffusion for both intact and pyrrolidine-disrupted virus (9), (ii) tube precipitin (1), and (iii) serologically specific electron microscopy (3).

Insect transmission.—Apterous *Myzus persicae* (Sulz.) adults were used to determine whether FMV might be transmitted in a stylet-borne or circulative manner to Pawnee wheat and *C. quinoa*. For stylet-borne transmission trials the aphids were starved 30 min prior to a 5 min access feeding period on FMV-infected wheat leaves. Ten aphids observed to probe within this time were transferred to each test seedling and removed 3 hr later. For persistent transmission trials, five to ten adults from an established colony on infected *C. quinoa* or *C. amaranticolor* were transferred to each young healthy *C. quinoa* plant and removed after 24, 48, or 72 hr.

The rice green leafhopper, *Nephotettix impicticeps* Ish. was used with the rice cultivars Taichung Native 1 and IR-20 as assay and production hosts. For nonpersistent transmission trials, male and female adults were starved for 2 hr then fed on infected rice leaves. After 30, 60, 120, and 180 min, two to five leafhoppers were transferred to seedlings. For persistent transmission trials, four to five instar nymphs were permitted to feed on infected IR-20 rice for 2 to 5 days. One to two leafhoppers were transferred to each test seedling using the test tube feeding technique (5). Daily serial transfers were made to healthy seedlings for 10 days.

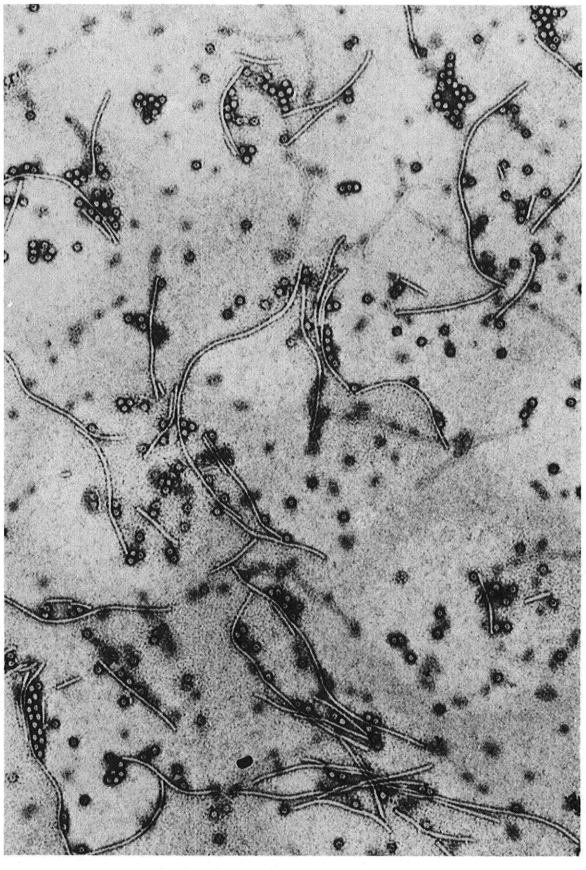

Fig. 1. Electron micrograph (× 55,000) of foxtail mosaic virus particles purified from Reno barley. Bean pod mottle virus was added as an internal standard and has a diameter of about 30 nm.

RESULTS

Host range, symptoms, and seed transmission.—Foxtail mosaic virus infected a wide host range among the grasses and dicotyledonous species (Table 1). Of 68 grasses tested, only 12 were found immune in repeated trials. They were *Agropyron smithii* Rydb., *Andropogon halli* Hack., *A. intermedius* Brown, *Arrhenatherum elatius* (L.) Mert. and Koch, *Digitaria horizontalis* Willd., *Lolium perenne* L., *Phalaris arundinacea* L., *Sisyrinchium bellum* Watts., *Sorghum halepense* (L.) Pers., *Sporobolus cryptandrus* Gray, *Stipa viridula* Trin., and *Zoysia japonica* Steud. Two other monocots, a *Commelina* sp. and *Cyperus esculentus* L., also were immune.

Reactions of the 56 susceptible grasses ranged from localized to systemic infections, with many as symptomless hosts (Table 1). On *Setaria* species and most susceptible grasses, the first symptoms were observed on emerging leaves as a chlorotic striping that persisted until the leaves became senescent. Wheat, rye, barley, and susceptible corn cultivars showed distinct light-green to yellow eyespots which became golden or ashy white in color at temperatures above 24 C.

Of 111 dicotyledonous species tested only 35 were found to be susceptible. Susceptible dicotyledonous species either were symptomless hosts or only became infected locally (Table 1). Lesions frequently became necrotic at temperatures below 22 C. Except for *Beta vulgaris* on which only local lesions were induced, all other species of Chenopodiaceae tested reacted with local and systemic infection. Only two other species, *Nicotiana glauca* and *N. megalosiphon* became systemically infected with visible symptoms. On these hosts, systemic symptoms as chlorotic rings and line patterns appeared 10-14 days after inoculation. The sequence of appearance of systemic symptoms on wheat, barley, rye, and related species, as well as on the *Chenopodium* spp. was characteristic. Local lesions as chlorotic spots developed on inoculated leaves in 5-6 days. Systemic symptoms appeared initially on the developed uninoculated leaves above the inoculated ones, and much later on emerging leaves as they expanded and matured. The symptomless emerging leaves, however, provided very infectious extracts.

Best symptoms were observed at temperatures above 24 C. At lower temperatures, local lesions frequently became slightly necrotic, and symptom appearance generally was delayed in all hosts.

Based on over 1,000 seedlings grown per species and assayed on *C. amaranticolor*, no seed transmission was detected in *C. amaranticolor*, *C. quinoa*, *Setaria italica*, *S. lutesecens*, *S. viridis*, *Panicum capillare*, Reno barley, or Pawnee wheat. About 2% transmission was observed in *Briza maxima* and 1% in Clintland oats (*Avena sativa*). On *B. maxima* some seedlings showed mosaic symptoms due to seed-transmitted FMV, but most did not. No

symptoms were observed on oat seedlings, but FMV infection was demonstrated by back inoculation to *C. amaranticolor*.

Physical properties.—Extracts prepared from infected Pawnee wheat or Reno barley were diluted in 0.03 M phosphate buffer, pH 8.0. A few local lesions were induced at 10^{-6}, but none at 10^{-7} dilution. Foxtail mosaic virus tolerated heating at 68 C but not 70 C for 10 min. Undiluted sap kept at 24-26 C was still infectious after 105 but not after 119 days. Some infectivity was lost when diced leaves were desiccated over $CaCl_2$. These samples were infectious only to 10^{-5} dilution after desiccation for 21 days at 4 C, but contained infectious virus after 5 yr, the longest time tested. Lyophilized and liquid-nitrogen frozen tissues prepared by the American Type Culture Collection (ATCC virus number PV-139) were also infectious for at least 1 yr.

Cross-protection tests.—Previous infection of *C. amaranticolor* and Golden Cross Bantam sweet corn by FMV did not protect such plants from subsequent infection by either brome mosaic (BMV) or barley stripe mosaic (BSMV) viruses. Similarly, infection by BMV or BSMV did not protect *C. amaranticolor* against FMV infection.

Purification and sedimentation coefficients.—Both purification techniques yielded opalescent preparations with $A_{260/280} = 1.2$. The preparations were highly infectious, producing 100 or more local lesions per half-leaf at $A_{260} = 0.03$. Some precipitation was apparent after 6 mo storage at 4 C, but preparations were still highly infectious.

None of several methods tried for purifying FMV resulted in total recovery of virus from the plant tissue. Considerable virus (measured by infectivity) was lost at each purification step and this was only partially prevented by adding Igepon T-73 and sucrose to the buffer. Two opalescent and UV-absorbing components were detected after density-gradient centrifugation of purified virus. These sedimented at 122S and 144S and both were infectious. Analysis of nucleic acid preparations revealed a single, infectious, UV-absorbing component sedimenting at 32S, and this component was obtained from both the 122S and 144S components. Infectivity of the nucleic acid preparations was eliminated by ribonuclease treatment. This suggests that FMV contains one single-stranded RNA species with a molecular weight of about 2.03 to 2.24×10^6 daltons (4, 11). In comparative experiments FMV-RNA sedimented slightly more rapidly than TMV-RNA which sediments at 31S and has a molecular weight of 2.05×10^6 daltons (2, 11).

Electron microscopy and serology.—Purified preparations of FMV contained particles that were rod-shaped and flexuous (Fig. 1). Several different lengths were observed, but the most common particles measured 430×15 nm. Of over 1,000 particles that were measured, 37% were this length. End-to-end aggregation of particles was very common in purified preparations. Leaf dip preparations also contained many 430×15-nm particles, but aggregation was less frequent.

Antiserum prepared to purified FMV possessed a titer of 1:1,280 when tested by the microprecipitin method with FMV at $A_{280} = 0.03$. In Ouchterlony agar diffusion tests antisera to BSMV, BMV, CYMV, panicum mosiac (PMV), PaMV, PVX, and tobacco mosiac (TMV) viruses were reacted with purified FMV and crude extracts of plants infected with the different viruses. In addition, crude extracts of plants infected with FMV, CYMV, PaMV, and PVX were mixed with an equal volume of 5% pyrrolidine and reacted similarly with antisera produced to protein subunits of CYMV, PaMV, and PVX. Precipitin bands formed only between homologous antigen:antibody combinations, except in the case of the two PVX antisera, which reacted to both intact and disrupted PVX. In tube precipitin tests 0.5 ml of a 1:4 dilution of all antisera was reacted with an equal volume of purified FMV or PVX (both 1 mg/ml) or 0.5 ml of an extract from healthy or FMV-infected barley at 37 C for 2 hr and then stored overnight at 4 C. The barley tissue was ground in 0.05 M Tris-HCl, pH 7.5, (5 g/10 ml), and the extract was filtered through cheesecloth and centrifuged (20 min, 30,000 *g*) before use. Abundant precipitate was observed in reactions between purified and crude FMV and FMV antiserum and also between PVX and its antiserum, but no other reactions were observed. Serologically specific electron microscopy was performed in all combinations using normal serum and antisera to CYMV, FMV, PaMV, and PVX and crude extracts as the source of each virus. Numerous virus particles were observed with all the homologous combinations. But with the heterologous combinations, few particles were observed and the number seldom exceeded that of the normal serum control.

Insect transmission.—No evidence of FMV transmission was obtained for either *M. persicae* or *N. impicticeps* by either the stylet-borne or persistent mechanism. In all experiments each test plant was back assayed to *C. amaranticolor* 3-4 wk after inoculation.

DISCUSSION

Many new hosts of FMV and their reactions are reported. Hosts of FMV include both monocotyledons and dicotyledons and its host range is extremely broad. However, FMV has not been isolated from crop plants and presently causes no known economic loss. Seed transmission occurred in two hosts, but neither host is widespread in Kansas. Therefore, FMV must persist from year to year in infected perennial hosts or be seed-transmitted in species we did not test. Neither the aphid nor leafhopper tested could vector FMV. Therefore, the natural vector or method of dissemination of this virus remains unknown.

Foxtail mosaic virus is extremely stable in both crude and purified preparations and reaches high concentrations in several hosts. The extreme longevity and dilution end point approach those reported for TMV, but the thermal inactivation temperature (70 C) is much lower than that of TMV (10).

There is a marked tendency for FMV to form end-to-end aggregates and this increases with the degree of purity. Aggregation can be reduced somewhat by including Igepon and sucrose in the purification buffers. Monomers or dimers generally were observed by electron microscopy. This observation and the recovery of a single 32S RNA species from both the 122S and 144S virus components suggest that the 144S component is a dimer of the 122S component. With TMV and other viruses,

aggregates of three, four, and often more particles are observed, but aggregates greater than dimers were not seen in preparations of FMV. Therefore, loss of considerable virus at each purification step may result from interaction of FMV with host constituents, which are later removed, rather than formation of large, readily sedimented, aggregates of FMV.

Our results indicate that FMV is distinct from other known sap-transmissible grass viruses, TMV, and the potex viruses. No serological relationship was demonstrated between FMV and these viruses nor was there cross protection between FMV and BSMV or BMV. Other data such as host range, physical properties, particle length, and sedimentation characteristics support the designation of foxtail mosaic virus as a distinct virus.

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