Figwort Mosaic Virus: Properties of the Virus and Its Adaption to a New Host

R. J. Shepherd, R. D. Richins, J. E. Duffus, and Mary K. Handley

First and second authors: Department of Plant Pathology, University of Kentucky, Lexington 40546; third author: U.S. Department of Agriculture, ARS Research Station, 1636 E. Alisal St., Salinas, CA 93905; fourth author: 609 South Eleventh St., Salina, KS 67401.
This research was supported by USDA CRGO grant CRCR-1-1743 and USDA CRGO grant CRCR-1-1505.
Accepted for publication 11 March 1987.

ABSTRACT

Shepherd, R. J., Richins, R. D., Duffus, J. E., and Handley, M. K. 1987. Figwort mosaic virus: Properties of the virus and its adaption to a new host. Phytopathology 77:1668-1673.

Properties and characterization of figwort mosaic (FMV), a caulimovirus, are described. The virus infects plants of the Scrophulariaceae, Chenopodiaceae, and Solanaceae, usually inducing chlorotic mottling symptoms. It is transmissible both mechanically and by aphids. Infected leaves contain amorphous inclusion bodies with an electron dense matrix in which isometric particles of about 50 nm are embedded. FMV is very distantly related serologically to cauliflower mosaic virus (CaMV). It has a slightly smaller sized DNA genome (about 7,750 base pairs vs. 8,031 for CaMV DNA), which exhibits the same conformational forms during gel electrophoresis as CaMV DNA. FMV DNA contains four single-stranded discontinuities as opposed to the three discontinuities in CaMV DNA. The discontinuities were mapped in relation to various restriction endonuclease cleavage sites. The physical map of the genome is distinctly different from that for any other caulimovirus. Virus maintained in *Datura innoxia* for a 2-yr period produced different symptoms on *D. stramonium* and reached a concentration in the latter nearly 10-fold higher than the original isolate from figwort. Nucleotide changes occurred in the gene VI portion of the genome of FMV. The gene VI sequence of FMV apparently mutates rapidly during adaptation of the virus to a new host.

The DNA viruses of plants make up two well-established groups, the geminiviruses with single-stranded DNA and the caulimoviruses with double-stranded genomes. About a dozen members of the latter group are recognized (12).

The type member of the caulimovirus group is cauliflower mosaic virus (CaMV). It is a well-characterized virus that has provided a useful system for studying the biology of DNA plant viruses. Its genome consists of relaxed circular DNA of about 8,000 base pairs, and several strains have been partially or entirely sequenced. Multiplication of the virus has received particular attention because of the novel mode of replication of its DNA by reverse transcription (reviewed by Hohn et al [9]).

In this report, we describe another member of the caulimovirus group, the figwort mosaic virus (FMV), and document some of the changes that occur in this virus during its adaptation to a new host species. FMV has many of the same advantages as CaMV for genetic manipulation by molecular cloning procedures. This makes it useful for studying eukaryotic gene regulation and viral pathogenesis in plants. Its relatively small double-stranded DNA genome can be easily isolated from plants and analyzed by restriction enzyme cleavage. The virus has one further advantage afforded by its host range. It infects a variety of solanaceous hosts, thus permitting its study in several currently defined plant protoplast and tissue culture systems.

MATERIALS AND METHODS

Host range. Two isolates of the virus (mild and severe) were collected in 1968 from figwort plants (*Scrophularia californica* Cham. & Schlecht.) growing along the Pajaro River near Watsonville, CA. The virus was maintained in naturally infected figwort propagated periodically by stem cuttings. Test plants were grown in a peat-sand-vermiculite mixture in 12.5-cm plastic pots in a greenhouse at 22–24 C.

Plants were fertilized with Osmocote 14-14-14 (Sierra Chemical Co., Milipitas, CA) by applying the slow-release pellets to the surface of each potted plant. Test plants were generally inoculated at the three- to four-leaf stage after dusting with 600-mesh Carborundum. Plants were usually maintained for about a month after inoculation to allow symptom development.

Datura innoxia Mill. infected with FMV during adaption of the virus to solanaceous plants was propagated by stem cuttings after dipping the lower tips into a commercial powder containing naphthalene acetic acid.

Aphid transmission. Transmission was tested with green peach aphids (*Myzus persicae* Sulz.) starved about 3 hr. Five to 10 aphids per plant were given a 5-min acquisition period on FMV-infected source plants followed by transfer with a camel's hair brush to healthy test seedlings. Aphids were left on plants for several hours and then killed with nicotine.

Microscopy. FMV-infected tissue was examined microscopically by methods similar to those used to examine CaMV-infected material (28).

Purification. FMV was isolated from chilled infected leaves in solutions maintained as cool (0–4 C) as possible. Starting material for virus purification consisted of 200 g of systemically infected *Nicotiana edwardsonii* Christie homogenized in a blender in 600 ml of grinding buffer (0.2 M Tris-Cl; 0.05 M ethylenediaminetetraacetic acid (EDTA); 1.5 M urea; 0.05 M sodium benzoate; and 2% (v/v) 2-mercaptoethanol, pH 7.6). After homogenization, 15 ml of Triton X-100 was added with stirring. About 30 min later, the extract was centrifuged for 20 min at 16,000 *g*. The supernatant was poured through four layers of Miracloth and the low-speed centrifugation and filtration steps were repeated. The extract was then ultracentrifuged in a Beckman type 42.1 or Ti60 rotor for 60 min at 38,000 rpm. The pellets were gently rinsed with cold distilled water and resuspended in 8 ml of TE (10 mM Tris, 1 mM EDTA, pH 7.6) with gentle stirring for 3–6 hr. After resuspension, the total volume was adjusted to 10.0 ml and 5.36 g of solid CsCl was added. The preparation was centrifuged in a Sorvall HB4 rotor for 20 min at 10,000 rpm. The supernatant was then carefully decanted through a single layer of Miracloth. The filtered supernatant was equally divided into 2–5 ml of Quick Seal Ultraclear centrifuge tubes (Beckman, Palo Alto, CA), and centrifuged at 40,000 rpm for 18–24 hr in a Beckman VTi65 rotor. The distinct light scattering band was carefully removed from the middle of the gradient with a needle and syringe. This solution was diluted to 25 ml with cold water and centrifuged in an angle rotor (type 42.1 or Ti60) as described previously. The virus pellet was resuspended overnight with very gentle stirring in 4.5 ml of cold water, then clarified by centrifugation (10,000 *g* for 10 min). The virus was then centrifuged through a 10–40% linear sucrose density gradient in a Beckman SW28 rotor for 2.5 hr at 24,000 rpm. A light

© 1987 The American Phytopathological Society

scattering band, located about halfway through the gradient was removed, diluted with cold water, and pelleted as before. The purified virus was resuspended in 1 ml of cold water overnight (without stirring). For long-term storage, the virus was kept in 50% (v/v) glycerol at −20 C.

Serology. Purified virus was injected into New Zealand White rabbits for preparation of antiserum. Injections were made subcutaneously at weekly intervals using about 0.3 mg of virus emulsified in Freund's complete adjuvant. After a prolonged series of injections, the titer of the antiserum reached 1/512 as determined by immunoprecipitin tests.

A modification of the enzyme-linked immunosorbent assay (ELISA) of Clark and Adams (2) was used to compare the serological relationship of FMV and CaMV (21). All determinations were carried out in triplicate on Immunlon 1 polystyrene plates (Dynatech, Alexandria, VA). Absorbance values (450 nm) were determined using a Titretek Multiscan spectrophotometer (Flow Laboratories, McLean, VA).

Nucleic acid. Viral DNA was isolated from infected leaves of either *D. stramonium* L. or *N. edwardsonii* by a modification of the single-leaf miniscreen procedure of Gardner and Shepherd (6). Infected leaves were processed in a similar manner as was used for purification of the virus through the first resuspension of high speed pellets in 8 ml of TE. After resuspension, the total volume was adjusted to 9.0 ml (with TE). One milliliter of 10× DNase I buffer (0.1 M Tris-Cl, pH 7.5; 0.5 M NaCl; 0.1 M $MgCl_2$) was added to the resuspended virus along with 0.1 ml of 1 mg/ml DNase I (Sigma, St. Louis, MO) and 0.02 ml of 10 mg/ml RNase A (Sigma). The preparation was incubated for 1 hr at 37 C after which another 0.1 ml aliquot of DNase I was added. After 1 hr of incubation at 37 C, Na EDTA and sodium dodecyl sulfate were added to give concentrations of 50 mM and 1% (w/v), respectively. Proteinase K (0.1 ml of a 10 mg/ml solution) was added to the solution and incubated for 1 hr at 50 C. Three phenol extractions, followed by a chloroform extraction and precipitation by ethanol (at 0 C) yielded DNA of sufficient purity to be cut by most commercially available restriction endonucleases. When further purity was required (e.g., for end labeling experiments), the DNA was sedimented through ethidium bromide-cesium chloride gradients, and/or into 5–20% sucrose density gradients.

Nucleic acid analysis and cloning used standard molecular biology protocols; see Maniatis et al (15). Viral DNA propagated in bacteria was isolated by the boiling procedure of Holmes and Quigley (11). The DNAs from the mild, severe, and adapted isolates of FMV were digested with a variety of restriction endonucleases (New England Biolabs, Beverly, MA). DNA of both the mild and severe strains contained a unique *Sal*I site, which was used for cloning the DNA into pBR322. The adapted (designated DxS) strain lacked this unique *Sal*I site but did possess a unique *Sac*I site used for its cloning into pUC13.

The infectivity of these clones (pFMV-M3 = severe isolate in pBR322; pFMV-M7 = mild isolate in pBR322; and pFMV-Sc3 = DxS isolate in pUC13) was demonstrated by excising each of the viral DNAs from their respective plasmid vectors followed by inoculation to seedlings of *D. stramonium* (pFMV-M3 and pFMV-M7) and/or *N. edwardsonii* or *N. bigelovii* S. Wats. (pFMV-Sc3). Plants to be inoculated with DNA were dusted with acid washed diatomaceous earth. Aliquots of 2–4 μl of DNA at concentrations of between 0.2 and 1.0 mg/ml were placed on a sterilized ground glass spatula followed by gentle abrasion of the leaf surface.

A restriction map for each of the clone DNAs was determined by analyzing single or multiple restriction enzyme digests of each clone on either agarose or acrylamide gels. Restriction fragments of pCaMV10 that has been sequenced (7) were used as molecular weight standards.

The polarity of the DxS isolate of FMV was determined by labeling the 5′ ends of native viral DNA with [gamma ^{32}P] adenosine triphosphate using the T4 polynucleotide kinase exchange reaction described by Maniatis et al (19). Labeled DNA was digested with various restriction endonucleases, then heat denatured and electrophoresed through agarose gels (13). Autoradiography of the dried gels was used to detect the positions of the labeled DNA fragments. Intensifying screens (Dupont Cronex Lightning Plus) were used (at −76 C) to enhance development of the Kodak X-Omat AR-5 film in these experiments.

Nucleic acid hybridization between FMV DNA and CaMV DNA was determined in a previous study (20).

RESULTS

Host range. Symptoms of the severe strain of FMV on naturally infected figwort consisted of a prominent chlorotic mottle (Fig. 1A). Chlorotic spots were also noted along the veins of younger plants. Occasionally the leaf margins were chlorotic as well. The mild strain caused a faint vein clearing with some chlorotic vein banding (Fig. 1A). Symptoms caused by either strain of the virus were most pronounced on the younger leaves. Neither strain caused noticeable stunting of the host.

Mechanical inoculation of infected (severe strain) figwort leaf extracts to seedlings of *D. stramonium* caused severe viruslike disease symptoms to appear within about 2 wk. The symptoms consisted of conspicuous vein clearing and puckering followed by necrotic spotting and necrosis of the veins, particularly near the base of the leaves (Fig. 1C). Eventually, the upper leaves and growing points of many plants were killed. Some plants survived with chronic symptoms consisting of dwarfed, malformed leaves with necrotic spots and streaks. *D. innoxia* was less severely affected by the virus and showed a chlorotic mottle without necrosis (Fig. 1B).

Various species of the Solanaceae were found to be susceptible. *N. clevelandii* Gray and *N. edwardsonii* developed a systemic chlorotic mottle. *N. tabacum* L. cultivar Xanthi-nc developed chlorotic local lesions but no systemic infection. *N. megalosiphum* W. Wats. and *Physalis wrightii* Gray developed a systemic mottle. *Nicandra physaloides* (L.) Gaertn. developed chlorotic local lesions.

Several chenopodiaceous species were also found to be susceptible including *Beta macrocarpa* Guss., *B. vulgaris* L., *Chenopodium capitatum* (L.) Asch., *C. murale* L., *C. quinoa* Willd., and *Spinacia oleracea* L. The latter produced prominent chlorotic local lesions followed by a chlorotic mottle.

The following hosts, grouped by family, were not susceptible to FMV after mechanical and aphid inoculations: Aizoaceae, *Tetragonia expansa* Thunb.; Asclepiadaceae, *Asclepias tuberosa* L.; Chenopodiaceae, *Chenopodium urbicum* L., *Monolepsis nuttalliana* (R. and S.) S. Wats.; Compositae, *Calendula officinalis* L., *Dahlia variabilis* (Wild.) Desf., *Lactuca sativa* L., *Picris echioides* L., *Sonchus oleraceus* L., *Verbesina encelioides* (Cav.) Bentr. and Hook., *Zinnia elegans* Jacq.; Cruciferae, *Capsella bursa-pastoris* (L.) Medik., *Lepidium sativum* L., *Lunaria annua* L., *Raphanus sativus* L., *Thaspi arvense* L.; Geraniaceae, *Geranium dissectum* L.; Linaceae, *Linum grandiflorum* Desf.; Malvaceae, *Gossypium hirsutum* L., *Hibiscus esculentus* L.; Scrophulariaceae, *Antirrhinum majus* L., *Digitalis purpurea* L.; Solanaceae, *Datura fastuosa* L., *Hyoscyamus niger* L., *Lycopersicon esculentum* Mill., *Nicotiana glutinosa* L., *N. rustica* L., *Physalis floridana* Rydbb., *P. alkekengi* L., *Solanum melogena* L.

Insect transmission. FMV was routinely transmitted by the green peach aphid. Although critical virus persistence studies were not conducted, the virus was acquired rapidly from a number of systematically infected hosts including figwort, sugarbeet, spinach, *D. stramonium*, and *N. clevelandii*.

Cytopathological effects. Intracellular inclusions typical of the caulimoviruses were found in naturally infected figwort and in inoculated *N. edwardsonii* and *N. bigelovii*. These bodies were observed in stripped epidermis (stained with 1% phloxine B) when examined with a light microscope and in leaf sections examined by electron microscopy. The inclusion bodies were roughly spherical and usually 5–10 μm in diameter and were never found in healthy plants.

The inclusion bodies consisted of a deeply staining, granular

matrix surrounding voids or vacuoles that contained spherical virus particles about 45 nm in diameter (Fig. 1D and E). Virions were also found embedded in the amorphous matrix; however, virions were rarely found in other parts of the cell. Ribosomes appeared to be clustered around the periphery of the inclusion bodies, especially the smaller ones (Fig. 1E). These inclusion bodies and embedded virus appeared to occur only in the cytoplasm of infected cells. In figwort, the cytoplasm surrounding the inclusion bodies frequently contained an unusual number of small vesicles (Fig. 1E).

Transformed plasmodesmata, characteristic of caulimovirus infected plants were also found in FMV-infected *N. edwardsonii* and *N. bigelovii*. These enlarged channel openings joining adjacent cells were about 65 μm in diameter and frequently contained a single file of virus-sized spherical particles. Cell walls surrounding these channels frequently showed an outgrowth protruding into the cell.

Immunological relationships. In heterologous ELISA tests in which CaMV IgG was reacted with FMV and FMV IgG with CaMV, a very weak heterologous reaction was obtained (Fig. 2). Hence, the viruses appear to have little serological similarity to one another.

Properties of viral nucleic acid. The native DNA of FMV exhibited forms characteristic of the knotted, closed circular (form II) and linear (form III) molecules of CaMV DNA when subjected to agarose gel electrophoresis (Fig. 3, lane B). *Sal*I (mild/severe strain) or *Sac*I (adapted strain) digested FMV viral DNA migrated in agarose gels at a rate nearly identical to linearized CaMV viral DNA. The approximate molecular weight for the FMV genome was estimated to be 5.16×10^6 (7,750 base pairs) compared with

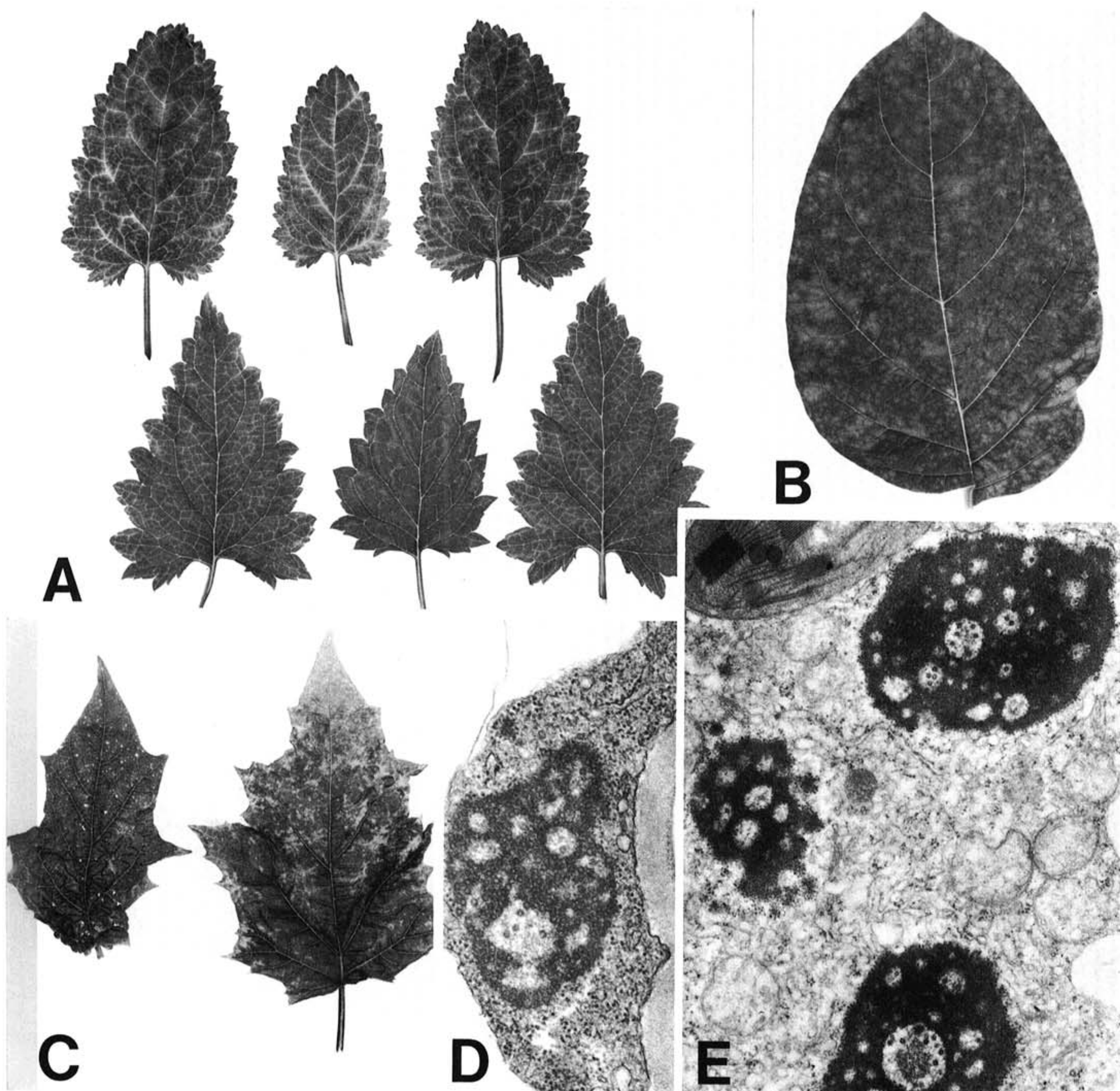

Fig. 1. Symptoms of figwort mosaic virus (FMV) infections on various hosts. **A,** Naturally infected figwort (*Scrophularia californica*). *Top*—severe strain; *bottom*—mild strain. **B,** Chlorotic mottle on leaf of *Datura innoxia*. **C,** Infected *D. stramonium*. Left—severe FMV infection with puckering and necrotic spots. Right—mild chlorotic mottle with adapted strain (DxS) taken from *D. innoxia* after 2 yr. **D,** Inclusion body in *Nicotiana edwardsonii* infected with severe strain adapted to *D. innoxia*. **E,** Inclusion bodies in naturally infected figwort.

5.32×10^6 (8,027–8,031 base pairs) for the CaMV genome (8,10).

Agarose gel electrophoresis of heat denatured viral FMV DNA gave four single-stranded components of approximately 7,750, 3,700, 2,050, and 2,000 base pairs (Fig. 3). By analogy with CaMV DNA, the 7,750 nucleotide component should represent the full-length α (minus) strand, while the other three components arise from denaturation of the β (plus) strand (25). These single-stranded discontinuities were mapped in relation to several restriction endonuclease cleavage sites to give the physical map shown in Figure 4. By convention, the map is presented with the unique α strand discontinuity placed at the zero (top) position with the orientation of the β strand reading clockwise 3′ to 5′ around the genome.

The physical map (Fig. 4) shows the cleavage sites for 10 restriction endonucleases. Enzymes that failed to cut the FMV genome include *Bst* EII, *Nar*I, *Pvu*I, and *Sph*I.

Adaption of the the virus to solanaceous hosts. During the course of this investigation, we observed that FMV seemed to adapt to some hosts to cause less severe disease symptoms during subsequent plant-to-plant transfers. Transmission of the original virus from figwort caused severe symptoms in *D. stramonium*. Transmission from figwort to *D. innoxia*, and then several weeks later to *D. stramonium*, produced greatly attenuated symptoms. The virus apparently changed during its culture in *D. innoxia*. Because host range determinants have been a subject of previous interest in the biology of the caulimoviruses (4,22), we tested the effects of prolonged culture in this new host.

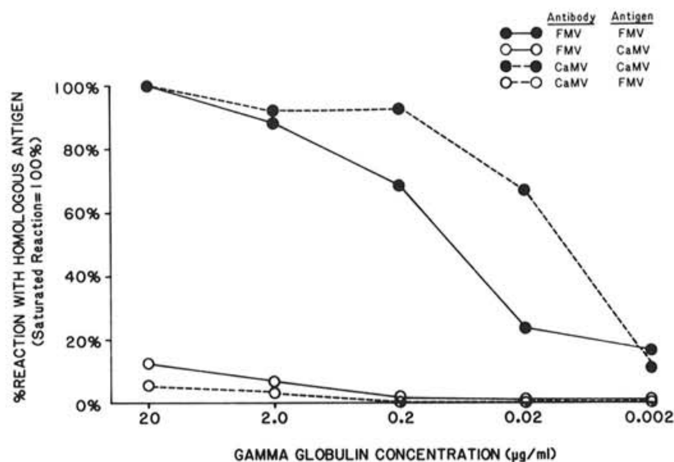

Fig. 2. Serological tests between figwort mosaic and cauliflower mosaic viruses with enzyme-linked immunosorbent tests. FMV-figwort mosaic virus and CaMV-cauliflower mosaic virus antigens and IgG used for coating and conjugate. All the reactions were corrected for the background reaction observed.

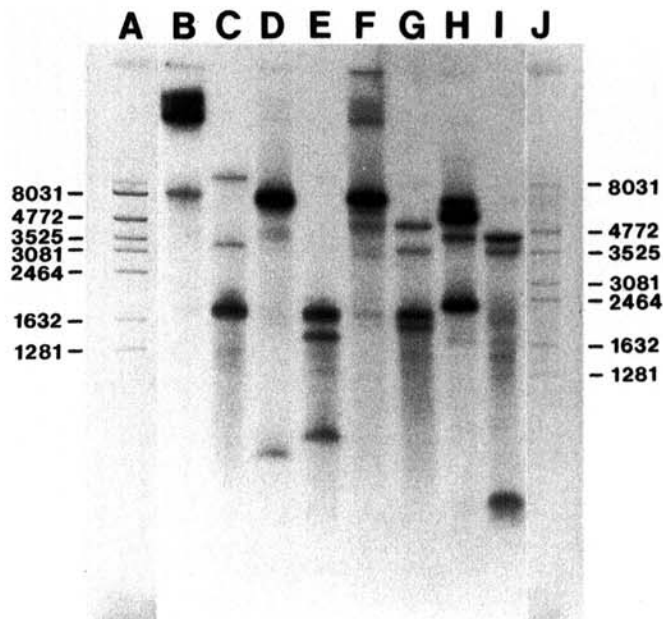

Fig. 3. Autoradiography establishing orientation of the figwort mosaic virus genome. DNA, isolated from virus as described in Materials and Methods, was purified further by centrifugation into a 5–20% sucrose density gradient. The 5′ ends at the discontinuities were labeled with ^{32}P using polynucleotide kinase. Labeled DNA was then cleaved by restriction endonuclease digestion and eletrophoresed into an agarose gel. Lanes A and J are ethidium bromide stained molecular weight standards from restriction digests of pCaMV10 DNA (7). A in 8 M urea but not heat denatured; J was heat denatured (100 C) in 8 M urea. All other lanes are FMV DNA detected by autoradiography: B/C—uncut; D/E—*Bam*HI cut; F/G—*Xho*I cut; H/I—*Pst*I cut. C, E, G, and I were denatured in 8 M urea at 100 C, then quickly cooled in an ice bath. B, D, F, and H were not heat denatured, although urea was added to a concentration of 8 M.

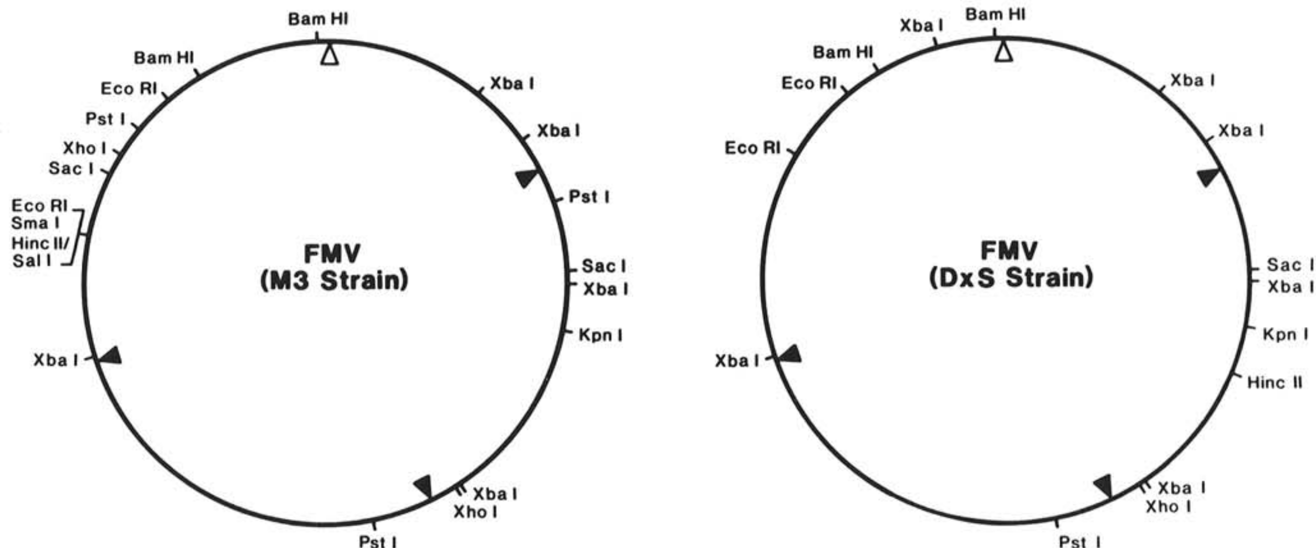

Fig. 4. Physical map of the figwort mosaic virus genome. The map on the left is that of FMV severe strain taken from figwort (virus DNA from pFMV-M3 clone). The map on the right is that of the severe strain adapted to *Datura innoxia*, DxS strain (virus DNA from pFMV-Sc3 clone). The small open triangle at the top of the map shows the location of the single discontinuity in the minus strand. The minus strand has a 3′ to 5′ clockwise orientation around the circular genome starting at this point. The small filled triangles show the locations of the three discontinuities in the plus strand.

D. innoxia was chosen as an experimental host for long-term culture of the virus because FMV causes mild reactions on this plant and because the plant can be easily propagated by vegetative means. The latter was desirable to avoid any chance for selective transfer of variants that arose early in the host adaption process.

The long-term culture of FMV in *D. innoxia* (2 yr) was started with the pFMV-M3 clone of the severe strain inoculated first to *D. stramonium* and, shortly thereafter, to *D. innoxia*. This clone was observed to induce typically severe symptoms on *D. stramonium*. The use of cloned virus to initiate this trial favored the presence of a single virus strain at the beginning of the experiment.

During the 2-yr culture of the virus in *D. innoxia*, there was no apparent indication of a change in the virulence of FMV to this host. However, when the adapted virus was inoculated to *D. stramonium*, the symptoms consisted of mild mottling (Fig. 1C) with none of the extensive necrosis or stunting associated with infection by the original strain (pFMV-M3 clone of severe strain).

D. stramonium plants sampled for ELISA tests 20 days after inoculation indicated that more than 10 times as much virus was present in plants with the adapted (DxS) strain compared with the original unadapted strain (pFMV-M3 clone). The concentration of the latter strain in plants was determined to be 0.33 ± 0.03 μg/g of fresh leaf tissue, whereas the concentration of DxS was 4.15 ± 0.86 μg/g. The virus concentration (severe strain) found in chronically infected figwort was 1.05 ± 0.07 μg/g of fresh leaf tissue.

Viral DNA from the DxS (adapted) strain FMV was isolated from *D. stramonium* by the miniscreen procedure and subjected to restriction endonuclease analysis. The results of this analysis are given in Figure 4. Several restriction sites that were present at specific locations in the original genome (pFMV-M3 clone of severe strain) were absent in the DxS clone (*Eco*RI, *Hinc*II, *Pst*I, *Sac*I, *Sma*I, *Xba*I, and *Xho*I).

The most striking feature of restriction site changes was their distribution in the genome. Eight of 10 changes occurred in a relatively small region of the genome, which by analogy with the CaMV genome would correspond to region VI. Recent sequencing data of the FMV genome (Richins, Scholthof, and Shepherd, *unpublished results*) confirm this region as analogous to region VI of CaMV, although little homology exists in this region between the two viruses. However, the region is about the same size as that of CaMV and has a small intergenic region immediately upstream from it.

DISCUSSION

Figwort mosaic virus appears to be typical of a caulimovirus in most of its properties. Its symptomatology, vector transmission, breadth of host range, and formation of cytoplasmic amorphous inclusion bodies in infected hosts are consistent with FMV being a member of the caulimovirus group.

The physical nature of FMV nucleic acid is also similar to that of the other caulimoviruses. The double-stranded DNA of both FMV and CaMV behaved similarly on nondenaturing agarose gels including the presence of two or three forms migrating more slowly than relaxed circular molecules. These forms are probably the knotted molecules described by Menissier et al (16). Analysis of the denatured nucleic acids of FMV and CaMV indicates that both contain single-stranded discontinuities in their viral genomes. The denatured fragment sizes varied somewhat between the viruses owing primarily to the presence of a third single-stranded discontinuity in the β (plus) strand of FMV (CaMV DNA contains only two such discontinuities in its β strand). Both viruses contain a unique single-stranded discontinuity in their α (transcribed) strand (8,13,25). The tentative strand designations used here (plus or minus) have been confirmed in a recent sequencing investigation on FMV DNA (Richins, Scholthof, and Shepherd, *unpublished*).

The discontinuities are probably triple-stranded regions in which a short, single-stranded tail hangs off the double helix (5). Ribonucleotides have also been observed in these regions of the genome and are likely present as the result of a previous priming event during replication of viral DNA by reverse transcription (9).

Hull and Donson (14) have also prepared a physical map of the FMV genome. Except for a few minor changes in some of the restriction cleavage sites, the map appears very similar to that of the severe (unadapted) strain described here. The restriction map of the DxS (adapted) strain, however, contains several changes indicating that an extensive series of mutations has likely occurred in the viral genome during its multiplication in *D. innoxia*.

Examples of changes in biological properties as a result of host passage are common among plant viruses (26), so the changes in virulence observed in this investigation by multiplication of FMV in *D. innoxia* are not unusual.

In most of the reports concerning changes in virulence due to host passage, however, it has not been determined whether the effect was due to a selection of a particular virus strain from an initial mixture or whether the virus actually mutated during the course of its propagation in the new host. Because the starting virus in this case was a bacterial clone, our observations would favor mutation as the probable explanation for the nucleotide changes. On two other occasions, when virus (taken from figwort) was inoculated to *D. innoxia* and then transferred to *D. stramonium* when the first systemic symptoms appeared on *D. innoxia*, the resulting disease on *D. stramonium* was not much different from that caused by virus taken directly from figwort. It was only after the virus was cultured in *D. innoxia* for several weeks or months that its transfer to *D. stramonium* gave mild symptoms. Moreover, the adaptation phenomenon was not observed in *D. stramonium*. Even during several sequential transfers by mechanical means, the infection caused severe reactions. However, no restriction analyses was made on DNA taken from these plants.

From these observations, one could argue for the change occurring slowly, perhaps through step-by-step mutation. Several examples of this sort have been documented with RNA viruses of animals (10). However, examples of genetic changes occurring among plant viruses have rarely been documented on the nucleic acid level, although Reddy and Black (19) showed that wound tumor virus lost certain segments of its multipartite genome when the virus was maintained for extensive periods in sweet clover (*Melilotus officinalis* (L.) Lam.) propagated by vegetative means.

The relatively rapid mutation rate during adaption of FMV to solanaceous hosts may occur as a natural consequence of the manner in which the DNA replicates. There is now good evidence for caulimoviral DNA replication by reverse transcription (19), and for the full-length RNA transcript serving as a replicative intermediate during this process. Two steps in this mode of replication are potentially error-prone. The first of these is the DNA→RNA transcriptional step carried out in the nucleus by DNA dependent RNA polymerase II (8). This is followed by an RNA→DNA reverse transcription step believed to occur in the cytoplasm. Misincorporation rates during each of these steps may be as high as one in 10^4 (18,24). In contrast, most DNA→DNA replication mechanisms have a very high fidelity with error rates as low as one in 10^{10} nucleotides (1,18). The high mutation rate observed in replication modes involving an RNA intermediate, when coupled to host selection pressure, probably enables rapid adaptation of reverse transcribing viruses to new host plants.

We are now aware, as the result of a separate DNA sequencing investigation with FMV (Richins, Scholthof, and Shepherd, *unpublished results*), that the upper left quadrant of the genome (Fig. 4) contains a putative coding region analogous to gene VI of CaMV. However, gene VI of FMV shares only limited nucleotide or amino acid homology with gene VI of CaMV. Nevertheless, like gene VI of CaMV, the FMV region is separated from the rest of the genome by two intergenic regions (immediately 5′ and 3′ of the coding region). The noncoding region immediately upstream to the gene has consensus sequences typical of many eukaryotic promoters and is probably transcribed as a separate subgenomic RNA equivalent to the 19 S RNA transcript of CaMV (3,17).

No molecular function for gene VI of the caulimoviruses has yet been revealed. Nevertheless, some biological roles for the gene have been discovered. Daubert et al (4) presented evidence that showed that CaMV region VI is largely responsible for the induction of chlorosis and mottling of infected plants and Schoelz et al (22) have shown that the gene VI of CaMV specifies a host

range determinant.

Unfortunately, none of these observations provides any specific clues as to the function of the region VI protein or why this region selectively mutates during adaptation of the virus to a new host. Discovery of the function of gene VI may explain how this occurs.

LITERATURE CITED

1. Baltimore, D. 1985. Retroviruses and retrotransposons: The role of reverse transcription in shaping the eukaryotic genome. Cell 40:481-482.
2. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
3. Covey, S. N., and Hull, R. 1981. Transcription of cauliflower mosaic virus DNA. Detection of transcripts, properties, and location of the gene encoding the virus inclusion body protein. Virology 111:463-474.
4. Daubert, S. D., Schoelz, J., Li, D., and Shepherd, R. J. 1984. Expression of disease symptoms in cauliflower mosaic virus genomic hybrids. J. Mol. Appl. Genet. 2:537-547.
5. Franck, A., Guilley, H., Jonard, G., Richards, K., and Hirth, L. 1980. Nucleotide sequence of cauliflower mosaic virus DNA. Cell 21:285-294.
6. Gardner, R. C., Howarth, A. J., Hahn, P., Brown-Luedi, M., Shepherd, R. J., and Messing, J. 1981. The complete nucleotide sequence of an infectious clone of cauliflower mosaic virus by M13mp7 shotgun sequencing. Nucleic Acids Res. 9:2871-2888.
7. Gardner, R. C., and Shepherd, R. J. 1980. A procedure for rapid isolation and analysis of cauliflower mosaic virus DNA. Virology 106:159-161.
8. Guilfoyle, T. J. 1980. Transcription of cauliflower mosaic virus genome in isolated nuclei from turnip leaves. Virology 107:71-80.
9. Hohn, T., Hohn, B., and Pfeiffer, P. 1985. Reverse transcription in cauliflower mosaic virus. Trends Biochem. Sci. 10:205-209.
10. Holland, J., Spindler, K., Horodyski, F., Gaabau, E., Nichol, S., and Vendepol, S. 1982. Rapid evolution of RNA genomes. Science 215:1577-1585.
11. Holmes, D., and Quigley, M. 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. 114:193-197.
12. Hull, R. 1984. Caulimovirus group. CMI/AAB Descriptions of Plant Viruses 295. 4 pp.
13. Hull, R., Covey, S. N., Stanley, J., and Davies, J. W. 1979. The polarity of the cauliflower mosaic virus genome. Nucleic Acids Res. 7:669-677.
14. Hull, R., and Donson, J. 1982. Physical mapping of the DNAs of carnation etched ring and figwort mosaic viruses. J. Gen. Virol. 60:125-134.
15. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
16. Menissier, J., de Murcia, G., Lebeurier, G., and Hirth, L. 1983. Electron microscopic studies of the different topological forms of cauliflower mosaic virus DNA. Knotted encapsidated DNA and nuclear minichromosomes. EMBO J. 2:1067-1071.
17. Odell, J. T., and Howell, S. H. 1980. The identification, mapping and characterization of mRNA for P66, a cauliflower mosaic virus-coded protein. Virology 102:348-359.
18. Reanney, D. 1984. Genetic noise in evolution. Nature (London) 307:318-319.
19. Reddy, D. V. R., and Black, L. M. 1977. Isolation and replication of mutant populations of wound tumor virus lacking certain genome segments. Virology 80:336-346.
20. Richins, R. D., and Shepherd, R. J. 1983. Physical maps of the genomes of dahlia mosaic virus and mirabilis mosaic virus—members of the caulimovirus group. Virology 124:208-214.
21. Richins, R. D., and Shepherd, R. J. 1986. Horseradish latent virus, a new member of the caulimovirus group. Phytopathology 76:749-754.
22. Schoelz, J., Shepherd, R. J., and Daubert, S. 1986. Region VI of cauliflower mosaic virus encodes a host range determinant. Mol. Cell. Biol. 6:2632-2637.
23. Shalla, T. A., Shepherd, R. J., and Petersen, L. J. 1980. Comparative cytology of nine isolates of cauliflower mosaic virus. Virology 102:381-388.
24. van Vloten-Doting, L., Bol, J.-F., and Cornelisson, B. 1985. Plant virus based vectors for gene transfer will be of limited use because of the high error frequency during viral RNA synthesis. Plant Mol. Biol. 4:323-326.
25. Volovitch, M., Drugeon, G., and Yot, P. 1978. Studies on the single-stranded discontinuities of the cauliflower mosaic virus genome. Nucleic Acids Res. 5:2913-2925.
26. Yarwood, C. E. 1977. Host passage effects with plant viruses. Adv. Virus Res. 25:169-190.