# A Severe Mosaic of Cucumbers in Lebanon Caused by Watermelon Mosaic Virus-1

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**ABSTRACT**

MAKKOUK, K. M., and D.-E. LESEMANN. 1980. A severe mosaic of cucumbers in Lebanon caused by watermelon mosaic virus-1. Plant Disease 64:799-801.

Watermelon mosaic virus-1 (WMV-1) was isolated from cucumber leaves with severe mottling, blistering, and malformation. The virus was readily mechanically transmitted and induced local lesions without systemic spread on *Chenopodium amaranticolor* and *C. quinoa* and systemic infection in cucumber, squash, pumpkin, and watermelon. The virus was transmitted by the green peach aphid, *Myzus persicae*, in the stylet-borne manner. In SDS-immunodiffusion tests, the virus isolate reacted with WMV-1 but not WMV-2 antiserum. Electron microscopy of negatively stained extracts from infected pumpkin revealed flexuous particles 750–800 nm long. Using the Derrick technique of immune electron microscopy, we observed strong specific trapping when grids were coated with WMV-1 antiserum, but no trapping with WMV-2 or bean yellow mosaic virus antisera. Using the decoration technique of immune electron microscopy, we observed a strong effect with WMV-1 antiserum, a weak effect with bean yellow mosaic virus antiserum, and no effect with WMV-2 antiserum. Examination of ultrathin sections of infected pumpkin leaves revealed pinwheel and scroll inclusions similar to those reported for WMV-1.

Additional key words: cucurbit virus, potyvirus

Cucumber and squash are among Lebanon's most important vegetables; in fact, cucumbers are the leading Lebanese greenhouse crop for both domestic and export markets. We began in 1977 to identify a virus disease causing serious losses to cucurbits.

Watermelon mosaic viruses (WMV) are known to affect cucurbit crops all over the world (1,4–6,10,13,15,18,19). Two viruses, WMV-1 and WMV-2 (20), often called strains, have been encountered most frequently. WMV-1 and WMV-2 can be distinguished by host range, serology, and cytopathology (3,9,14,20). Recent work, however, indicates that WMV isolates should be classified into at least three groups (6,14,16,20).

In Lebanon, cucumber mosaic virus has been reported to affect cucurbits (12). We report the isolation of WMV-1 from cucumbers in Lebanon and present some evidence on the relationship of WMV-1 to WMV-2 and bean yellow mosaic virus.

Partial contribution of the Faculty of Agricultural and Food Sciences, American University of Beirut, as Journal Article 540B.

This study was supported in part by a grant from the Deutscher Akademischer Austauschdienst to the senior author.

0191-2917/80/08079903/$03.00/0


## MATERIALS AND METHODS

**Host range.** Infected cucumber leaves with severe mottling, blistering, and malformation served as the original inoculum source. The virus was maintained in the greenhouse in *Cucurbita pepo* L. 'Small Sugar.' Mechanical inoculations using crude extracts prepared in 0.01 M phosphate buffer, pH 7.2, containing 2.5% Celite, were made in the following plant species: *Capsicum annuum* L. 'Yolo Wonder,' *Chenopodium amaranticolor* Coste & Reyer, *C. quinoa* Willd., *Cucumis sativus* L. 'Beit Alpha,' *Citrullus vulgaris* Schrad. 'Crimson Sweet,' *Cucurbita pepo* 'Small Sugar,' *Datura stramonium* L., *Gomphrena globosa* L., *Lupinus albus* L. local cultivar, *Lycopersicon esculentum* Mill. 'Maramande,' *Nicotiana clevelandii* Gray, *N. glutinosa* L., *N. tabacum* L. 'Havana 423' and 'Xanthi-nc,' *Petunia hybrida* Vilm., *Phaseolus vulgaris* L. 'Bountiful,' *Physalis floridana* Rydb., *Pisum sativum* L. local cultivar, *Vicia faba* L. local cultivar, *Vigna unguiculata* Savi. 'California Black Eye No. 5.'

**Insect transmission.** Transmission tests were done with nonviruliferous, apterous *Myzus persicae* (Sulzer) adults raised on radishes. Aphids were starved for 2 hr,

transferred to infected pumpkin leaves to feed for 1 min, and then moved to healthy cucumber and squash plants for inoculation feeding of 1–2 min.

**Serology.** Antisera to WMV-1, WMV-2, cucumber mosaic virus, and squash mosaic virus were provided by D. E. Purcifull, University of Florida; antiserum to bean yellow mosaic virus by R. Koenig, Braunschweig, Federal Republic of Germany; and antiserum to zucchini yellow fleck virus by C. Vovlas, Bari, Italy.

Plates for immunodiffusion tests were prepared by dissolving in water 0.8% Noble agar, 0.5% sodium dodecyl sulfate (SDS), and 1% sodium azide. Crude extracts of infected tissue placed in the peripheral wells were extracted in 1 or 1.5% SDS. D. E. Purcifull performed the SDS-immunodiffusion tests.

**Electron microscopy.** For detection of particles, crude extracts from infected pumpkin leaves were negatively stained with 2% uranyl acetate. A Siemens Elmiskop 1a electron microscope was used to study the cytology of infected cells in ultrathin sections from infected pumpkin leaves embedded in Epon as described previously (17).

**Immune electron microscopy.** Two methods were employed. In the Derrick technique (2,7), grids were coated with antiserum diluted 1:1,000 before incubation with the virus suspensions; in the decoration technique (11), antiserum diluted 1:10 was incubated with virus particles adsorbed to the grids.

## RESULTS

**Host range.** The virus produced local lesions on *C. quinoa* and *C. amaranticolor* without systemic spread and systemically invaded all cucurbit species tested. Symptoms were more severe on squash than on cucumber. None of the noncucurbitaceous hosts showed symptoms.

**Insect transmission.** The virus was transmitted in the stylet-borne manner by *M. persicae*. All 10 plants of both cucumber and squash were infected when 10–15 aphids per plant were used.

**Serology.** No reaction was obtained between our isolate and WMV-2 (Florida), cucumber mosaic virus, squash mosaic virus, or zucchini yellow fleck virus antisera when crude extracts of infected leaf tissue were used in SDS-immunodiffusion tests. A precipitin line was observed only when our virus isolate was reacted with WMV-1 (Florida) antiserum; the precipitin line fused with that of the homologous antigen without spur formation (Fig. 1).

**Electron microscopy.** Filamentous particles approximately 750–800 nm long were observed in crude extracts of infected tissue. Examination of sections of infected pumpkin leaves showed pinwheel and scroll inclusions (Fig. 2A,B) but no laminated aggregates. Amorphous, electron-dense masses (Fig. 2C) were seen occasionally in the cytoplasm of infected cells, as reported previously (3,8,16).

**Immune electron microscopy.** Using the Derrick technique, the number of virus particles trapped on grids coated with WMV-1 antiserum was much greater than the number trapped by normal serum, WMV-2 antiserum, or bean yellow mosaic virus antiserum (Table 1). Using the decoration technique, particles of the cucurbit virus were heavily decorated with WMV-1 antiserum (Fig. 3A), less decorated with bean yellow mosaic virus antiserum (Fig. 3B), and very slightly, if at all, decorated with WMV-2 antiserum (Fig. 3C) compared with normal serum (Fig. 3D).

**Table 1.** Quantitative assay of cucurbit virus particles trapped on grids coated with antisera to watermelon mosaic virus-1 (WMV-1), WMV-2, and bean yellow mosaic virus (BYMV)

| Grid coated with antiserum against: | Particles trapped[a] per 250 $\mu m^2$ | |
|---|---|---|
| | Experiment 1 | Experiment 2 |
| WMV-1 | 230 | 129 |
| WMV-2 | 14 | 2 |
| BYMV | ...[b] | 4 |
| Normal serum | 10 | 5 |

[a] Means from 50 random viewing fields on each of duplicate grids; × 40,000 magnification.
[b] Not tested.

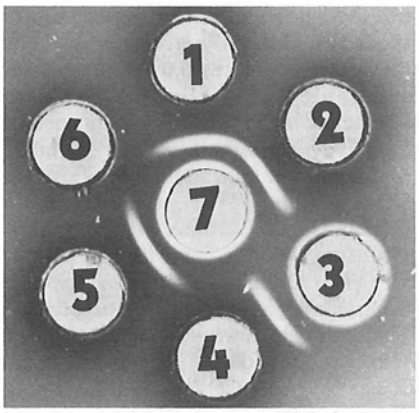


**Fig. 1.** Serologic relationship of watermelon mosaic virus-1 (WMV-1, Florida) and the cucurbit virus from Lebanon. The antigens were freshly prepared or lyophilized leaf extracts prepared in sodium dodecyl sulfate. The wells contain: 1, WMV-1 (Florida); 2, cucurbit virus (Lebanon); 3, WMV-2 (Florida) antiserum; 4, WMV-2; 5, cucurbit virus (Lebanon); 6, healthy *Cucurbita pepo*; 7, WMV-1 (Florida) antiserum.

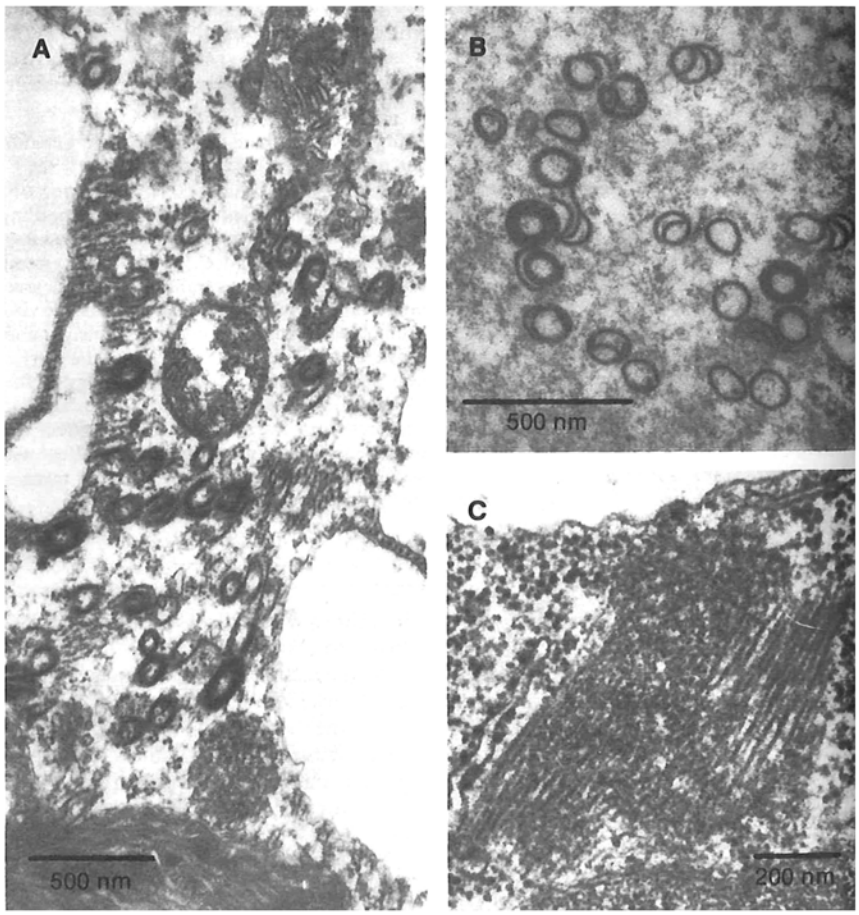


**Fig. 2.** Ultrathin sections of pumpkin leaves infected with the cucurbit virus from Lebanon, showing cytoplasmic pinwheel, tube, and scroll inclusions **(A,B)**, which in **(A)** are associated with filamentous particles, and **(C)** electron-dense amorphous inclusion associated with filamentous particles.

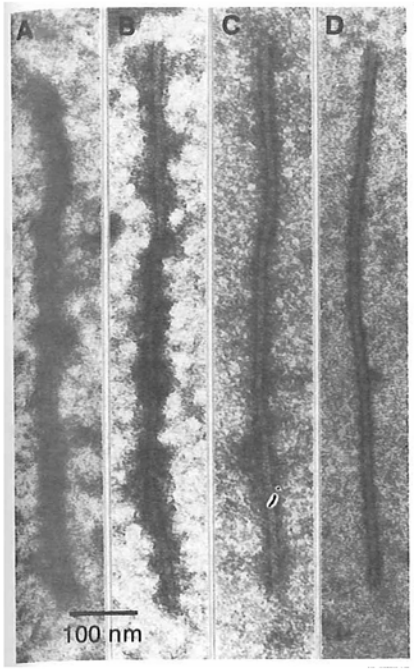


**Fig. 3.** Decoration test with virus particles in crude extracts of cucumber leaves infected with the cucurbit virus from Lebanon, using **(A)** watermelon mosaic virus-1 (WMV-1) antiserum, **(B)** bean yellow mosaic virus antiserum, **(C)** WMV-2 antiserum, and **(D)** normal serum.

## DISCUSSION

Based on host range, immunodiffusion tests, immune electron microscopy, and structures of induced cellular inclusions, the curcurbit virus was identified as WMV-1. This is the first report of WMV-1 occurring on cucurbits in Lebanon.

Many studies have classified WMV isolates into either WMV-1 or WMV-2 (9,19,20). Purcifull and Hiebert (14) in a recent investigation of serologic distinctions between WMV isolates from different sources suggested that such classification may not adequately define all WMV isolates. Milne and Grogan (9), reporting a close serologic relationship between WMV-1 and WMV-2, considered them strains of one virus, but earlier (20) and later (14,16) researchers found that WMV-1 and WMV-2 are not serologically related. Immunodiffusion tests and immune electron microscopy indicated that WMV-1 from Lebanon is serologically distinct from WMV-2 from Florida. The immune electron microscopy studies using the Derrick technique showed that WMV-2 (Florida) is not more closely related to WMV-1 (Lebanon) than to bean yellow mosaic virus, and the decoration technique indicated that bean yellow mosaic virus could be more closely related to WMV-1 (Lebanon) than to WMV-2 (Florida).

Our results support the view (6,16,20) that WMV-1 and WMV-2 are two separate viruses; this view is corroborated by direct serologic comparison of WMV-1 and WMV-2 and also by the different heterologous relatedness of both to other potyviruses (14). The two virus types are clearly separated cytopathologically because of the kind of cylindrical inclusions induced (3) and the amorphous cytoplasmic inclusions (16). Edwardson (3) grouped WMV-1, WMV-2, and bean yellow mosaic virus in separate subdivisions of the potyvirus group. Our observations support the view that "retention of the same name (watermelon mosaic virus) for two different viruses seems hardly justified" (16).

### ACKNOWLEDGMENT

The excellent technical assistance of Ursula Herzberg is gratefully acknowledged.

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