Turnip, Cucumber, and Ribgrass Mosaic Viruses Isolated from *Hesperis matronalis* in British Columbia

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ABSTRACT

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Viruslike leaf mosaic and petal color-breaking symptoms were observed in the perennial ornamental Dame's rocket (*Hesperis matronalis* [Cruciferae]) collected from eight locations in Vancouver, BC. Three viruses, cucumber mosaic (CMV, To serotype), a necrotic strain of ribgrass mosaic (RMV), and turnip mosaic (TuMV) were isolated (TuMV most often). Gel electrophoresis of genomic and replicative RNAs and molecular hybridization with cDNA were used in addition to serology to characterize the CMV strain. Seedlings of *H. matronalis* mechanically inoculated with either CMV or RMV alone remained symptomless. TuMV alone caused mosaic of leaves, but plants inoculated with TuMV simultaneously in mixed infections with CMV and/or RMV showed a more severe mosaic, distortion, and occasional necrosis. CMV was the only virus isolated from immature ovules, but no seed transmission of any of the viruses occurred in seedlings grown from seed produced by infected *H. matronalis*. This is the first report of the natural occurrence of either RMV or CMV in *H. matronalis* and only the second report of TuMV in British Columbia. *H. matronalis* may be a reservoir host for one or more of these viruses in certain vegetable or crop production areas.

Ornamentals and weeds have been implicated as reservoirs of viruses, and suggestions were made 60 yr ago for eradication of plants shown by circumstantial evidence to be bridging hosts to economically important crops (11). *Hesperis matronalis* L., known as Dame's violet or Dame's rocket, is a perennial crucifer commonly grown in gardens as an ornamental or found as an escape along roadsides, in thickets, and in open woods in certain parts of Canada and the United States (1). It was observed in Iowa in 1968 and in British Columbia in 1985 with viruslike symptoms of mosaic in leaves and color-break patterns in petals. McWhorter (22) attributed mosaic and color breaking in petals of *H. matronalis* in Oregon to serious outbreaks of mosaic diseases in crucifers, but the virus(es) associated with the symptoms was not identified.

The most commonly reported virus occurring naturally in *H. matronalis* is turnip mosaic virus (TuMV) (5,34). *H. matronalis* is susceptible after inoculation with several other viruses (7,32), including cucumber mosaic virus (CMV) (5,24).

Herein we report the isolation and identification of three viruses, CMV, ribgrass mosaic virus (RMV), and TuMV from *H. matronalis* in British Columbia, the symptoms caused by virus combinations inoculated to this host, and an assessment of the potential seed transmissibility of these viruses.

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MATERIALS AND METHODS

Sources of virus isolates. The three viruses isolated and characterized were from specimens of naturally infected *H. matronalis* growing both as cultivated plants near private residences in Vancouver, BC, or as wild plants in natural habitats on the University of British Columbia Endowment Lands, Vancouver. Collections of four to 10 plants, from each of eight separate locations, were made from early June through late July 1985 and maintained in greenhouse cultures. For discussion purposes, the viruses isolated from *H. matronalis* are referred to as CMV-Hm, RMV-Hm, and TuMV-Hm. Both TuMV-Hm and RMV-Hm were isolated from one collection, TuMV-Hm only from six collections and all three, TuMV-Hm, CMV-Hm and RMV-Hm, from one collection.

Host range. All plants were grown from seed at the Agriculture Canada Research Station in insect-free greenhouses provided with supplemental standard fluorescent light to ensure a day length of about 12 hr. Seed of *H. matronalis* was collected in late July-early August 1985 from symptomless plants that were virus-free based on infectivity assays on indicator plants. Five to eight seedlings from such seed were inoculated with each of the virus isolates individually and in all possible combinations. Plant species tested for susceptibility and as virus indicators are listed in Table 1. Plants with three or four expanded leaves were inoculated by rubbing inocula on leaves previously dusted with Carborundum. Inocula were freshly harvested leaves from field- or greenhouse-grown plants ground in 0.01 M potassium phosphate buffer, pH 7.0, or were samples at various stages of purification in 0.005 M borate, pH 8.5, or other buffers.

Serology. Polyclonal antisera (rabbit) to the following viruses from stock supplies at the Agriculture Canada Research Station were used: CMV (To serotype, obtained from M. Hollings), tobacco mosaic virus (TMV), RMV, tomato mosaic virus (ToMV), and TuMV. ELISA (enzyme-linked immunosorbent assay) was done by a double-antibody sandwich method (9) in Linbro Titertek plates (Flow Laboratories, Inc., McLean, VA) with either horseradish peroxidase or alkaline phosphatase and using antigens in purified virus or in crude sap from experimental plants.

Immunoglobulins for use in ELISA tests and for conjugation were purified from polyclonal antisera of CMV and TuMV by $(NH_4)_2SO_4$ precipitation. Standard agar gel diffusion tests were done in 1.5% Noble agar prepared in distilled water. The degree of serological relationship was determined by a serological differentiation index (SDI) (36) in standard microprecipitin tests (2).

Seed transmission. Both infectivity assays and ELISA were used to test for the presence of CMV and TuMV in both immature and mature ovules. Ovules were dissected from siliques at immature to mature stages of seed development, triturated individually in one or two drops of 0.01 M potassium phosphate, pH 7.0, and inoculated to *Chenopodium quinoa* Willd. ELISA was also used to assay for TuMV and CMV in seedlings grown from seed collected from naturally

infected *H. matronalis*.

Electron microscopy. Leaf dips were made by crushing 2–3 mm^2 of tissue in buffer, then placing a droplet on a Formvar-coated grid for 5–10 sec, staining either with 2% phosphotungstic acid (PTA), pH 7.0, or with freshly prepared 4% glutaraldehyde/2% uranyl acetate (UA), pH 5.0, and rinsing with PTA or UA. Grids were observed with either a Philips 300 or a Hitachi H600 transmission electron microscope at 60 kV. Similarly, viruses in situ were observed after fixation in glutaraldehyde and dehydration of infected tissue, embedment in epoxy, sectioning by a diamond knife with an LKB microtome, and staining with 4% UA followed by Reynold's lead citrate.

Purification. Each of the viruses was purified using standard published protocols for CMV (18), for TuMV (37), and for RMV (27).

Nucleic acid extraction. Genomic, single-stranded RNAs (ssRNAs), and replicative, double-stranded RNAs (dsRNAs) corresponding to CMV-Hm were extracted from purified virus preparations (27) and infected tissues (23), respectively, and compared by agarose gel electrophoresis (38) with those of the following cucumoviruses: CMV-Q (To serotype, supplied by J. H. Tremaine) and two Hungarian CMV isolates, Nt80/3 and Vf13 from *Nicotiana tabacum* L. and *Vicia faba* L. (3), respectively; peanut stunt virus (PSV-Tp, isolated from *Trifolium pratense* in Hungary [4]); and tomato aspermy virus (TAV, supplied by R. Stace-Smith). dsRNA was also extracted from tissue infected with cowpea ringspot virus (CpRSV, supplied by R. Hull) and a seed-transmitted cucumolike virus (Cp) from cowpea grown in Hungary. Leaf ribosomal RNAs were used as molecular weight standards for ssRNAs (28).

Molecular hybridization analysis. Preparation of cDNA to CMV-Hm and the details of molecular hybridization analysis were as described previously (27).

RESULTS

Host ranges and symptoms. Symptoms of color break in flowers and mosaic in leaves of *H. matronalis* collected in early June 1985 suggested virus infection (Fig. 1). Three mechanically transmissible viruses were isolated from *H. matronalis* after inoculation of test plants. A flexuous virus, later shown to be TuMV, was isolated from discrete local lesions on *N. tabacum* 'Xanthi-nc' and successfully transferred after several attempts to *Brassica perviridis* (L. H. Bailey) L. H. Bailey 'Tendergreen,' in which the virus was subsequently maintained. On occasion, i.e., July, it was possible to isolate TuMV directly from *H. matronalis* by inoculation of Tendergreen. A rigid rod virus typical of the tobamovirus group was readily isolated from necrotic local lesions on Xanthi-nc and *N. glutinosa* L. and maintained in *C. amaranticolor* Coste & Reyn. or *N. debneyi* Domin. An isometric virus, later shown to be CMV, was isolated from ringspot lesions after inoculation of Xanthi-nc and was transferred to *N. glutinosa* for maintenance.

The symptoms induced by CMV-Hm and TuMV-Hm appeared similar to those induced by other CMV and TuMV strains in their hosts (Table 1). Symptoms induced by RMV-Hm were distinct from those of other RMV isolates in that the virus induced necrotic local lesions in standard Turkish tobacco plants and very rarely spread systemically in these hosts (Table 1).

Symptoms in seedlings of *H. matronalis* inoculated with each virus alone and in all possible combinations were as follows: 1) no symptoms were induced by CMV-Hm or RMV-Hm for 2 mo after inoculation, although the viruses replicated well based on infectivity assays on *C. quinoa* and *N. glutinosa*, respectively; 2) a severe mosaic symptom that persisted as the leaves aged was induced by TuMV-Hm; 3) more severe symptoms such as stunting and leaf curling were induced by simultaneous

Table 1. Partial host range of turnip mosaic (TuMV), cucumber mosaic (CMV), and ribgrass mosaic (RMV) viruses isolated from *Hesperis matronalis* in British Columbia[a]

Host	TuMV	CMV	RMV
Amaranthaceae			
Gomphrena globosa L.	LLn/–	LLn/s	LLrSp/–
Chenopodiaceae			
Chenopodium amaranticolor Coste & Reyn.	LLc/–	LLn/–	LLn/R,mo
C. quinoa Willd.	LLc/–	LLc/–	LLcr(n)/–
Compositeae			
Verbesina encelioides (Cav.) Benth. & Hook	–/–	Ls/s	···
Cruciferae			
Brassica perviridis (L. H. Bailey) L. H. Bailey 'Tendergreen'	Lmt/mt	–/–	···
B. pekinenis (Four.) Rupr. 'Pe-Tsai'	Lmt/mt	–/–	···
Hesperis matronalis L.	LLr/mo	Ls/s	Ls/s
Matthiola incana (L.) R. Br.	Ln/mo	···	···
Cucurbitaceae			
Cucumis sativus L.	···	LLw,cSp/vc,mo,st	LLn/–
Papilonaceae (= Leguminoseae)			
Phaseolus vulgaris L. Black Turtle	–/–	–/–	···
Bountiful	···	–/–	···
Vicia faba L.	···	LLn/–	···
Vigna unguiculata (L.) Walp. subsp. *unguiculata*	···	LLn/–	···
Solanaceae			
Nicotiana clevelandii Gray	···	LnrSp/	LLn/n,st
N. debneyi Domin.	Lc/c,mo,mt	LLn/vc,R,n,st	LLn/vc,R,n,st
N. glutinosa L.	LLd/–	Ls/mt,st,c	LLn/–
N. megalosiphon Heurk & Muell. Hungary selection		LLnrSp/Sp,mo,st	LLn/–
Canada selection		Ls/mo	LLn/–
N. tabacum L. Haronova	LLn/–	LLnr/vc,mo	LLn/–(nlp)
Samsum	LLg/–	LLcr/mt	LLrSp/(nr)
Xanthi	LLn/–	LLnrs/mt,er,s	LLn/–

[a] Symptom abbreviations: c = chlorosis, e = etch, g = green island, l = line, L = local, LL = local lesion, mo = mosaic, mt = mottle, n = necrosis, p = pattern, R = leaf roll, r = ring, s = symptomless, Sp = spot, st = stunt, vc = veinclearing, w = wilt, y = yellow, ? = uncertain, and – = no infection detected based on back-inoculation. Symptoms on inoculated leaves to the left of the slash and symptoms in systemically infected leaves to the right of the slash. Symptoms within parentheses indicate seldom but occasionally observed. All host data are supported by back-inoculations to an assay host (TuMV = *C. quinoa*, CMV = *Vigna unguiculata* subsp. *unguiculata* 'Blackeye' cowpea, and TMV = *N. glutinosa*) for doubtful symptoms or symptomless plants.

Table 2. Recovery of infectivity of cucumber (CMV) or turnip (TuMV) mosaic viruses from plant parts of *Hesperis matronalis* by assay on several half-leaves of *Nicotiana tabacum* 'Xanthi' and *Chenopodium amaranticolor*

Plant part[a]	CMV	TuMV
Leaves	+	+
Petals	+	+
Anthers	+	ND[b]
Siliques	+	+
Ovules	+	–

[a] Eighty-five individual ovules and 12-24 samples of each of the other tissue types were tested.
[b] No data.

inoculation with TuMV-Hm and either CMV-Hm or RMV-Hm; and 4) marked stunting and occasional necrosis followed inoculation of all three viruses. None of the seedling plants have flowered in the 18 mo since inoculation with the viruses, so we cannot report which virus(es) or combinations caused the flower-breaking symptoms in nature.

Seed transmission in *H. matronalis*. Five hundred seedlings grown from seed of infected mother sources were symptomless, and no virus was recovered from nearly 100 of these seedlings selected for both infectivity assays and ELISA. Only CMV-Hm was recovered from 85 ovules examined (Table 2). No virus was detected by infectivity assays of extracts from at least 25 seedlings grown from seed harvested from a source plant infected with all three viruses.

Electron microscopy. Flexuous (690–760 nm) and rigid (290–300 nm) particles (Fig. 2A,B), typical of potyviruses and tobamoviruses, respectively, were observed in negatively stained leaf-dip preparations from various specimens of *H. matronalis*. At least 50 particles were measured from each preparation. These viruses were morphologically identical to those from tobacco infected with the virus from the respective *H. matronalis* plant when observed under the same conditions. Similar tobamovirus particles were also observed in 1968 in the Iowa sample of *H. matronalis* (R. E. Ford, *unpublished*). No viruslike particles typical of CMV were unequivocally seen in leaf-dip preparations, but they were visible in partially purified preparations (Fig. 2C) after fixation in glutaraldehyde (15) and staining in UA.

All three viruses could be recognized in thin sections of leaf cells of experimentally

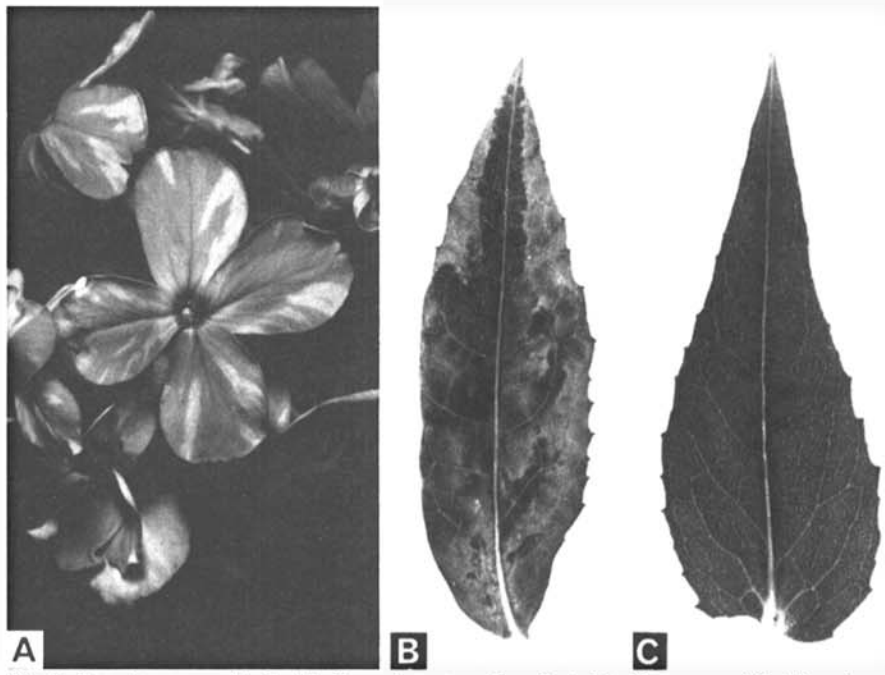

Fig. 1. Symptoms associated with *Hesperis matronalis* collected in Vancouver, BC: **(A)** color-break symptoms in a flower from an infected plant, **(B)** mosaic-infected leaf, and **(C)** leaf from healthy plant.

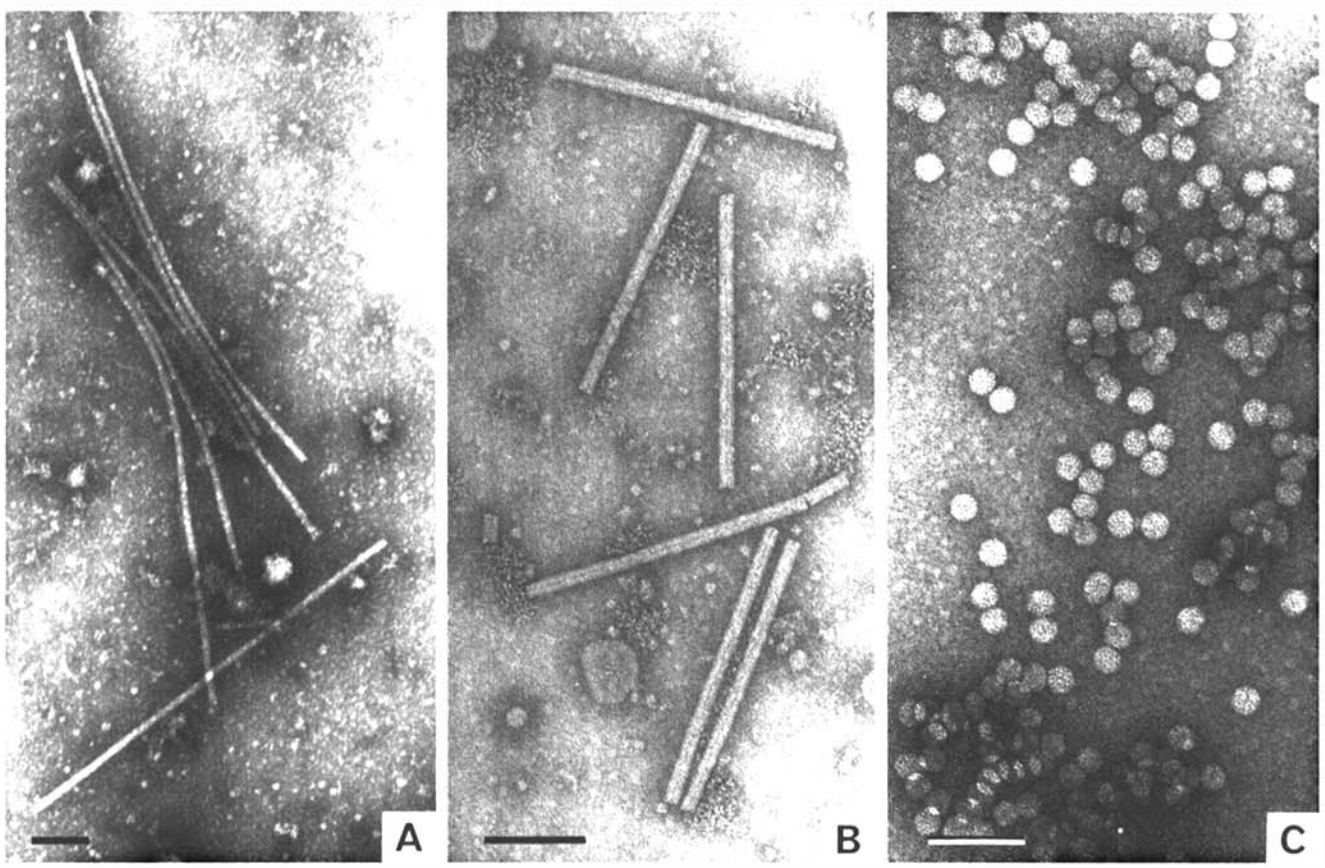

Fig. 2. Virus particles associated with naturally infected *Hesperis matronalis*: **(A)** turnip mosaic virus negatively stained with 2% phosphotungstic acid (PTA), **(B)** ribgrass mosaic virus (BC isolate) negatively stained with 2% PTA, and **(C)** cucumber mosaic virus negatively stained with glutaraldehyde-uranyl acetate. Virus particles in A and B were in leaf dips directly from *H. matronalis*, and particles in C were in a partially purified preparation from tobacco. Scale bar = 100 nm.

infected *H. matronalis* (Fig. 3A–C). The scroll-like and laminar inclusion bodies of TuMV-infected cells (Fig. 3A) are diagnostic of potyvirus subgroup III to which TuMV belongs (13). The uniseriate array of TuMV virions is unusual for this virus, but it was frequently observed.

Serology. The identity of each virus was confirmed by serological tests. The potyvirus reacted only with a TuMV antiserum in ELISA, and preliminary immunodiffusion tests showed that the cucumolike virus reacted only with a stock CMV antiserum (To serotype). In subsequent immunodiffusion tests using antisera to the To and D serotypes of CMV (3), the CMV-Hm antigen:antibody precipitin band fused directly with that of a To serotype but spurred strongly with that of CMV Nt80/3 (D serotype), indicating that CMV-Hm belongs to the To serotype. In microprecipitin tests, the tobamovirus was related closely to several isolates of RMV (SDI = <0.5; six antisera, three replicates) and distantly related to ToMV (SDI = 3.0; two antisera, three replicates) and TMV-U_1 (SDI = 5.0; two antisera, three replicates).

Gel electrophoresis of cucumovirus replicative and genomic RNAs. The dsRNA patterns corresponding to the various cucumoviruses are shown in Figure 4. Four migrating dsRNAs, corresponding to those of the three genomic RNAs, and the subgenomic m-RNA for coat protein (16) were usually obtained, but differences in the staining intensities of the bands allowed for distinguishing isolates and strains. The intensity of dsRNA-3 of CMV-Hm was characteristically the strongest of the four dsRNAs, whereas of the four dsRNAs of Cp, dsRNA-1 was the strongest and dsRNA-4 could only be seen in overloaded gels. This difference was consistent in our hands, regardless of age of infection or variability in growth conditions. The genomic RNA patterns of CMV-Hm and other strains of CMV were essentially identical. Four ssRNA species corresponding to three genomic RNAs and one subgenomic RNA (16) were observed (Fig. 5). No evidence was obtained for the association of a satellite RNA (Carna-5) with CMV-Hm, but one was observed readily in the ssRNA isolated from CMV-Nt80/3 (Fig. 5, lane d), as previously reported (8).

Molecular hybridization analysis. cDNA to CMV-Hm hybridized strongly with CMV-Q RNA (serotype To), weakly with genomic RNAs of CMV-Nt 80/3 (serotype D), Cp, and CpRSV, and not at all with healthy plant RNA, under high stringency conditions (Fig. 6).

DISCUSSION

We report, for the first time, natural infection of *H. matronalis* by CMV and RMV. Moreover, this is only the second report of naturally occurring TuMV in British Columbia; it was first reported in this province in rhubarb in 1967 (31). We suspect that it could be present in other hosts, possibly weeds.

TuMV-Hm is similar to isolates obtained from other crucifers and described by Evans (14) as nonpathogenic to *N. glutinosa*. However, TuMV-Hm did infect inoculated leaves of *N. glutinosa* (inducing slight depressions at the inoculation site). Moreover, TuMV-Hm seems to differ from the TuMV isolate observed in rutabaga (*Brassica napobrassica* [= *B. napus* var. *napobrassica* (L.) Reichb.]) in Ontario in 1946 (6), a not-unexpected conclusion. The Hm isolate infected Pe-Tsai (*B. pekinensis* (Lear) Rupn.) and Tendergreen with difficulty (causing only local depressions on the inoculated surface), did not systemically infect *N. glutinosa*, and induced leaf distortion in addition to mosaic in another ornamental crucifer (*Matthiola incana* (L.) R. Br.). No mention was made by Berkeley and Weintraub (6) of difficulty in infecting Pe-Tsai with the Ontario isolate; *N. glutinosa* exhibited local and systemic symptoms, and both primary and systemic ring lesions or spots were noted in *Matthiola*. Tompkins (33) reported a

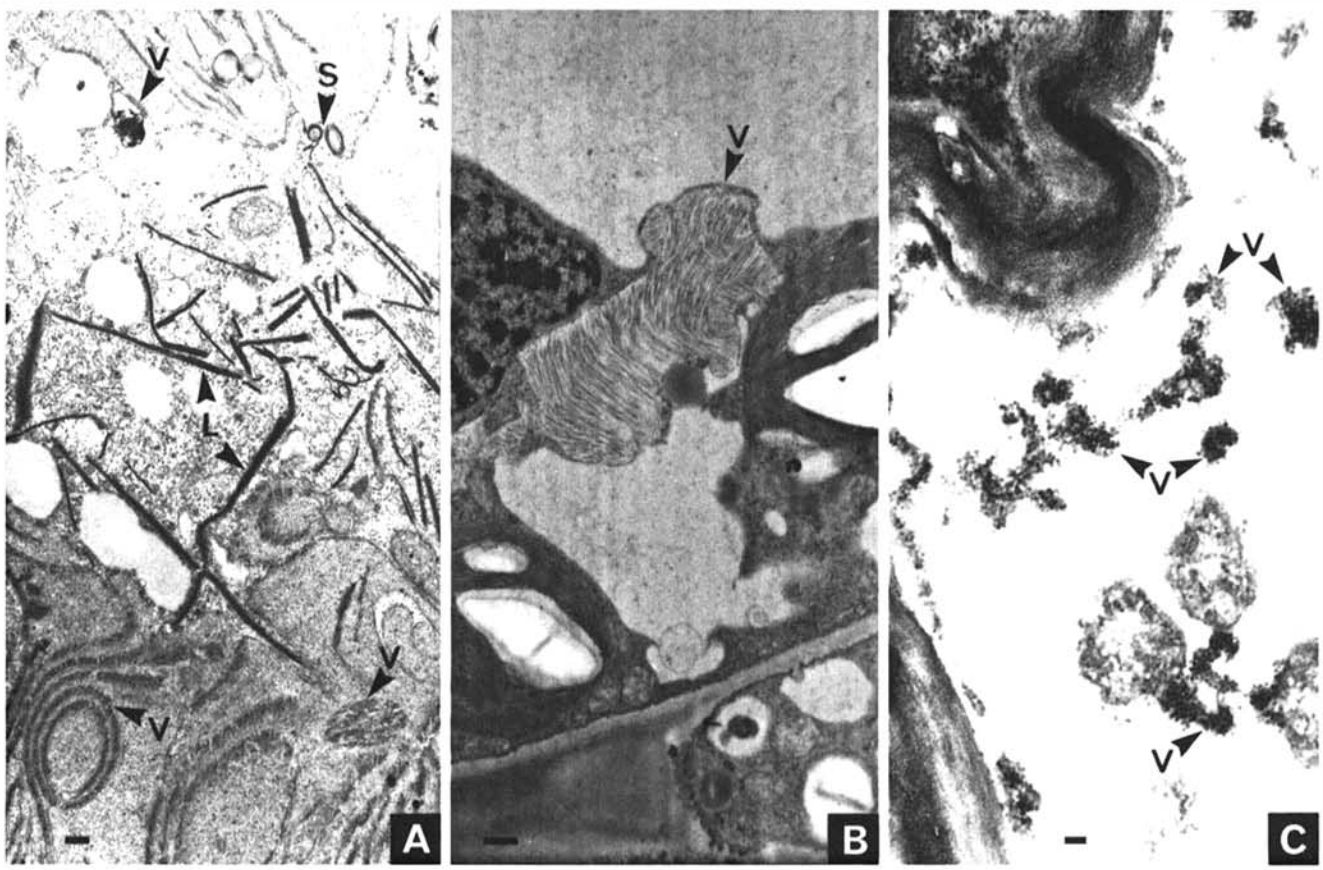

Fig. 3. Virus particles in leaf cells of *Hesperis matronalis*: **(A)** turnip mosaic virus-infected cell showing scroll-like (S) and laminar aggregate (L) inclusion bodies and virions (V), **(B)** ribgrass mosaic virus-infected cell showing large aggregate of virions (V) in cytoplasm, and **(C)** cucumber mosaic virus-infected cell showing small aggregates of virions (V) in cytoplasm. Scale bar = 100 nm.

color-breaking symptom in petals of naturally infected *Matthiola*, later shown to be infected with TuMV (34). Moreover, McWhorter (22) probably had observed TuMV-infected *H. matronalis*, although CMV could have been involved.

The cytoplasmic inclusion bodies in sections of *H. matronalis* leaves infected with TuMV-Hm appear to be identical to those observed in *B. perviridis* infected with another TuMV isolate (TuMV-D) from *H. matronalis* in Ontario (2U).

The properties of RMV-Hm will be reported elsewhere (L. Beczner et al, *unpublished*). Some of its properties differ from those of other described strains. For example, RMV-Hm induced local lesions and only rarely became systemic in *N. tabacum* 'Samsun' and 'Haronova,' which support systemic infection of most of the tobamoviruses, including the described RMV isolates. Although we have a record of the Iowa tobamovirus in electron micrographs of leaf-dip preparations from leaves of *H. matronalis* showing color-added flower-breaking and leaf mosaic symptoms, it was not further characterized and the isolate is not available. Close examination of the electron micrographs suggests that CMV-like particles may also have been present. The erratic isolation of RMV-Hm from *H. matronalis* doubly infected with CMV may be due to the repressive effect of CMV on the replication of RMV, as has been reported for double infections of CMV and TMV (19).

CMV, which probably has the largest host range of the known plant viruses (29), often infecting without symptoms, was isolated for the first time from naturally infected *H. matronalis*. Those plants were showing color break in the flowers, but the role of CMV in the induction of this symptom in this host is not known. Petal color-break patterns, either color-added or color-subtracted, may be diagnostic for certain viruses including CMV (21,30). For example, brighter symptoms of viola mottle occurred in *Viola odorata* coinfected with CMV and viola mottle virus (VMV) than in plants infected with VMV alone (17). In our study, CMV infection did not cause symptoms in *H. matronalis* maintained in the greenhouse for 2 mo after inoculation, but mild mosaic symptoms appeared by 3 mo in a few of the infected plants. Several reports indicate that CMV is symptomless in many hosts including *H. matronalis* (5,29); however, Pound and Walker (24) observed that the bright yellow symptoms of CMV infection in mechanically inoculated *H. matronalis* did not occur until 2 mo after infection.

The Hm isolate of CMV is the B strain (To serotype) based on published descriptions of the ringspot or etched-ring symptoms in Xanthi tobacco (25,26), the strong reaction with To antiserum (10) and the intense spur formation between CMV-D and CMV-Hm and corresponding antisera in reciprocal immunodiffusion tests. The cDNA data also support this conclusion as judged by the strong hybridization between CMV-Hm cDNA, and CMV-Q RNA (Fig. 6).

A new isolate of CMV, CMV-Hm2, was separated from a complex of CMV-Hm and RMV-Hm in Haronova and Samsun tobacco, in which it caused severe stunting, necrosis, and near death. It differed from the Hm isolate of CMV as follows: 1) it did not cause necrotic symptoms on inoculated leaves of Xanthi; 2) it caused pronounced white necrotic ringspot local lesions with several concentric rings in *N. clevelandii* A. Gray; and 3) it occasionally caused necrotic local lesions on bean (*Phaseolus vulgaris* L. 'Bountiful' and 'Black Turtle Soup'). The new CMV isolate also differed from the typical CMV-Hm in inducing brown necrotic local lesions in French bean and many more local lesions in *N. clevelandii*. However, its serology and dsRNA species were identical to those of CMV-Hm (Fig. 4, lanes a and j).

Although differences were found in the intensity of staining among the dsRNA species of the CMV strains, the limited number of CMV strains and other cucumoviruses that were compared precludes concluding that dsRNA analysis is a general method of distinguishing strains and/or members of this group. However, the method seems applicable for distinguishing members and strains of some groups of helical viruses (35).

Seed transmission in *H. matronalis* of both CMV and TuMV may be possible since they were isolated from various flower parts. If at all, chances for seed

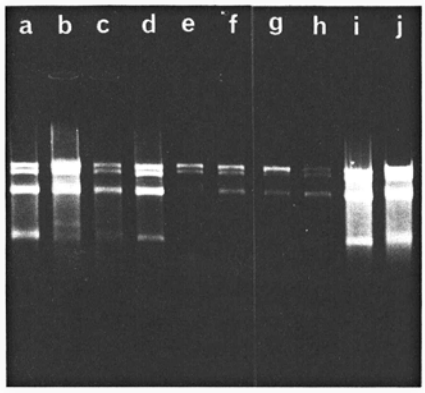

Fig. 4. Agarose gel (0.6%) electrophorogram of dsRNA isolated from tissues infected with cucumoviruses. Lanes a and j = cucumber mosaic virus (CMV) isolate Hm2, lanes b and e = tomato aspermy virus (TAV), lanes c and h = CMV-Hm, lane d = peanut stunt virus (PSV-Tp), lane f = cowpea ringspot virus (CpRSV), lane g = cowpea seed-transmitted virus (Cp), and lane i = CMV-Vf13. Gel was loaded with approximately equivalent amounts of dsRNA (about 1 μg); electrophoresis was for 2 hr at 20 mA, 40V (constant); staining was with ethidium bromide.

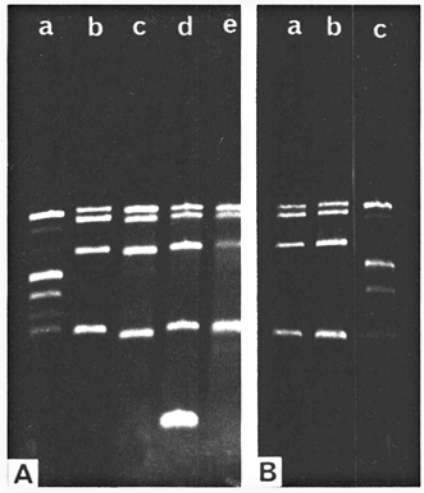

Fig. 5. Agarose gel (0.6%) electrophorograms of ssRNA isolated from purified cucumoviruses: **(A)** lane a = M_r standards (spinach leaf RNA of 2.9, 2.6, 2.0, 1.56, 1.3, and 1.0 kbar); lane b = cucumber mosaic virus (CMV) isolate Hm; lane c = CMV-Vf13; lane d = CMV-Nt80/3; and lane e = cowpea seed-transmitted virus (Cp). **(B)** Lane a = CMV-Hm; lane b = CMV-Q; and lane c = spinach leaf RNA (as in A). Gels were loaded with equivalent amounts of RNA (100 μg); electrophoresis was for 1 hr at 3.5 mA, 10V (constant); staining was with ethidium bromide.

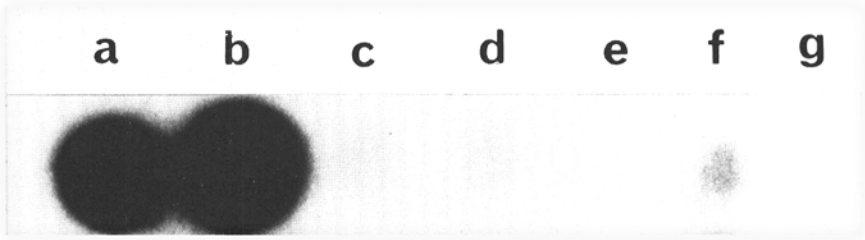

Fig. 6. Dot-blot hybridization between ^{32}P-labeled cDNA to cucumber mosaic virus (CMV) isolate Hm and RNA of various cucumoviruses immobilized on nitrocellulose: a = CMV-Q; b = CMV-Hm; c = CMV, D serotype; d = cowpea seed-transmitted virus (Cp); e = PSV-Tp; f = cowpea ringspot virus (CpRSV); and g = tomato aspermy virus. Nitrocellulose was spotted with 100 ng of viral RNA and hybridized with labeled probe at 42 C in 50% formamide, 1 M NaCl for 16–20 hr, and then washed in 2× SCC (1× SSC is 0.15 M NaCl, 0.015 M Na citrate) at 60 C and successively lower concentrations of SSC to 0.1× SSC at 60 C. Exposure for autoradiography was for 7 days at −85 C.

transmission seem more likely for CMV than for either TuMV or RMV because CMV was recovered from ovules at all stages of development. However, in our test sample, no seed transmission of CMV was observed in seedlings grown from mature ovules. *H. matronalis*, because of its perennial habit in areas where it occurs commonly, may provide a reservoir of CMV or TuMV, thus causing potential disease problems in localized and concentrated vegetable-growing regions (12,39). However, we have no experimental evidence of the natural movement of any virus from *H. matronalis* to vegetable crops.

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