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Ultrastructural Aspects of the Mixed Infections of Watermelon Mosaic Potyvirus Isolated from Pumpkin and Cucumber Green Mottle Mosaic Tobamovirus from Watermelon

Jeom Deog Cho[1], Jeong Soo Kim[2]*, Hong Soo Choi[3], Yong Jun La[1] and Kyung Soo Kim[4]

[1]*School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea*
[2]**Vegetable Breeding Division, National Horticultural Research Institute, Suwon 441-440, Korea*
[3]*Plant Pathology Division, National Institute of Agricultural Sciences and Technology, Suwon 441-707, Korea*
[4]*Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, USA*

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Mixed infection of watermelon mosaic potyvirus II isolated from pumpkin (WMV-P) and cucumber green mottle mosaic tobamovirus from watermelon (CGMMV-W) caused extremely severe symptoms such as progressive wilting and death of watermelon plants. Single infections of either WMV-P or CGMMV-W on the same hosts produced only vein clearing and/or mosaic on the upper leaves. In cells infected with WMV-P, potyvirus-characteristic inclusions of pinwheels, scrolls and cylindrical inclusions were present in the cytosol. Parallel arrays of virus particles in the tonoplast were also common. In cells infected with CGMMV-W, virus particles occurred as stacked-bands or scattered randomly in the cytosol and vacuoles in all type cells. Many cells also contained vesiculated mitochondria with fibril-containing vesicles. Cells infected mixedly with WMV-P and CGMMV-W contained structural features that were not observed in cells infected singly with the two viruses. A particle of WMV-P potyvirus was surrounded by evenly spaced nine particles of CGMMV-W tobamovirus, which made a unique nonagon ring. The angled layers having 60°-135° were alternating layer, herringbone, crosshatching and ladder figures.

Keywords: CGMMV, WMV, mixed infection, nonagon, angled layer aggregate

Specific arrangements of the different virus particles were reported between the related or unrelated different viruses in plant cells. The arrangements that might be concerned with the synergism of virus disease in symptom expression were hexagon, octagon and nonagon. Hexagon in bean dwarf disease was that one particle of bean yellow mosaic potyvirus was surrounded by 6 particles of cowpea mosaic comovirus (Carr and Kim, 1983). Octagon in cowpea stunt disease was figured that one particle of cucumber mosaic cucumovirus was surrounded by 8 particles of black eye cowpea mosaic potyvirus (Anderson et al., 1994). Nonagon in watermelon necrosis disease had the shape that one particle of watermelon mosaic potyvirus was surrounded by 9 particles of cucumber green mottle mosaic tobamovirus (Cho, 1998).

Nonagon, a specific ultrastructure produced in the mixedly infected cells of watermelon and cucumber plants, was induced by interaction of the two pathogens for WMV and CGMMV but not by host specificity. The host specificity of pathogen will be generally adopted in synergism, therefore, the relationships of the synergism and the specific ultrastructure will be studied when the isolate of WMV in double infection is changed.

Materials and Methods

Viruses used. Watermelon mosaic potyvirus was isolated from pumpkin (WMV-P) and it was used for the partner virus in mixed infection with cucumber green mottle mosaic tobamovirus (CGMMV-W) which used as a counterpart virus in mixed infection with WMV-W isolate (Cho, 1998).

Mixed infection. Four kinds of Cucurbits for watermelon, cucumber 'Jangilibchu', melon 'Euncheon' and pumpkin 'Aihobag' were used for the making of mixed infection. The cultivar of watermelon was 'Geumno' and 'Dalgona'. Mixed infection was made by mechanical inoculation with maceration of leaf samples for the same weight of pumpkin and cucumber infected singly with WMV-P and CGMMV-W in a 4 vol. of 0.01M Na-phosphate buffer, pH 7.0.

Electron microscopy. Infected tissues passed 2 weeks after inoculation were used for ultrastructural studies. Tissue samples, 1×3 mm^2, were taken from systemically infected leaves and fixed with 2.5% glutaraldehyde in Millonig's phosphate buffer, pH 7.3, for 2 hrs. Dehydration was done with 50-100% ethyl alcohol in six steps after treatment of 2% osmium tetroxide. The dehydrated tissues were embedded in Epon 812 and hardened at 60°C for overnight and then at 90°C for 2 hrs. Ultrathin sectioning of 80 nm

*Corresponding author.
Phone) +82-31-290-6178, FAX) +82-31-290-6100
E-mail) kjs@nhri.go.kr

Table 1. Symptomatology of WMV-P[a], WMV-W[b] and CGMMV-W[c] in the indicator plants

Plant used	Symptoms[d] of		
	WMV-P	WMV-W	CGMMV -W
Chenopodium amaranticolor	CS/–	CS/–	CS/–
C. quinoa	CS/–	CS/–	CS/–
Datura stramonium	–/–	–/–	–/–
Gomphrena globosa	–/–	CS/–	–/–
Physalis floridana	–/–	–/–	–/–
Nicotiana benthamiana	–/–	CS/M	–/M
N. clevelandii	*	–/–	–/–
N. glutinosa	–/–	CS/M	–/–
N. occidentalis	–/–	–/–	–/–
N. rustica	–/–	–/–	–/–
N. tabacum 'Ky-57'	–/–	CS/–	–/–
N. tabacum 'Bright yellow'	*	–/–	–/–
Tetragonia expansa	*	–/–	–/–
Cucumis sativus 'Jangilibchu'	–/SM,MAL	–/CS,SM	–/M
C. melo 'Euncheon'	–/SM,ST	–/CS,SM	–/M
Cucurbita moschata 'Aihobag'	–/VB,M	–/VB,M	–/–

[a] Watermelon mosaic potyvirus isolated from pumpkin.
[b] Watermelon mosaic potyvirus isolated from watermelon.
[c] Cucumer green mottle mosaic tobamovirus isolated from watermelon.
[d] CS; Chlorotic spot, M; Mosaic, SM; Severe mosaic, MAL; Malform, ST; Stunt, VB; Vein banding, –; Non reaction, *; Not test, Inoculated leaves/Upper leaves.

thickness was done with a diamond knife. The sections were stained with uranyl acetate and lead citrate for 5 min and 7 min, respectively.

Results

Characteristics of WMV and CGMMV. The reactions on the indicator plants for two different isolates of WMV from watermelon and pumpkin were compared in Table 1. WMV-P could infect locally *Chenopodium amaranticolor* and *C. quinoa,* and systemically Cucurbits. The symptoms of severe mosaic and malformation, or stunt on Cucurbits were produced, but vein banding was produced on the upper leaves of *Cucurbita moschata* 'Aihobag'. WMV-P could not produce any symptoms on *Gomphrena globosa, Nicotiana benthamiana* and so forth. However, WMV-W could infect *G. globosa, N. benthamiana, N. occidentalis* and so forth. Host range of WMV-W was wider than that of WMV-P, however, the pathogenicities of WMV-P on Cucurbits as the symptoms of malformation and stunt were severer than those of WMV-W. CGMMV-W could produce the mosaic symptom on *Citrullus vulgaris, Cucumis sativus* and *C. melo,* however, it could not produce any symptoms on *Cucurbita moschata* 'Aihobag' (Table 1).

Symptom expression by single and mixed infection

Watermelon. The symptoms of wilt, bud necrosis and plant death were shown at 5-7 days after double infection with WMV-P and CGMMV-W (Fig. 1A). WMV-P produced vein clearing and mosaic symptoms on the upper leaves at 7 days after inoculation, followed by severe mosaic symptom (Fig. 1B). CGMMV-W produced mild mosaic symptom on the upper leaves (Fig. 1C). Healthy plant was shown in Fig. 1D.

Melon. The cultivar of 'Euncheon' infected mixedly with WMV-P and CGMMV-W was shown the various symptoms of vein clearing, severe mosaic, yellowing and wilt, followed by death (Fig. 1E). WMV-P produced severe mosaic and malformation on the upper leaves after showing chlorotic spots and vein clearing at 10 days after inoculation (Fig. 1F). However, CGMMV-W could produce only mild mosaic on the upper leaves at 7 days after mechanical inoculation (Fig. 1G).

Cucumber. Symptoms in singly and mixedly infected cucumbers were almost same. Mild mosaic was produced by CGMMV-W (Fig. 1J) and mosaic was produced by WMV-P at 10 days after mechanical inoculation (Fig. 1I), however, the doubly infected cucumbers were stunted slightly with mild mosaic symptom (Fig. 1H).

Pumpkin. Vein banding was produced on the upper leaves of 'Aihobag' at 8 days after mechanical inoculation with WMV-P. In the double infection with WMV-P and CGMMV-W, no synergistic symptoms were produced because CGMMV-W could not produce any symptoms.

Ultrastucture of WMV-P single infection. WMV-P produced the typical cytoplasmic inclusions of pinwheels, scrolls, laminated aggregates and tubes in cells of watermelon, melon and cucumber. Virus particles of WMV-P presented at inner part of scroll or pinwheel inclusions and tonoplast (Cho, 1998).

Ultrastructures of CGMMV-W single infection. CGMMV-W had the typical ultrastuctures for tobamovirus as the stacked-band structure in cytosol and also caused the membrane vesiculation of mitochondria. In xylem vessels, the virus particles presented lineally or dispersedly. The typical ultrastructures of CGMMV-W were shown in the cells of watermelon, melon and cucumber (Cho, 1998).

Ultrastructures of WMV-P and CGMMV-W mixed infections. The two unrelated viruses made a specific arrangement of nonagon, one particle of WMV-P potyvirus in center and 9 particles of CGMMV-W in round. They shared two CGMMV-W particles with the adjacent nonagons (Fig. 2A). The nonagons were formed in the central area of CGMMV-W crystal that sectioned parallel with the axis of virus particles. The large number of nonagons was formed near or in the stacked-band structure of CGMMV-W in cytosol and vacuoles, and also produced at inner part of scroll inclusions that were the specific ultra-

Fig. 1. Watermelon of 'Geumno' cultivar infected mixedly with two different viruses of WMV-P and CGMMV-W was killed at 5-7 days after mechanical inoculation (A). In single infection of WMV-P, the virus produced mosaic symptom on the upper leaves (B). The symptoms produced by CGMMV-W were difficult to judge because it was very mild mosaic (C) when compared with the healthy plant (D). The mixed virions of WMV-P and CGMMV-W killed the melon 'Euncheon' cultivar (E), but WMV-P produced mosaic symptom on the upper leaves after producing vein clearing and chlorotic spots on the inoculated leaves (F). On cucumber 'Jangilibchu', no synergistic symptom was produced (H) and WMV-P produced mild mosaic symptom on the upper leaves (I). CGMMV-W caused the mild mosaic symptom on the upper leaves of melon (G) and cucumber (J).

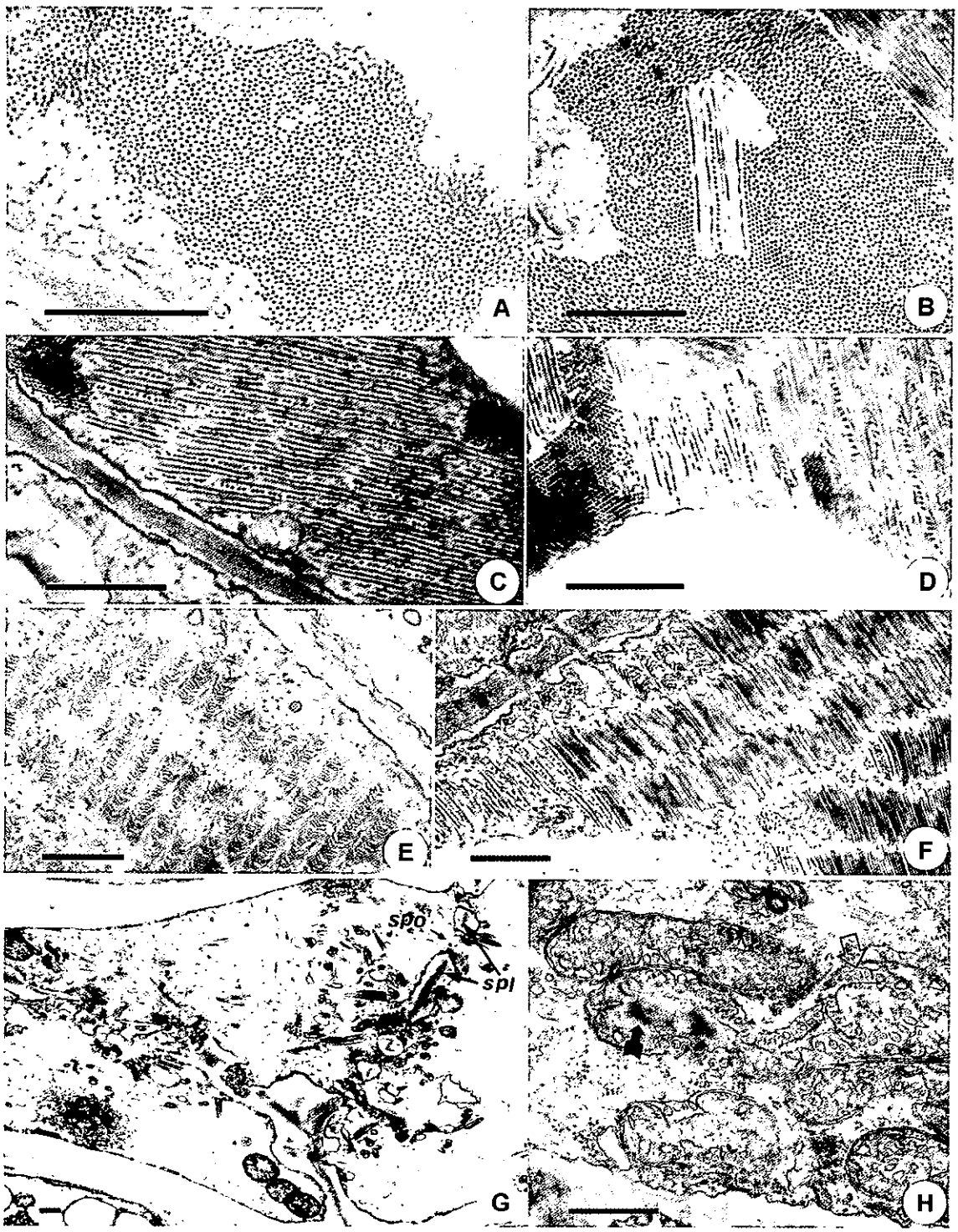

Fig. 2. Two unrelated viruses of WMV-P and CGMMV-W made nonagons, a particle of WMV-P in center and 9 particles of CGMMV-W in round (A). Alternating layers of 1:3 were presented in inner part of nonagon crystal (B) and cytosol (C). Herringbone structure having 75°-90° between two virus particles formed from alternating layers by sections (D). Ladder figures having 120°-135° were made by 3 particles of tobamovirus (E). The four different structures of angled-layer aggregates were observed simultaneously at the ends of the stacked-band structures for CGMMV-W (F). Sieve tubes for watermelon infected mixedly had the potyvirus inclusions of pinwheel scrolls, tobamovirus particles, and nonagons (G). CGMMV-W caused severe vesiculation (empty arrow) on mitochondrial membrane (H). The mitochondria had the virus particles of CGMMV-W sectioned crossly (black arrow in H). Bars represent 500 nm.

Table 2. Symptom development by WMV-P, CGMMV-W or their mixed inoculum

Plant	Symptoms[a] produced by		
	WMV-P (W)	CGMM V-W (C)	W+C
Citrullus vulgaris 'Geumno'	–/VC,M	–/MM	–/W,D
C. vulgaris 'Dalgona'	–/VC,M	–/MM	–/W,D
Cucumis melo 'Euncheon'	CS,VC/VC,M	–/MM	–/VC,SM,W
C. sativus 'Jangilibchu'	–/VC,MM	–/MM	–/ST,MM
Cucurbita moschata 'Aihobag'	–/VC	–/–	–/ST,VB

[a] VC; Vein clearing, M; Mosaic, MM; Mild mosaic, SM; severe mosaic, W; Wilt, D; Death, CS; Chlorotic spot, VB; Vein banding, ST; Stunt, –; Non reaction, Inoculated leaves/Upper leaves. Symptoms were investigated on 6 plants each treatment at 7-10 days after mechanical inoculation.

structures produced only by the potyvirus (Adwardson et al., 1994). Another ring structure was made by 9-12 virus particles of CGMMV-W surrounded a closed single-layered ring of potyvirus inclusion.

In mixed infections of WMV-P and CGMMV-W, two types of aggregations were observed. The first one was the stacked-band structure and the other structure was angled-layer aggregates. The two different structures were located simultaneously in a cell. The ultrastructures of angled-layer aggregates (ALA) in this thesis have not been observed in the single infection of CGMMV-W.

ALA was classified as four types morphologically by the orientation of sections. The first one was the alternating layer; one layer of cross sectioned virus particles and 3 or 4 layers (mostly 3 layers) of parallel sectioned virus particles for CGMMV tobamovirus (Fig. 2B, 2C). Second type was the herringbone figure that two tobamovirus particles had 75°-90° between layers of virus particles. This structure was presented with the alternating layers at the margin of itself (Fig. 2D). Third one was crosshatching figure. The two virus particles crossed one horizontal particle with 120° as symmetrical figure. The last figure of ALA was ladder made by three particles (Fig. 2E). The angle was 120°-135°. The ladder figures were also presented between two parallel sectioned virus particles. The four kind figures of ALA related with the stacked-band structure and those were shown at the end of the stacked-band structure of tobamovirus (Fig. 2F).

Tobamovirus particles and potyvirus inclusions were easily observed in sieve tubes. Those were located abundantly at the near of sieve plates (spl), and there was shown as little problem in translocation through the sieve pores (spo) of sieve plates. The nonagons also presented in sieve tubes with potyvirus inclusions (Fig. 2G).

The mitochondrial vesiculation (arrow) was heavier in the mixed infection of WMV-P and CGMMV-W (Fig. 2H) than that in single infection. The vesiculated mitochondria also had the tobamovirus particles sectioned vertically and parallel with the axis of virus particle (black arrow in Fig. 2H).

Discussion

WMV-P potyvirus particles in or near potyvirus inclusions were mostly substituted for CGMMV-W tobamovirus particles in the mixed infection. The proteins of potyvirus inclusions were involved in the replication of coat proteins and nucleic acid of the potyvirus particles (Ammar et al., 1994). Therefore, from the close affinity of two unrelated viruses, the potyvirus inclusions might be a role of helper component in the replication of tobamovirus, or *vice versa*. The nonagon of 1:9 arrangement was formed at inner parts of scroll potyvirus inclusions and small ring tubes having single layer. The nonagons in scroll and small ring might be made at the same time with the formation of potyvirus inclusions.

Among tobamoviruses, CGMMV and cucumber virus 4 had the ultrastructural characteristics of mitochondrial membrane vesiculation (Hatta and Ushiyama, 1975; Hollings et al., 1975). The membrane vesiculation of mitochondria in the mixed infection with CGMMV-W and WMV-W from watermelon was occurred slightly with embedding virus particles of CGMMV-W (Cho, 1998). However, CGMMV-W produced severe membrane vesiculation on mitochondria in cells infected mixedly with the pumpkin isolate of WMV-P in this paper.

Virus particles in the ALA sectioned with various orientations might be made by an angle of 120°-135° or 45°-90°. The ALA had been reported in acuba strain of tobacco mosaic virus (TMV) (Warmke, 1974), U5 strain of TMV (Shalla, 1968), peanut clump virus (Thouvenel et al., 1981), beet necrotic yellow vein virus (Tomata, 1975) and odontoglossum ringspot virus (Francki et al., 1985). Despite the CGMMV-W did not induce the ALA ultrastructurally, the CGMMV-W in double infection with WMV-P produced the ALA, however, the CGMMV-W in double infection with WMV-W, watermelon isolate, did not induce the ALA (Cho, 1998). From this study, two unrelated viruses of WMV-P and CGMMV-W were influenced each other and the CGMMV-W might be changed in protein and/or nucleic acid compositions, followed by having the characteristics of ALA which was a specific ultrastructure of U5-TMV and so forth. The ALA of U5-TMV had the angles of 120° or 60° having 1:2 alternating layers for one layer of crossly sectioned virus particles and 2 layers of parallel sectioned virus particles, but ALA by WMV-P and CGMMV-W in this paper were ladder shape having 1:3 alternating

layers for transverse sections : parallel sections. The ALA in this paper was reported newly in the mixed infection and the difference with ALA reported previously was obscure.

The ultrastructures of nonagons, tobamovirus particles in potyvirus inclusions and so forth were observed in the cells of cucumber infected mixedly with WMV-P and CGMMV-W like in watermelon and melon. However, the mixedly infected cucumber did not produce the synergistically external symptoms. Actually CGMMV-W could infect cucumber without symptoms, but the cytopathic effects of CGMMV-W could be observed.

References

Ammar, E.D., Rodriguez-Cerezo, E., Shau, J.G. and Pirone, T.P. 1994. Association of virions and coat protein of tobacco vein mottling potyvirus with cylindrical inclusions in tobacco cells. *Phytopathology* 84:520-524.

Anderson, E.J., Kline, A.S., Kim, K.S., Socke. S.C. and Albriton, C.W. 1994. Identification of cowpea stunt disease in south central Arkansas. *Ark. Farm Res.* Vol. 43(2):14-15.

Carr, R.J. and Kim, K.S. 1983. Ultrastructure of mixed plant virus infection; bean yellow mosaic virus with cowpea severe mosaic virus or cowpea mosaic virus in Bean. *Virology* 124: 338-348.

Cho, J.D. 1998. Ultrastructural aspects of watermelon necrosis disease caused by mixed infection with watermelon mosaic potyvirus and cucumber green mottle mosaic tobamovirus. A thesis for the degree of Master of Science, Seoul National University.

Edwardson, J.R. 1974. Some properties of the potato virus Y group. Florida Agricultural Experiment Station, Monograph series 4, 398pp.

Francki, R.I.B., Milne, R.G. and Hatta, T. 1985. Atlas of Plant Viruses Vol. I. CRC Press, Boca Raton, FI. 222pp.

Hatta, T. and Ushiyama, R. 1975. Mitochondrial vesiculation associated with cucumber green mottle mosaic virus-infected plant. *J. Gen. Virol.* 21:9.

Hollings, S.M., Komuro, Y. and Tochihara, H. 1975. Cucumber green mottle mosaic virus. *CMI/AAB Description of plant viruses* No. 154.

Pio-Ribeiro, G., Wyatt, S.D. and Kuhn, C.W. 1978. Cowpea stunt: a disease caused by a synergistic interaction of two viruses. *Phytopathology* 68:1260-1265.

Shalla, T.A. 1968. Virus particles in chloroplasts of plant infected with the U5 strain of tobacco mosaic virus. *Virology* 35:194-203.

Thouvenel, J.C. and Fauquet, C. 1981. Peanut clump virus. *CMI/AAB Description of plant viruses* No. 235.

Tomata, T. 1975. Beet necrotic yellow vein virus. *CMI/AAB Description of plant viruses* No. 144.

Warmke, H.E. 1974. Direction of rotation of acuba (TMV) angled-layer aggregates. *Virology* 59:591-594.