Characterization of Iris fulva Mosaic Virus

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ABSTRACT

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An *Iris fulva* × *I. brevicaulis* hybrid had faint mosaic symptoms. A virus was transmitted from this hybrid to *Belamcanda chinensis* on which a chronic mosaic developed. The virus, designated Iris fulva mosaic virus, also was transmitted to *Amaranthus caudatus* and *Chenopodium quinoa*. In *B. chinensis* sap infectivity was lost after aging 4 days, heating at 60 C for 10 min, or diluting to 10^{-5}. In leaf-dip preparations, virus particles had a normal length of 767 nm. The particles appeared swollen in sodium phosphotungstate stain, but not in ammonium molybdate. They were flexuous in $MgCl_2$ but straight in EDTA. Cytoplasmic inclusions were composed of scrolls, tubes, laminated aggregates, pinwheels, and bundles. In leaf-dip serology the virus was not related to other known iris viruses.

A mosaic of *Iris fulva* Ker. was reported by Travis (18) in 1957. The virus, which was isolated from the diseased *I. fulva,* infected *Belamcanda chinensis* DC. and was aphid-transmitted. We also found a mosaic disease in a hybrid of *Iris fulva* and *I. brevicaulis* Raf. (= *I. foliosa* Mackenzie & Bush). The virus isolated from this hybrid (2, 3) is characterized in this report and is tentatively named Iris fulva mosaic virus (IFMV).

MATERIALS AND METHODS

An Apogon (beardless) iris hybrid (*Iris fulva* and *I. brevicaulis*) with faint mosaic symptoms was obtained in 1971, several years after the cross was made. The virus was maintained in the original rhizomes. The virus is deposited with the American Type Culture Collection (PV-202). Routine mechanical inoculations and in vitro property tests were done as described by Barnett et al. (4).

Purified virus was obtained by three methods. The first was that used by Barnett et al. (4) to purify bearded iris mosaic virus (BIMV). The second was a modification of procedures from Jones and Tolin (11) and Knesek and Hampton (13) as follows: *B. chinensis* leaves (between 200 and 400 g of tissue) showing mosaic symptoms were harvested, chilled, and homogenized in 2.5 volumes of 0.1 M sodium citrate plus 0.5% 2-mercaptoethanol, pH 7.9. The homogenate was expressed through cheesecloth and centrifuged 10 min at 10,000 *g*. The supernatant liquid was emulsified with 0.5 volume of cold chloroform and centrifuged for 5 min at 4,000 *g*. The aqueous upper phase was held at 4 C for 3 hr and then centrifuged 15 min at 12,000 *g*. Polyethylene glycol (PEG-6000, Union Carbide, South Charleston, WV 25303) and sodium chloride were added to the supernatant solution (4% and 2% by weight, respectively, of the supernatant volume). The mixture was stirred for 1 hr at 4 C and then centrifuged 20 min at 10,000 *g*, after which the pellet was resuspended in 0.005 M sodium citrate buffer, pH 7, overnight. The resuspended pellet was centrifuged 20 min at 10,000 *g* and the supernatant liquid centrifuged 40 min at 269,000 *g*. The pellet was suspended in 0.005 M citrate, pH 7, and centrifuged 20 min at 10,000 *g*. The resulting 1 ml was placed on top of 15 ml of a solution of 30% sucrose, 4% PEG, and 0.12 M sodium chloride and centrifuged 2 hr at 80,900 *g* in an SW 27.1 rotor. The pellets were suspended in 0.005 M citrate buffer, pH 7. The third method was adapted from the procedures used by Huttinga (10). The adapted procedure is the same as that used for BIMV (3).

An antiserum to IFMV was made by injecting a rabbit intramuscularly with virus preparations made by method 1 or 2 mixed with an equal volume of Freund's complete adjuvant. Ten injections, with an average of 1.8 mg virus per injection, were given over a 10-mo period. Serum from each bleeding was diluted with an equal volume of glycerin, and NaN_3 (1%) was added prior to storing the sera separately at 4 C. Microprecipitin tests were made with virus purified by method 3; final readings were made after 4 hr at room temperature (22 C). Ouchterlony gel diffusion with sodium dodecyl sulfate (SDS) added was done according to Gooding and Bing (7). Leaf-dip serology was done by the method of Ball and Brakke (1) as modified by Langenberg (14). Antiserum was diluted 1:250 with 0.001 M ammonium acetate. Antisera against mild iris mosaic virus (MIMV) and severe iris mosaic virus (SIMV) were supplied by A. A. Brunt. Chlorotic local lesions in *Tetragonia expansa* Murr. and *Chenopodium quinoa* Willd. were used as sources of

MIMV and SIMV for leaf-dip serology (5).

Virus particles were mounted on carbon-coated collodion membranes and stained with 2% sodium phosphotungstate (PTA), pH 7.0, 1% uranyl acetate, pH 3, or 2% ammonium molybdate, pH 7. Purified virus was placed on the grid and excess liquid was removed with filter paper. Purified virus was treated with $MgCl_2$ or EDTA by dialysis prior to putting the virus on the grid. Generally the stains were left on the grid for no longer than 15 sec, but when more intense staining effects were desired, exposure times were extended to 45-60 sec. Leaf-dips were made by cutting the edges of small tissue pieces and touching the cut edge to a drop of stain on the grid. This procedure was repeated 10 to 20 times prior to removal of the excess stain with filter paper. Usually the stain was removed as soon as the dips were completed, but to accentuate the stain effects, stain was left on the grid 30 min in a moist chamber (stain effects for leaf-dips required longer times than for purified particles). To treat with $MgCl_2$ or EDTA, the leaf-dips were made directly into the solutions instead of into stain. The solutions remained on the grid 30 min to 1 hr before the excess was removed and the stain was applied for 15 sec. Normal lengths were calculated after measuring the particles from prints with a calibrated wheel.

Tissue was fixed in 5% glutaraldehyde and postfixed in 1% OsO_4 in 0.1 M cacodylate buffer, pH 7.2, then stained with 1% uranyl acetate overnight at 4 C, dehydrated in ethanol, embedded in Spurr's (15) low-viscosity medium, sectioned on a Porter-Blum MT2-B microtome with glass knives, stained with lead citrate, and observed with a Philips 300 electron microscope at 60 KV.

RESULTS

Occurrence and symptoms in iris.—In the original *I. fulva* × *I. brevicaulis* hybrid only faint mosaic symptoms occurred. The virus was transmitted by mechanical inoculation from the iris hybrid to *Belamcanda chinensis*. Seedlings produced from the iris hybrid seed were mechanically inoculated with IFMV from *B. chinensis*. A faint mosaic consisting of fairly wide yellowish streaks, or, on some leaves, faint general yellowing, developed on one of four seedlings inoculated.

To determine the prevalence of IFMV in other Apogon (beardless) irises, two plants of *B. chinensis* were inoculated from each of 15 iris species or cultivars (usually one plant per species or cultivar), most of which had virus symptoms. These 15 plants were collected from various regions of the USA and included the following:

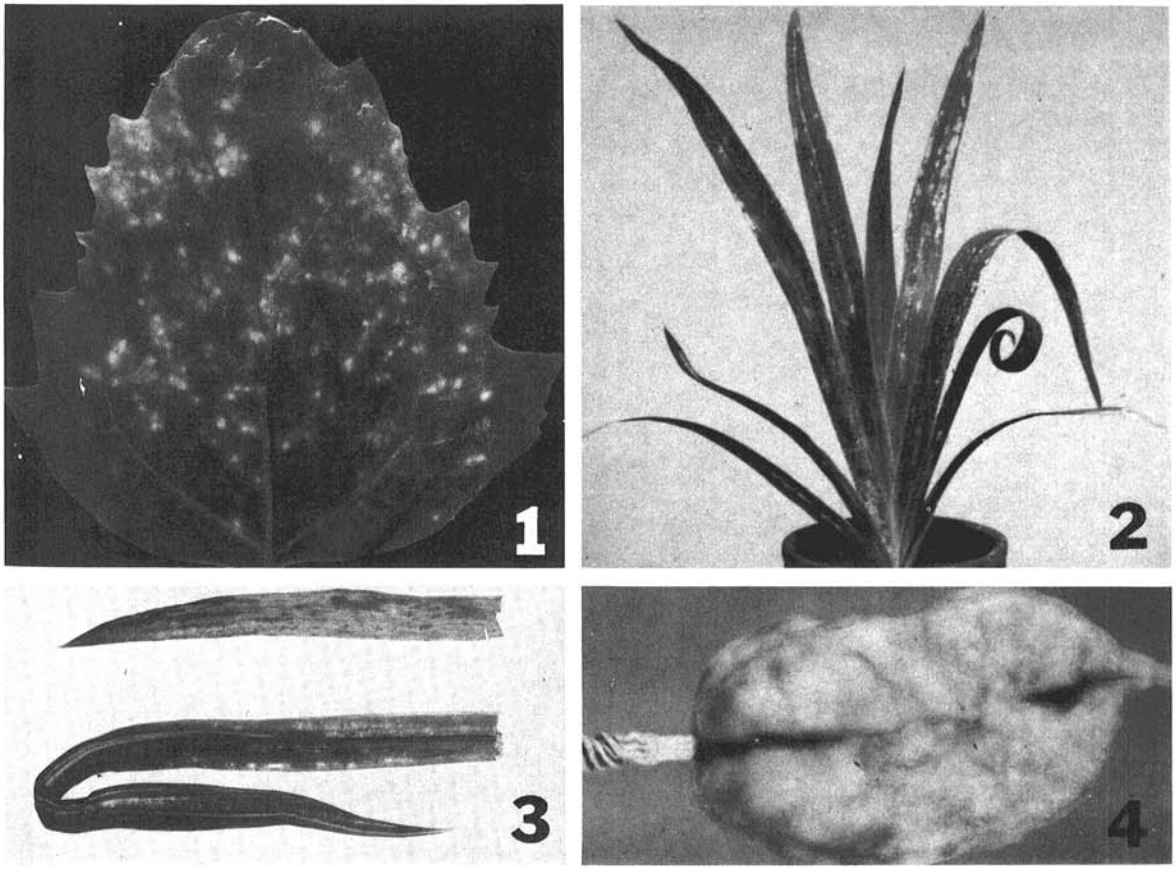


Fig. 1-4. Symptoms of Iris fulva mosaic virus. **1)** Chlorotic local lesions on *Chenopodium quinoa*. **2)** Mosaic and leaf curling on *Belamcanda chinensis*. **3)** Enlargement of leaves of *Belamcanda chinensis;* upper: mosaic; lower: mosaic, necrotic streak, and curling. **4)** *Belamcanda chinensis* seed pod deformity.

Spuria group–cultivars Archie Owen, Morningtide, Counter Point, Coal Dust, and Lord Wolseley; Siberian group–cultivar Ugly Duckling; California group–*I. douglasiana* Herb.; Louisiana group–*I. giganticaerulea* Small 'Petunia Butterfly'; Laevigata group–*I. kaempferi* Sieb., *I. virginica* L., *I. pseudacorus* L.; Miscellaneous–*I. ensata* Thunb., *I. setosa* Pall., *I. foetidissima* L., *I. unguicularis* Poir. Iris fulva mosaic virus was not detected from any of these species or cultivars. Bearded iris mosaic virus was found in *I. setosa*, Morningtide, and Lord Wolseley. Tobacco ringspot virus was found in *I. kaempferi*. Chlorotic local lesions occurred on *Chenopodium quinoa* when inoculated with extracts from Archie Owen, *I. virginica*, *I. pseudacorus*, *I. ensata*, *I. foetidissima*, and *I. unguicularis*. The virus that caused these symptoms was not identified, but it was not IFMV since *B. chinensis* was not infected.

TABLE 1. Comparison of Iris fulva mosaic virus absorbance ratios with different purification procedures

Purification method[a]	Absorbance ratio[b] (corrected for scattering) 260/280	260/min	Uncorrected[b] minimum
1	1.60 ± 0.10	1.44 ± 0.11	243 ± 1.0
2	1.43 ± 0.03	1.62 ± 0.20	242 ± 0.4
3	1.19 ± 0.03	1.17 ± 0.02	247 ± 0.4

[a]The solvents were 0.05 M sodium citrate-citric acid buffer, pH 7, for method 1; 0.005 M sodium citrate-citric acid buffer, pH 7, for method 2; and distilled water or 0.1 M Tris, pH 9, for method 3.

[b]Means followed by the standard error of the mean calculated from 4, 3, and 4 preparations for procedures 1, 2, and 3, respectively.

Host range.—On *B. chinensis*, IFMV caused a few local symptoms but a chronic mosaic developed systemically. Often some of the younger leaves were curled downward or twisted as a result of necrotic streaks (Fig. 2, 3). Seed pods on IFMV-infected *B. chinensis* were deformed (Fig. 4). Seedlings from these seed showed no symptoms of virus infection, but symptoms developed on all of the 139 seedlings after inoculation with IFMV from *B. chinensis*, which indicated no seed transmission of IFMV.

Several virus host range indicators and species of Iridaceae were mechanically inoculated with IFMV from *B. chinensis*. At least three plants of each species were inoculated and after several weeks each plant was assayed

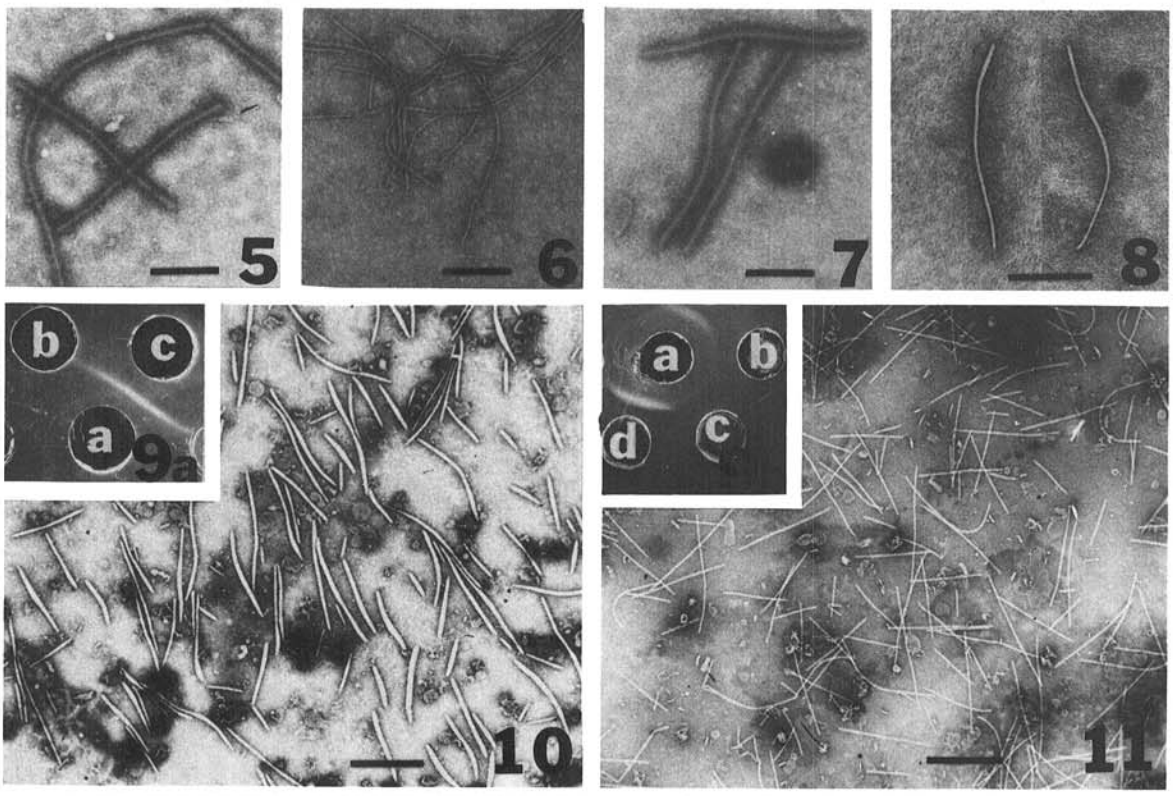
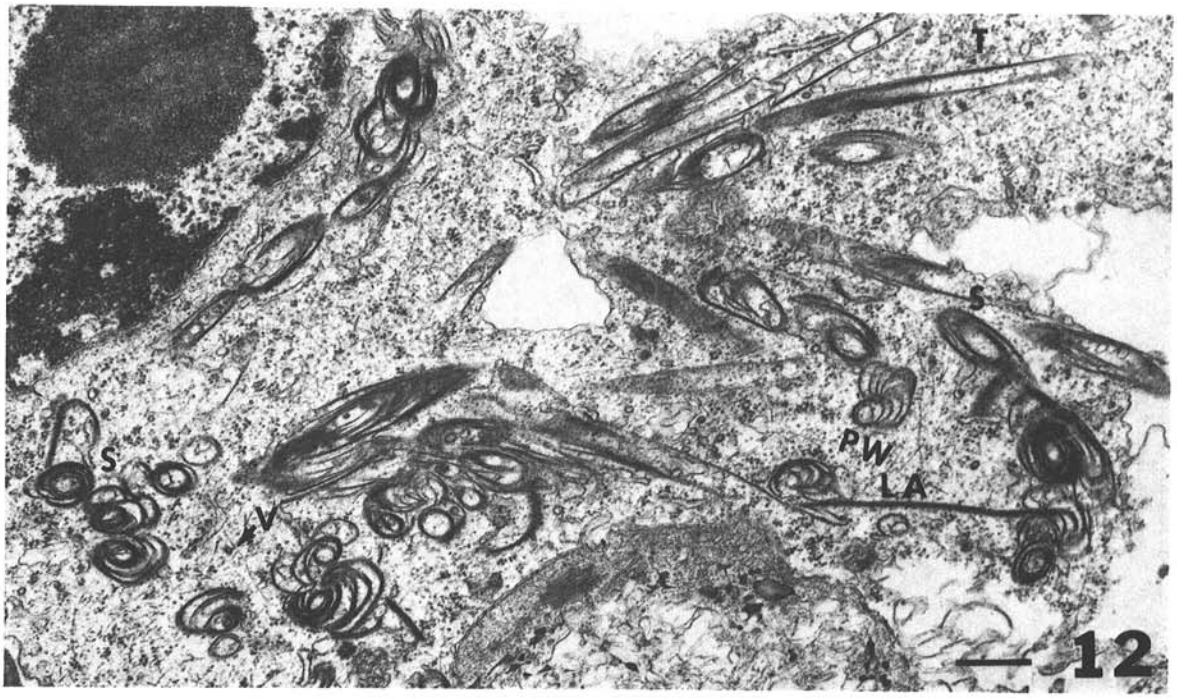


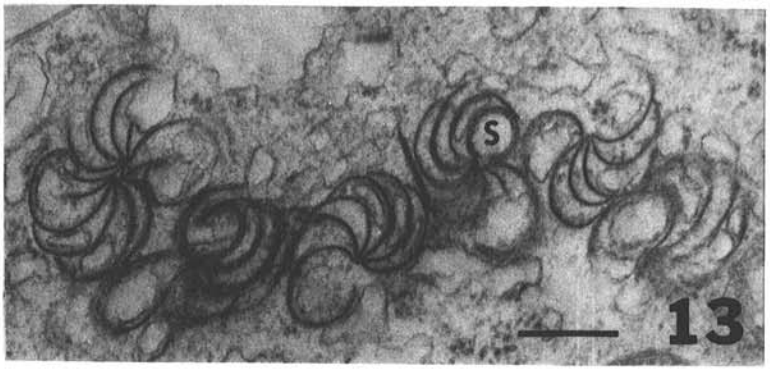
Fig. 5-11. *Leaf-dip serology*. **5)** Positive reaction of Iris fulva mosaic virus (IFMV) antiserum with IFMV. **6)** Negative reaction of IFMV antiserum with bearded iris mosaic virus (BIMV). **7)** Positive reaction of BIMV antiserum with BIMV. **8)** Negative reaction of BIMV antiserum with IFMV. (bars = 250 nm). *Gel diffusion serology*. **9)** Ouchterlony gel diffusion serology. a) IFMV antiserum. Antigens treated with 5% pyrrolidine: b) BIMV, c) IFMV. **9b** Ouchterlony gel diffusion with sodium dodecyl sulfate added. a) IFMV antiserum. Antigens in plant sap: b) BIMV, c) healthy *Belamcanda chinensis*, d) IFMV (note that the reaction with IFMV spurs the reaction with plant protein from wells b and c). *Effect of negative stains on purified particles of IFMV*. **10)** Stained with sodium phosphotungstate. **11)** Stained with ammonium molybdate. (bars = 500 nm).

to *B. chinensis*. Of the indicator hosts that were inoculated only *Amaranthus caudatus* L. and *Chenopodium quinoa* were infected. Both plants exhibited chlorotic local lesions only in certain seasons (Fig. 1). No virus infectivity could be demonstrated by inoculation with extracts from either *A. caudatus* or *C. quinoa* to *B. chinensis*, but a positive reaction with IFMV antiserum was obtained by leaf-dip serology. The following plants showed no virus symptoms and no symptoms developed when assayed to *B. chinensis*: *C. amaranticolor* Coste & Reyn., *Gomphrena globosa* L., *Nicotiana clevelandii* Gray, *N. tabacum* L. 'X-73', *Pisum sativum* L. 'Alaska', *Phaseolus vulgaris* L. 'Bountiful', *Tetragonia expansa* Murr., and *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'California Blackeye'.

Seedlings of several iridaceous species also were inoculated. Besides *B. chinensis* and the seedlings from the *I. fulva* × *I. brevicaulis* hybrid, only seedlings of *I. sibirica* L. became infected. On *I. sibirica* initial symptoms consisted of yellow streaks soon followed by necrosis. Two of the three plants inoculated died soon after the necrotic symptoms developed. The assay of *I. sibirica* to *B. chinensis* resulted in typical IFMV symptoms. Other iridaceous species which did not become infected were: *Gladiolus* sp. hybrids, *I. spuria*, and *Neomaracus gracilus* Sprague.

Properties of IFMV in vitro.—*Belamcanda chinensis* tissue inoculated 14-20 days earlier was used in these tests with each property test repeated three times. *B. chinensis* was used as the assay plant. Crude extracts of IFMV were infective at 2 days, but not at 4 days, when stored at greenhouse temperature (about 27 C). Virus was infective





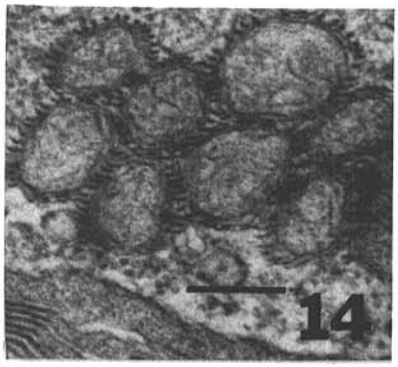


Fig. 12-14. Thin sections of *Belamcanda chinensis* infected with Iris fulva mosaic virus. **12)** Inclusion bodies, predominantly scrolls (S) and tubes (T) with a few laminated aggregates (LA) and pinwheels (PW). Virus particles (V) are scattered in the cytoplasm. (bar = 500 nm) **13)** Another area where pinwheels predominate. Some of the pinwheels have scrolls attached. (bar = 250 nm) **14)** Aggregate of mitochondria with associated rods. (bar = 250 nm)

after being heated for 10 min at 50, but not 60 C, and after diluting to 10^{-4}, but not after dilution to 10^{-5}.

Purification.—The first purification method did not give preparations of consistent purity. The $A_{260/280}$ and $A_{260/min}$ ratios varied considerably among IFMV preparations (Table 1). Virus particles also were aggregated. The second procedure utilized PEG precipitation instead of acid precipitation and, for further purification, precipitation through sucrose instead of exclusion chromatography. The $A_{260/280}$ and $A_{260/min}$ ratios (Table 1) were more consistent, but much host plant material (based on UV-absorbing material near the top of density-gradient tubes) was still in the preparations and the particles were aggregated. The third method adapted from Huttinga (10) was simple and fast and gave an average $A_{260/280}$ of 1.19 and $A_{260/247}$ of 1.17 (Table 1). These ratios were consistent and agreed with those of other long flexuous rods (16). The virus preparation was not aggregated so that density gradients could be used for the final purification step.

Virus purified by the third procedure was analyzed in an AN-D rotor in a Beckman Model E analytical ultracentrifuge. The density and viscosity of the 0.1 M Tris-thioglycolic acid buffer, pH 9, were 1.00153 g/ml and 1.0388×10^{-3} N·s/m^2, respectively. In this buffer the $S^0_{w,20}$ of IFMV was 141. The virus sedimented as a single peak which tended to be symmetrical, though broad, near the end of the run. The extent of peak-broadening indicated a highly heterogeneous preparation. Particles of this preparation were stained with phosphotungstic acid and photographed. Many particles were broken and no definite peak for length was observed. The normal length of 76 particles measured was 529 nm.

Serology.—The serum prepared against IFMV isolated from *B. chinensis* reacted with healthy protein preparations (serum titer to plant protein 1/128). Leaf-dip serology was used to be sure that any serological reaction was with the virus and not with contaminating host components (1, 14).

In leaf-dip serology, IFMV reacted with homologous serum as shown by the accumulation of antibodies along the virus particle with the resulting buildup of negative stain (Fig. 5). No reaction occurred between IFMV and antisera against BIMV (Fig. 6), MIMV, or SIMV. When BIMV was used in leaf-dip serology, a positive reaction occurred with antiserum against BIMV (Fig. 7), but not with IFMV (Fig. 8), MIMV, or SIMV antisera. Also, when SIMV was used, it reacted only with SIMV antiserum and not with antisera against IFMV, BIMV, or MIMV. Too few local lesions developed on *T. expansa* to compare the antisera with MIMV.

The serological reactions found by leaf-dip serology were confirmed by microprecipitin, latex precipitin, and agar gel diffusion (see Fig. 9-a, 9-b) tests.

Electron microscopy.—In thin-sections of IFMV-infected *B. chinensis,* inclusions consisted of both the tubular and laminated aggregate types (Fig. 12). Most areas of the tissue resembled Fig. 12, with the scrolls and tubular inclusions predominating. Other areas were seen in which pinwheel inclusions predominated, but even there some of the pinwheel arms terminated in scrolls (Fig. 13).

Virus particles were abundant in the cytoplasm (Fig. 12). No aggregates of particles were seen, but occasionally mitochondria were aggregated with rods surrounding the mitochondria (Fig. 14). The rods around mitochondria were identified as virus particles in the case of henbane mosaic virus (12). Not all mitochondrial aggregates in IFMV-infected tissue had associated rods.

Leaf-dip preparations with PTA from infected *B.*

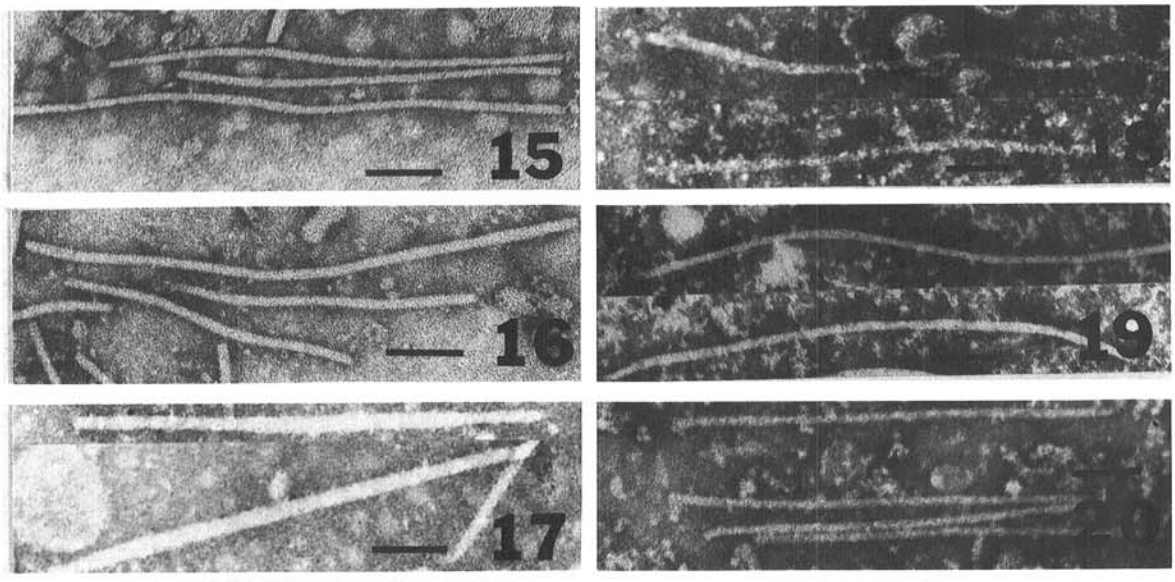


Fig. 15-20. Effect of magnesium chloride and ethylenediaminetetraacetate on Iris fulva mosaic virus particles. Stained with sodium phosphotungstate. **15)** Purified particles in 0.1 M Tris-thioglycolate buffer, pH 8.9. **16)** Purified particles treated with 0.05 M magnesium chloride. **17)** Purified particles treated with 0.05 M EDTA. **18)** Leaf-dip with no additives. **19)** Leaf-dip into $MgCl_2$. **20)** Leaf-dip into EDTA. (bars = 100 nm)

chinensis leaves contained long flexuous particles (Fig. 18); of 45 particles measured, lengths ranged from 673 to 816 nm with the peak near 775 nm (Fig. 21). If only the particles 50 nm either side of the peak were considered, the 37 particles had a normal length of 767 nm. Thus, Iris fulva mosaic virus is morphologically similar to three other viruses from irises, BIMV, MIMV, and SIMV.

Particle measurements of several purified preparations negatively stained with PTA (Fig. 15) gave mean lengths between 504 and 547 nm. The particle lengths ranged widely, from 250 up to 775 nm, and were distributed across the whole range with no peak. The effect of 0.05 M $MgCl_2$ or 0.05 M EDTA on purified virus was examined next. In either Tris or borate buffer the particle mean lengths were still in the 520 to 560 nm range after treatment with either $MgCl_2$ or EDTA. The particles, however, were flexuous in $MgCl_2$ (Fig. 16) and most of the particles were stiff in EDTA (Fig. 17). The effect of $MgCl_2$ or EDTA on the rigidity of IFMV is opposite that reported for other viruses (8), so we tried leaf-dip preparations in $MgCl_2$ or EDTA to find the effect of the chemicals on particle length. Leaf-dips into a drop of either $MgCl_2$ or EDTA stained with PTA gave particles with a mean length of 769 nm in $MgCl_2$ with a definite peak near the mean and 568 nm in EDTA with a much wider spread of particle lengths and no peak (Fig. 21). The particles in the dip preparations still were flexuous in $MgCl_2$ and stiff in EDTA (Fig. 19, 20).

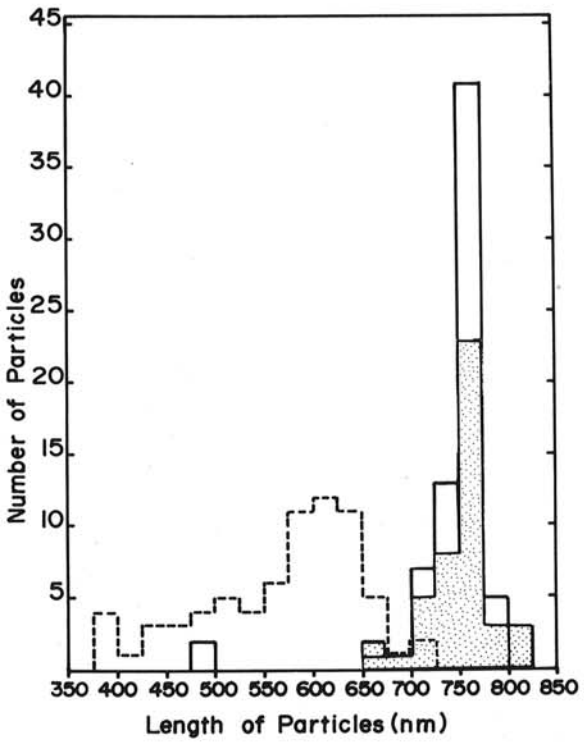


Fig. 21. Histogram of virus particles from leaf-dip preparations stained with sodium phosphotungstate (see Fig. 18-20). The solid line represents the length distribution of virus particles with no additives. The broken line represents the length distribution of particles treated with EDTA prior to staining. The shaded area represents the length distribution of particles treated with $MgCl_2$ prior to staining.

Occasionally during measurement of particle lengths, several images of apparently swollen particles were observed. Sometimes many particles on a grid looked swollen; the stain was suspected of being the cause. Purified IFMV in Tris buffer was stained for 45 sec with PTA, uranyl acetate, and ammonium molybdate. Most of the particles looked swollen in PTA (Fig. 10) and many particles looked swollen in uranyl acetate, but no apparent swelling was seen in particles stained with ammonium molybdate (Fig. 11). This was repeated by making leaf-dips directly into PTA or ammonium molybdate and allowing the virus to stand in the stain 30 min. Again the particles looked swollen in PTA and not in ammonium molybdate.

DISCUSSION

The virus disease of *I. fulva* reported by Travis (18) was caused by a virus which was found only in *I. fulva*. It was mechanically transmissible and aphid transmissible by *Myzus persicae* (Sulz.) and *Macrosiphum euphorbiae* (Thos.), but not by *Aphis fabae* (Scopoli) and it infected *B. chinensis*. The virus reported here was found only in the *I. fulva* × *I. brevicaulis* hybrid and was mechanically transmitted to *B. chinensis*. As no exhaustive survey of the Apogon irises was attempted, IFMV later may be found in other plants of the species mentioned here or in other species of the subsection. The IFMV was not transmitted in repeated trials with *M. persicae, M. euphorbiae,* and *A. craccivora* (Koch). The lack of aphid transmission was probably due to the characteristics of this particular isolate of IFMV. Because of the infection of *I. fulva* and *B. chinensis,* the designation of IFMV was chosen because it agrees closely with the disease name reported by Travis.

This virus, IFMV, seems to be different from other potyviruses reported on iris. The IFMV-inclusion morphology places this virus in Edwardson's (6) subdivision III of the potyvirus group. Inclusions of BIMV are different from those of IFMV and place BIMV in subdivision II. The inclusions of SIMV were indistinct and not grouped (6) and there are no reports of the morphology of MIMV inclusions. [The virus called SIMV in this paper was that defined by Brunt (5)]. The host ranges of these viruses also differ. The IFMV and BIMV readily infect *B. chinensis*, but the symptoms are usually different. The MIMV and SIMV do not infect *B. chinensis*. Both IFMV and SIMV infect *C. quinoa* locally, but SIMV infects *C. quinoa* with ease throughout the year whereas IFMV will infect *C. quinoa* only at certain times of the year. The MIMV does not infect *B. chinensis* or *C. quinoa*, but it does infect *T. expansa* (5). Further work needs to be done to determine if IFMV and BIMV will infect bulbous irises. Serologically IFMV was unrelated to BIMV. This was shown by the leaf-dip serology and confirmed by microprecipitin, latex, and Ouchterlony serological tests. There also were no indications that IFMV or BIMV were related serologically to SIMV or MIMV. All of the heterologous reactions between IFMV, BIMV, and SIMV in leaf-dip serology were negative. The evidence would be stronger if MIMV had been available for comparative trials, but

under our conditions local lesions of MIMV on *T. expansa* developed poorly and bulbous irises which were infected with MIMV also were infected with SIMV.

Particles of IFMV apparently were broken by PTA stain in the absence of divalent cations, because, in leaf-dip preparations first treated with EDTA then negatively stained with PTA, particle lengths were distributed over a broad range. This did not occur either in leaf-dip preparations without EDTA or in leaf-dip preparations with $MgCl_2$. It is interesting that pea seedborne mosaic virus has anomalous particle lengths unless it is fixed with glutaraldehyde (9) and that this virus also occurs in subdivision III based on inclusion morphology (6).

Possibly, the PTA stain also was responsible for the apparent swelling of IFMV particles. Tosic et al. (17) found that maize dwarf- and sugarcane mosaic viruses swell in PTA stain only if purified in chloroform concentrations of 25% or greater but not in 3-5% chloroform. With IFMV, virus particles from purifications utilizing 17% chloroform appear to be swollen when negatively stained with PTA, but when stained with ammonium molybdate they apparently are normal. The IFMV particles also swell when leaf-dip preparations in PTA were made which did not involve any contact with chloroform.

The S value obtained on purified virus is not as high as those of several other viruses of the potyvirus group (16), but it does agree with those reported by Huttinga (10). These differences may result from different degrees of fragmentation or differences in the rigidity of the virus particles. Because the viruses in this group fragment easily, particle measurements should be made on the same preparations that are used in S value determinations.

Note added in proof: Mild iris mosaic virus (5) as discussed in this paper is apparently synonymous with narcissus latent virus, a member of the carlavirus group (Brunt, A. A. 1976. Narcissus latent virus. No. 170 *in* Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. 4 p).

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