Properties of an Unusual Strain of Cauliflower Mosaic Virus

J. E. Schoelz, R. J. Shepherd, and R. D. Richins

Department of Plant Pathology, University of Kentucky, Lexington 40546.
Research supported by National Science Foundation Grant PCM-8342878 and U.S. Department of Agriculture CRGO Grant CRCR-1-1505.
Accepted for publication 31 October 1985 (submitted for electronic processing).

ABSTRACT

Schoelz, J. E., Shepherd, R. J., and Richins, R. D. 1986. Properties of an unusual strain of cauliflower mosaic virus. Phytopathology 76: 451-454.

A virus from naturally infected *Brassica campestris* systemically infected *Datura stramonium* and certain other solanaceous plants. The virus, designated D4, induced atypical veinal necrosis on *Brassica* species. D4 was transmitted by aphids. It infected a variety of cruciferous and solanaceous species but no plants in other families after mechanical inoculation. Purified virus particles were spherical and about 50 nm in diameter. It reacted with cauliflower mosaic virus antiserum and had inclusion bodies characteristic of a caulimovirus. The viral DNA was purified from turnips, then cloned to the *E. coli* vector pBR322 and propagated in bacteria. The cloned virus was infectious on *B. campestris* when excised from the vector and it induced the same necrotic symptoms as the original virus. The restriction endonuclease map of the cloned viral DNA was similar to that of cauliflower mosaic virus. It was concluded that the virus is a strain of cauliflower mosaic virus that induces unusual symptoms and has an unusual host range.

Cauliflower mosaic virus (CaMV) causes economically important diseases of crucifers, on which it induces mottling symptoms. It is the best characterized representative of the caulimoviruses, a group of double-stranded DNA viruses of great interest as model systems for gene expression in higher plants.

The caulimoviruses have become popular subjects for genetic analysis because their simple genomes can be propagated by molecular cloning in bacteria. This greatly simplifies mutagenesis, recombination, and restructuring of viral genomes so that specific DNA sequences can be associated with biological activity. Strains with distinctive symptoms and host range are especially useful for genetic mapping of biological functions. For this reason, we describe herein an unusual strain of CaMV. It induces symptoms unlike other strains of CaMV and has the unusual capacity to systemically infect solanaceous plants. The in vitro recombination of this strain, designated D4, with more conventional CaMV strains has led to the definition and mapping of nucleotide sequences which determine symptoms (2) and host specificity (16).

MATERIALS AND METHODS

Virus and host manipulation. After transfer from systemically infected *Datura stramonium* L., the virus was maintained in turnip (*Brassica campestris* L. 'Just Right'). Virus was purified from turnip as described by Hull et al (11). For the host range tests, inoculum was prepared by grinding infected turnip tissue in 0.05 M phosphate buffer, pH 7.5, at a tissue:buffer ratio of about 1:10. All plants were grown in a peat-sand-soil (5:2:1, v/v) mixture in 12.5-cm-diameter plastic pots. At least six to eight seedlings of each host were inoculated in the three- to six-leaf stage. Symptoms were observed for at least 1 mo after inoculation. In most cases, transmission tests were made from inoculated plants to turnip seedlings to confirm either the presence or absence of positive infection.

Serological tests were performed in a 0.7% agarose medium containing 0.85% sodium chloride and 0.04% sodium azide in 0.01 M tris, pH 7.5. Ten milliliters of agarose were used per plate and wells 7 mm in diameter and 5 mm apart were cut just before the first reagents were added. Virus was added to the wells 8 hr before the antiserum so precipitin lines formed away from the antigen well. Antisera to CaMV were those prepared previously (5).

Aphid transmission tests were performed with green peach aphids (*Myzus persicae* Sulzer) (three aphids per test plant) that were starved for 3 hr prior to the acquisition access feeding period of 5 min. Infected turnip and *D. stramonium* were virus source plants. Turnip seedlings in the cotyledon stage were the test plants. Aphids were caged on the latter overnight before they were removed by spraying the plants with nicotine sulfate.

Construction of the D4 clone. DNA of D4 was isolated by the miniscreen procedure (4) and cloned at its unique *Sal*I site into the plasmid vector pBR322 by procedures similar to those used previously (3,14). The cloning host was *Escherichia coli* K12 strain 71-18 (VΔ (*lac-pro AB*) *sup* F^1 *thi F lac lq Z*Δ *M15 pro* A_+ B_+). The bacterial strain was maintained on M9 minimal solid medium. For pBR322 plasmid propagation, the bacterium was grown in DYT (15) broth containing 100 μg of ampicillin and 20 μg of tetracycline per milliliter.

In these experiments, *Sal*I-cleaved viral DNA was mixed with *Sal*I-cleaved DNA of pBR322 and the mixture was ligated overnight at 12 C (T4 ligase). Cells of *E. coli* made competent by $CaCl_2$ as described by Cohen et al (1) were transformed with ligated DNA. Transformed cells were spread on DYTA plates containing ampicillin. After overnight incubation at 37 C, several colonies were screened on additional plates for ampicillin resistance and tetracycline sensitivity. Selected colonies were cultured in DYT broth, their plasmid DNA was isolated as described by Holmes and Quigley (8) and screened for full-length viral DNA by gel electrophoresis in 1% agarose. A clone containing full-length viral DNA was identified by comparing restriction digests of cloned DNA to native viral DNA on 1% agarose gels.The isolated full-length clone, shown to be infectious by DNA inoculation to turnip seedlings, was designated pCaMV-D4.

To test infectivity of pCaMV-D4, isolated plasmid DNA was cleaved with *Sal*I and inoculated to 12-day-old turnip seedlings. Each seedling was inoculated with approximately 2 μl of DNA at 0.5 μg/μl.

Restriction enzyme mapping. The conditions for use of each restriction endonuclease were as recommended by the supplier (New England Biolabs, Beverly, MA). Restriction sites on pCaMV-D4 were mapped by comparing single and double digests of its DNA with those of pCaMV-10 (3), an infectious clone of CaMV strain CM1841. The fragment order of CM1841 is known from its complete DNA sequence (3). DNA fragments were separated on either 1.0% agarose or 6.0% polyacrylamide gels in tris-borate-EDTA buffer (13). The single-stranded discontinuities in native D4 virus DNA were mapped by the procedure of Volovitch et al (18).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

RESULTS

Isolation of and symptoms produced by the virus. The D4 virus was derived from an inoculum containing an ordinary mottle-inducing strain of CaMV. The latter, designated the "Fordham isolate," was discovered in an infected turnip in a vegetable garden in Davis, CA. This isolate produced mottling typical of CaMV on seedlings of *B. campestris*. Systemic symptoms of the disease appeared 10–12 days after inoculation, typical of CaMV infections. Systemic symptoms caused by RNA viruses generally appear 5–7 days after mechanical inoculation.

When young seedlings of *D. stramonium* were inoculated with the Fordham isolate from turnip, prominent symptoms of systemic disease appeared 3–4 wk later. The most conspicuous symptoms were yellowing of the major veins and puckering of the interveinal tissue (Fig. 1C). This led to a coarse mottling and distortion of the leaves.

When the virus was transferred from *D. stramonium* back to turnip, the symptoms consisted almost exclusively of veinal necrosis (Fig. 1A and B). The smaller veinlets of the leaves developed the most severe necrosis. The larger veins and midribs did not become necrotic. This symptom developed initially only on

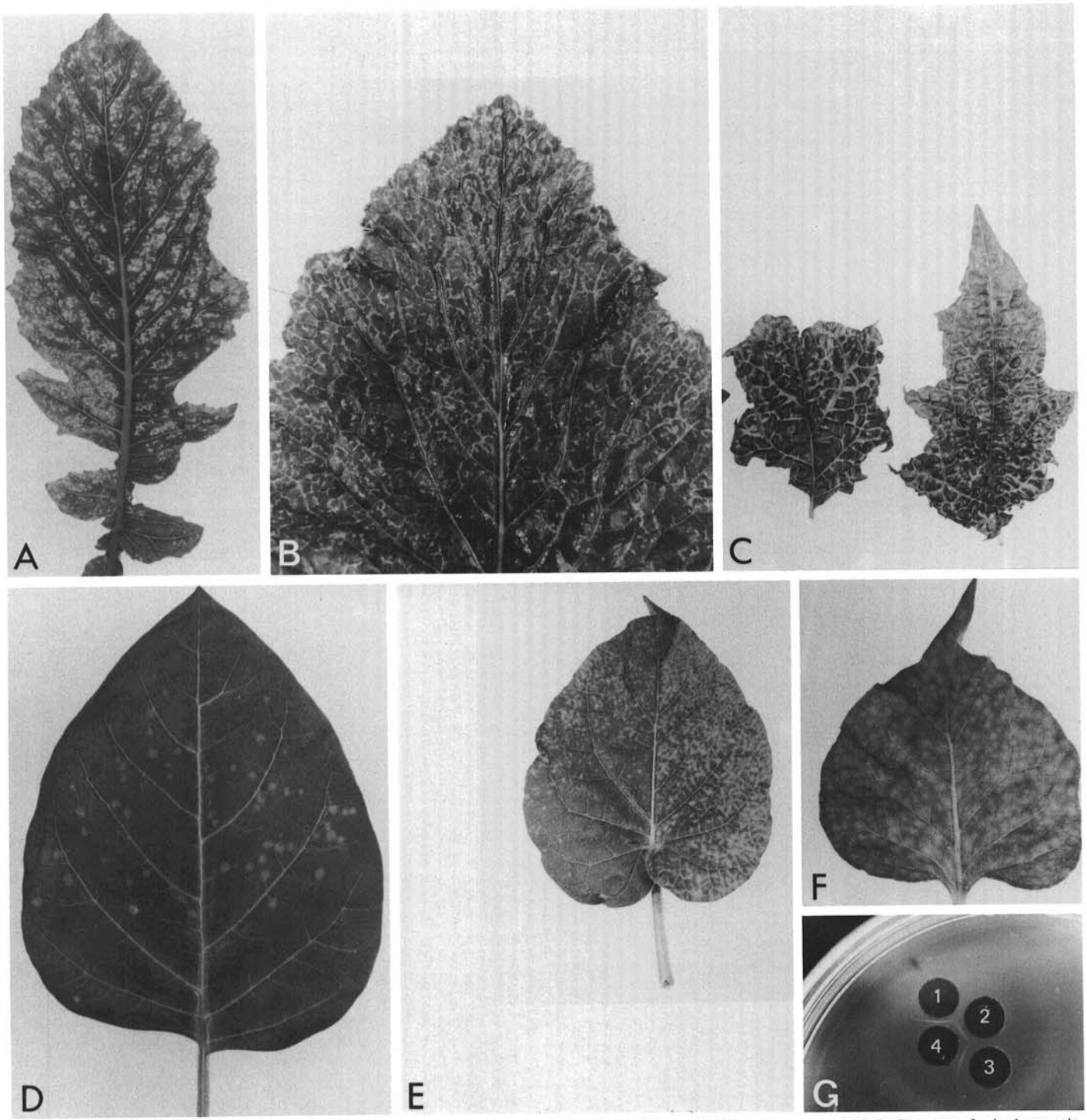

Fig. 1. Symptoms and serology of virus D4. **A,** Veinal necrosis symptoms on cultivar Just Right turnip (*Brassica campestris*). **B,** Close-up of veinal necrosis on *B. campestris*. **C,** Systemic symptoms on *Datura stramonium*. **D,** Local chlorotic lesions on *Datura innoxia*. **E,** Systemic vein yellowing on *Nicotiana glutinosa*. **F,** Systemic chlorotic mottle on *Nicotiana edwardsonii*. **G,** Agar gel-diffusion test with purified D4 with antiserum to CaMV: Well 1, purified CaMV strain CM1841 (homologous antigen) at 0.1 mg/ml; wells 2 and 3, purified D4 at 0.1 mg/ml; well 4, antiserum to CaMV (placed in the well 8 hr after the viral antigens were added).

leaves 4–6 cm long. The smaller leaves were generally symptomless. Only a few of the leaves developed veinal necrosis. Many that never showed symptoms contained virus; this was shown by test inoculations to other plants. In fact, symptomless leaves contained as much virus as symptomatic leaves.

Young leaves of turnip that showed veinal necrosis developed until they reached almost normal size. Plant growth rate and size were unaffected by infection.

The virus from *D. stramonium* was designated D4 because of its unusual disease-inducing properties and to distinguish it from the Fordham isolate. The latter, which we presume accompanied D4 in the original infection in *B. campestris*, induced mottling diseases typical of CaMV in crucifers.

D4 induced more severe symptoms at cooler temperatures. Symptoms at 20 C were prominent on both turnip and *D. stramonium*. Symptoms were largely masked at 28 C and above.

Host range of D4. The virus had a cruciferous host range typical of CaMV. It infected many cultivars of *B. campestris, Brassica oleracea* L., and *B. napus* L. but not *B. nigra* (L.) Koch or *Raphanus sativus* L.. Among solanaceous species, it infected *Datura innoxia* Mill. locally (Fig. 1D) but not systemically. It induced systemic infections on *Nicotiana clevelandii* Gray, *N. glutinosa* L. (Fig. 1E), *N. bigelovii* S. Watson and *N. edwardsonii* Christie & Hall (1F). D4 did not infect *N. tabacum* L., *N. glauca* Graham, *N. debneyi* Domin, *N. plumbaginifolia* Viviani or *N. benthamiana* Domin. It also did not infect potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), or eggplant (*Solanum melongena* L.).

None of the following species in other families were infected: *Vigna unguiculata* (L.) Walp. subsp. *unguiculata, Phaseolus vulgaris* L., *Pisum sativum* L., *Beta vulgaris* L., *Spinacia oleracea* L., *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., *C. capitatum* L., *Tetragonia expansa* Thunb., *Cucumis sativus* L., *C. melo* L., *Cucurbita pepo* L., or *Zinnia elegans* Jacq..

Insect transmission. The virus was readily transmitted by green peach aphids from either *D. stramonium* or turnip. When starved green peach aphids were allowed a 15-min acquisition feed on infected plants of *D. stramonium* and an overnight inoculation feed on turnip seedlings, 45 of 81 test plants (55%) became infected. In a similar test with systemically infected turnip used as the source plant, 28 of 77 plants (37%) became infected.

Serology. In agar gel diffusion tests with CaMV antiserum, D4 showed a strong reaction (Fig. 1G). The lines of precipitation coalesced with only a slight indication of a heterologous reaction less intense than the homologous virus antigen. This consisted of a less sharp line of precipitate than the homologous antigen (Fig. 1G).

Electron microscopy of virus and virus inclusion bodies. Purified preparations of D4 sedimented in 10–40% sucrose density gradients at the same rate as CM1841. Purified virus, after being stained with uranyl acetate, consisted of isometric virus particles about 50 nm in diameter. Purified tobacco mosaic virus was used as an internal size standard. Particles in situ are illustrated in Shalla et al (17).

The inclusion bodies in turnip were smaller than those of most CaMV strains. They consisted of small patches of electron-dense matrix with virions embedded in the matrix. The inclusion bodies in infected *D. stramonium* were much larger with prominent amounts of matrix and virions. These differences have been illustrated by Shalla et al (17) in a comparative study of the inclusion bodies of CaMV strains.

Restriction mapping of the DNA genome. A physical map of the virus genome was constructed by using 11 different restriction endonucleases. The viral DNA showed restriction patterns very similar to that of CaMV strain CM1841 (Fig. 2). The two viruses had 14 cleavage sites in common, out of a total of 28 sites mapped. Nine restriction sites were unique to CM1841 while five sites were unique to D4. Denaturing gel electrophoresis showed that D4 viral DNA has three single-stranded interruptions. These three

TABLE 1. Nucleotide positions of restriction endonuclease sites in the DNA genomes of D4 and CM1841[a]

Restriction enzyme	Site unique to CM1841	Site common to both viruses	Site unique to pCaMVD4[b]
*Bgl*I	6656[c]	3408	
*Bst*EII		126	
*Cla*I	1783, 2681, 7987	820, 3957	
*Eco*RI	6105	408, 2414, 2474, 5646	6040
*Eco*RV	2834, 7340	5708	
*Hgi*AI		5822, 7025	3750
*Hpa*I	5514		3060
*Pvu*II		6318	4000
*Sac*I		5822	3750
*Sal*I		4833	
*Xho*I	1642		

[a] The nucleotide positions have been taken from those of CM1841, whose complete DNA sequence has been determined (4).
[b] pCaMVD4 is an infectious clone of D4.
[c] Nucleotide position relative to the single-interruption in the alpha strand. Nucleotide base pairs are numbered consecutively in clockwise position from the alpha strand interruption.

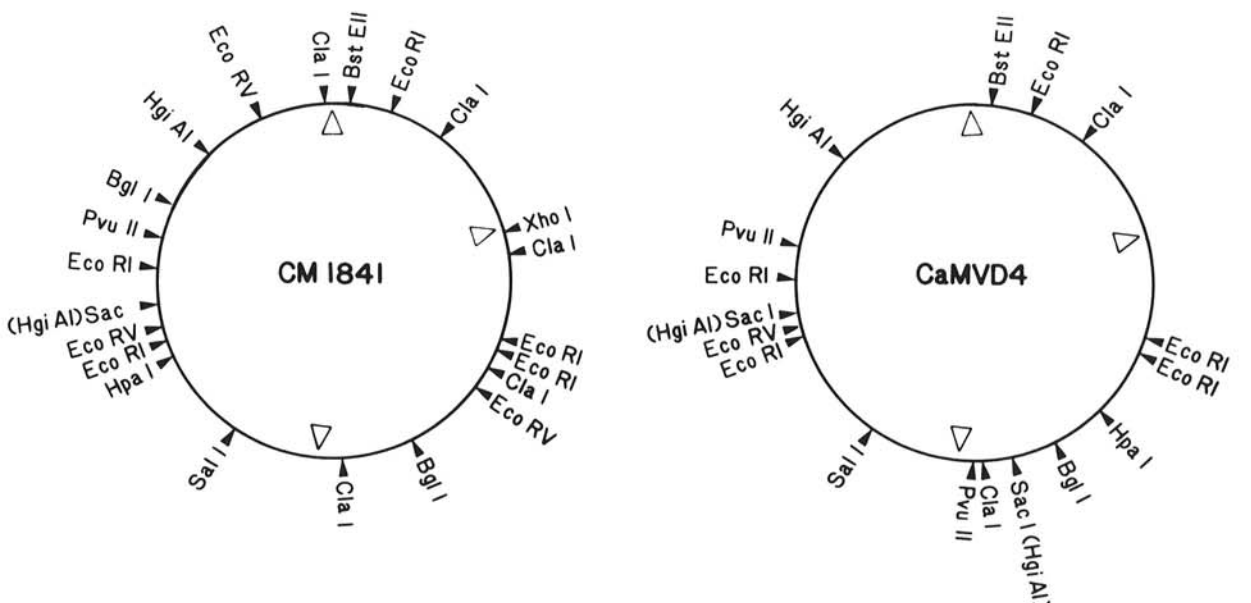

Fig. 2. A comparison of the physical maps of CaMVD4 and strain CM1841. The positions of the single-stranded discontinuities are indicated by a triangular pointer. The exact positions of restriction endonuclease cleaveage sites are given in Table 1.

interruptions were in approximately the same positions as the three interruptions of CM1841.

DISCUSSION

D4 is a strain of CaMV that causes unusual symptoms in turnips and has a broader host range than most CaMV strains. D4 has many typical caulimovirus features. For example it requires about 10 days to develop systemic symptoms following mechanical inoculation, it is transmitted by aphids, and its morphology and inclusion bodies are characteristic of caulimoviruses (6) Its serology and restriction endonuclease map establish that it is a strain of CaMV.

The relationship between D4 and the Fordham strain was not pursued in this study. The genotypic differences which account for the altered symptoms and host range probably could not be detected at the restriction enzyme level. CM1841 was chosen for comparison rather than the Fordham strain because its genome has been more completely characterized (2,3).

To a certain extent, restriction endonuclease cleavage sites reflected nucleotide sequence differences between CM1841 and D4. Of the 28 restriction sites summarized in Table 1, half were unique to one virus or the other and half were common to both viruses. In this regard, D4 exceeds the differences shown among other CaMV strains (9). However, the restriction map of D4 is much more similar to CaMV strains than to other caulimoviruses. The restriction maps of figwort mosaic virus, carnation etched ring virus, dahlia mosaic virus, and mirabilis mosaic virus have essentially no similarity to that of CaMV (10,15).

Although D4 has properties typical of CaMV, it has a broader host range and it induces radically different symptoms in turnips. Most strains of CaMV have a host range limited to the Cruciferae, but some strains infect *N. clevelandii* systemically (7) or induce local necrotic lesions on *D. stramonium* (12). No previously reported strains systemically infect a variety of solanaceous plants, including *D. stramonium* as does D4. The virus also is unusual in provoking veinal necrosis rather than chlorosis and mottling of crucifers.

The D4 strain of CaMV provides a new genotype useful in mapping virus functions responsible for symptoms and host specificity. Evidence from recombinant viruses constructed between D4 and CM1841 indicate that the coding region of gene VI of CaMV mediate chlorosis and mottling in crucifers. Recombinants between strains in which D4 contributes gene VI cause few if any symptoms on turnips. The veinal necrosis symptom of D4 on crucifers is associated with some portion of the genome other than gene VI (2).

LITERATURE CITED

1. Cohen, S. N., Chang, A. C. Y., and Hsu, L. 1972. Nonchromosomal antibiotic resistance in bacteria: Genetic transformation of *Escherichia coli* by R-factor DNA. Proc. Nat. Acad. Sci. USA 69:2110-2114.
2. 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.
3. 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 M13 mp7 shotgun sequencing. Nucleic Acids Res. 9:2871-2888.
4. Gardner, R. C., and Shepherd, R. J. 1980. A procedure for rapid isolation and analysis of cauliflower mosaic virus. Virology 106:159-161.
5. Ghabrial, S. A., and Shepherd, R. J. 1980. A sensitive radioimmunosorbent assay for the detection of plant viruses. J. Gen. Virol. 48:311-317.
6. Harrison, B. D., Finch, J. T., Gibbs, A. J., Hollings, M., Shepherd, R. J., Valenta, V., and Wetter, C. 1971. Sixteen groups of plant viruses. Virology 45:356-363.
7. Hills, B. J., and Campbell, R. N. 1968. Morphology of broccoli necrotic yellows virus. J. Ultrastruct. Res. 24:134-144.
8. Holmes, D., and Quigley, M. 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. 114:193-197.
9. Hull, R. 1980. Structure of the cauliflower mosaic virus genome III. Restriction endonculease mapping of thirty-three isolates. Virology 100:76-90.
10. 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.
11. Hull, R., Shepherd, R. J., and Harvey, J. D. 1976. Cauliflower mosaic virus: An improved purification procedure and some properties of the virus particles. J. Gen. Virol. 31:93-100.
12. Lung, M. C. Y., and Pirone, T. P. 1972. *Datura stramonium*, a local lesion host for certain isolates of cauliflower mosaic virus. Phytopathology 62:1473-1474.
13. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
14. Meagher, R. B., Shepherd, R. J., and Boyer, H. W. 1977. The structure of cauliflower mosaic virus. I. A restriction endonuclease map of cauliflower mosaic virus DNA. Virology 80:362-375.
15. Richins, R. D., and Shepherd, R. J. 1983. Physical maps of the genomes of dahlia mosaic virus and mirabilis mosaic virus—Two members of the caulimovirus group. Virology 124:208-214.
16. Schoelz, J. E., Daubert, S. D., and Shepherd, R. J. 1984. Gene VI of cauliflower mosaic virus controls systemic spread in solanaceous hosts. (Abstr.) Phytopathology 74:861.
17. Shalla, T. A., Shepherd, R. J., and Petersen, L. J. 1980. Comparative cytology of nine isolates of cauliflower mosaic virus. Virology 102:381-388.
18. Volovitch, M., Dumas, J. P., Drugeon, G., and Yot, P. 1976. Single-stranded interruptions in cauliflower mosaic virus DNA. Colloq. 26. Pages 635-641 in: Nucleic Acids and Protein Synthesis in Plants. L. Bogorad, and J. H. Weil, eds. Centre National de la Recherche Scientifique, Paris.