Ultrastructural Cytology of Soybean Infected with Mild and Severe Strains of Soybean Mosaic Virus

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ABSTRACT

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Two soybean mosaic virus (SMV) strains from Virginia, VA/G1 and OCM/G3, and two SMV strains from Illinois, IL/G1 and IL/G3, were compared biologically and ultrastructurally in soybean (*Glycine max* 'Essex'). Strain VA/G1 induced severe mosaic symptoms on soybean and abundant pinwheel inclusions in the cytoplasm of infected cells. Strains OCM/G3, IL/G3, and IL/G1 induced extremely mild symptoms with both pinwheels and cytoplasmic strands. Cytoplasmic strands contained virus particles and traversed the vacuole of infected cells. The four strains were closely related in serological tests. In a time-sequence study, pinwheels were detected in leaves infected with all of the strains 9 days after inoculation. Cytoplasmic strands were first observed at 10 days in cells infected with strain OCM/G3 or IL/G3 and at 11 days with IL/G1. No cytoplasmic strands were observed in cells infected with the severe strain, VA/G1. Production of cytoplasmic strands was concluded to be an intracellular virus localization mechanism leading to the tolerant reaction of Essex soybean to the three mild strains of SMV.

Strains of soybean mosaic virus (SMV), differing in symptom expression on soybean (*Glycine max* (L.) Merr.) and in host range, have been reported since 1948 (2,4,11–15). Two SMV strains (VA and OCM), isolated in Virginia, were classified into the G1 and G3 strain groups, respectively (6,8), based on their virulence in resistant soybean cultivars (3). In Essex, a susceptible soybean cultivar, these two strains were distinguished by symptom severity and ultrastructural characteristics (8). The G1 strain, SMV-VA, induced typical severe mosaic symptoms and abundant pinwheels in the cytoplasm of infected cells, whereas the G3 strain, SMV-OCM, induced extremely mild symptoms, pinwheels, and cytoplasmic strands. These strands contained virus particles and traversed the vacuole of infected cells.

The objective of this study was to determine if other strains in these two groups induced similar symptoms and ultrastructural changes in Essex soybean, and if these changes were associated with strain group or symptom severity. A preliminary report was presented (7).

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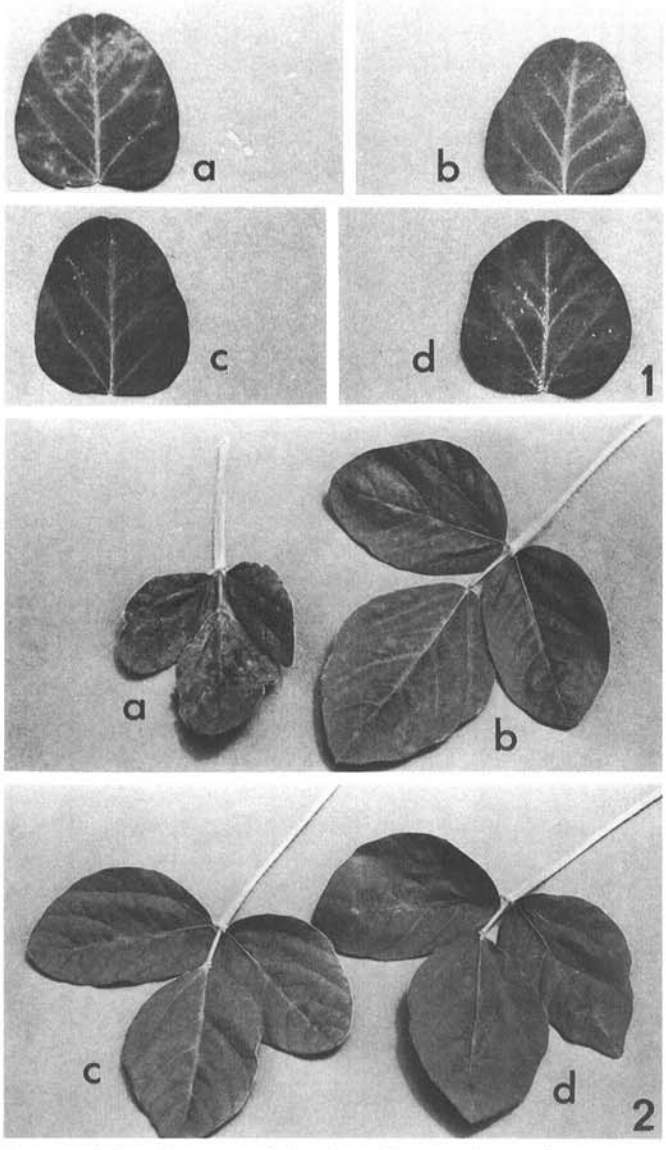
MATERIALS AND METHODS

Source and maintenance of the strains. The SMV-VA (VA/G1) and SMV-OCM (OCM/G3) strains were originally isolated in

Virginia and have been previously described as severe and mild strains on Essex soybean (6,8). Two SMV isolates were obtained from R. M. Goodman (University of Illinois, Urbana). One isolate (SMV-IL-019-2) was a representative of the G1 strain group (IL/G1) and the other isolate (SMV-IL-83-2) of the G3 strain group (IL/G3). All four strains were mechanically transmitted to Essex soybean plants as described previously (8) and were maintained in these plants under greenhouse conditions.

Serology. The virus strains were tested serologically in Ouchterlony double diffusion tests. The medium consisted of 0.6% Ionagar No. 2 (Oxo Limited, London, England) containing 0.2% sodium dodecyl sulfate, 0.55% sodium chloride and 0.1% sodium azide. Undiluted antiserum against SMV-VA, made in 1980, was used in all tests (8). Antigen was crude sap extracted by grinding soybean primary and trifoliolate leaf tissue in a mortar and pestle with an equal amount (w/v) of distilled water. Double diffusion tests were also conducted with twofold dilutions of the antigen extracts and undiluted antiserum against SMV-VA.

Electron microscopy. Inoculated Essex primary leaves were sampled for transmission electron microscopy daily for 9–14 days and at 28 days after inoculation. The samples were fixed and embedded as reported previously (8).



Figs. 1 and **2. 1,** Symptoms induced on Essex soybean primary leaves inoculated with soybean mosaic virus (SMV) strains. Local necrotic lesions present on leaf inoculated with **a,** SMV-VA/G1. Veinal chlorosis on leaves inoculated with **b,** SMV-IL/G1; **c,** SMV-OCM/G3; and **d,** SMV-IL/G3. **2,** Systemic symptoms induced on Essex soybean trifoliolate leaves by soybean mosaic virus (SMV) strains: **a,** SMV-VA/G1; **b,** SMV-IL/G1; **c,** SMV-OCM/G3; and **d,** SMV-IL/G3.

RESULTS

Biological characteristics of the SMV strains. All four strains were readily transmissible to Essex soybean plants. However, macroscopic symptoms induced in Essex by the strains were not identical. Strain VA/G1 induced vein-clearing on the first expanding trifoliolate leaf 6–7 days after inoculation. Necrotic lesions developed on the inoculated primary leaves within 8–10 days (Fig. 1). A severe mosaic and rugosity (Fig. 2) was observed on the trifoliolate leaves at later stages of infection. Infected plants were generally stunted. Plants inoculated with OCM/G3, IL/G3, or IL/G1 also developed vein-clearing on the first expanding trifoliolate leaf 6–7 days after inoculation. However, this symptom was transitory in this leaf and was followed by the development of an extremely mild mosaic on the first and subsequent trifoliolate leaves (Fig. 2). A faint veinal chlorosis developed on the inoculated primary leaves (Fig. 1). Stunting of the Essex plants was not evident.

Serology. In the Ouchterlony double diffusion tests, all four strains produced precipitin bands upon reaction with antiserum to SMV-VA. These bands were of comparable intensity and fused completely, indicating a close serological relationship between the strains. The dilution end point of all four strains was 1:8, indicating that the concentration of virus antigen in leaf tissues was relatively equal.

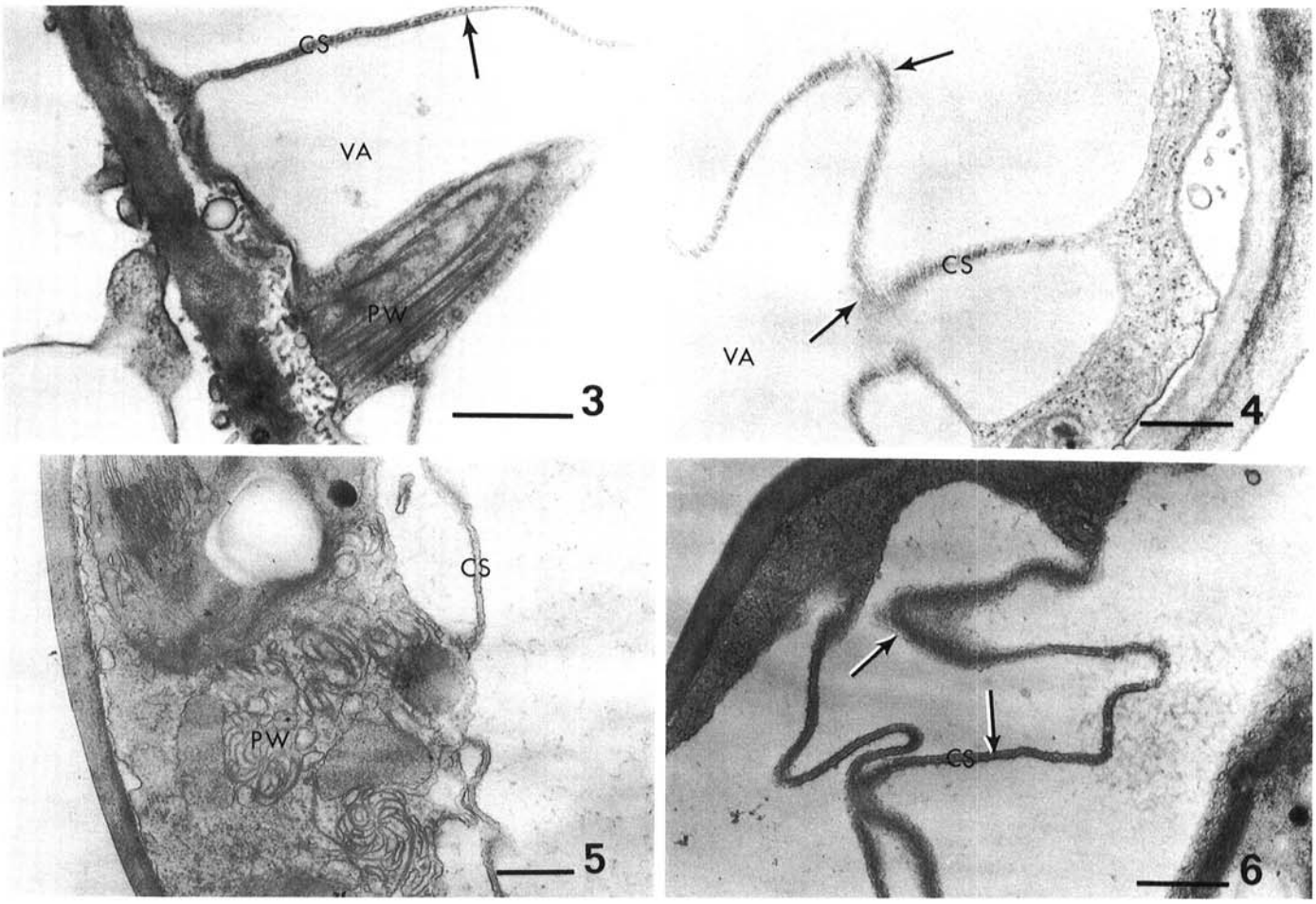
Ultrastructural characteristics. The ultrastructure of Essex primary leaf cells infected with VA/G1 and OCM/G3 28 days after inoculation has been described previously (10). Similar cells infected with either IL/G3 or IL/G1, sampled at 28 days, contained pinwheel cytoplasmic inclusion bodies in the form of scrolls or tubes (Fig. 3) and cytoplasmic strands (Figs. 3 and 4), which protruded into the vacuole of the cell. These strands contained viruslike particles (Fig. 4) and appeared similar to strands induced by OCM/G3 at 28 days (8).

A time-sequence study was conducted to determine when cytoplasmic strands were formed within infected cells of Essex primary leaves. Pinwheels were observed in cells infected with any one of the four strains at 9 days after inoculation and at all subsequent sampling times. Cytoplasmic strands were first observed in cells infected with OCM/G3 or IL/G3 at 10 days, but not until 11 days with IL/G1. Cytoplasmic strands induced by any of the three strains were observed in leaves sampled daily up to 14 days after inoculation, and did not appear to differ in structure from those present in primary leaves sampled at 28 days (Figs. 3 and 4). The strands were found in several cell types and ranged from short loops near the cytoplasm (Fig. 5) to extensive, convoluted structures, which, because of the observance of both transverse and longitudinally oriented virus particles, appeared to have an undulating form (Fig. 6).

Vesiculated areas, composed primarily of endoplasmic reticulum (ER), were also observed in the cytoplasm of cells infected with OCM/G3 (Fig. 7), IL/G3 (Figs. 8 and 9), and IL/G1 (Figs. 10 and 11). Pinwheels were frequently associated with this ER (Figs. 7–10). The pinwheels appeared to be forming from these areas, usually from one side of a double-membrane sac of ER (Figs. 7 and 9 [arrows]). In cells infected with the three mild strains, cytoplasmic strands were sometimes, but not always, observed near these pinwheel-forming areas (Fig. 9). Pinwheels observed next to the cell wall were frequently near areas of numerous small vesicles (plasmalemmasomes) between the cell wall and cytoplasm (Fig. 11). Cells infected with the severe strain, VA/G1, showed similar vesiculation and pinwheel-forming areas (Fig. 12), but there was no evidence of virus-containing cytoplasmic strands. Virus particles were frequently observed as masses in the cytoplasm (Fig. 12).

DISCUSSION

The four SMV strains produced two different symptom patterns



Figs. 3–6. Ultrastructure of Essex soybean primary leaf cells infected with soybean mosaic virus (SMV) strains. **3** and **4,** Mesophyll cell samples 28 days after inoculation with SMV-IL/G1, with pinwheel (PW) and cytoplasmic strands (CS) protruding into vacuole (VA). Virus particles (arrows) in **3,** cross and **4,** longitudinal section. **5,** Pinwheels (PW) and cytoplasmic strands induced by strain IL/G1, sampled 11 days after inoculation. **6,** Cytoplasmic strand (CS) in cross section and longitudinal section containing virus particles (arrows) induced by SMV-OCM/G3 in tissue sampled 10 days after inoculation. Bars = 0.5 μm.

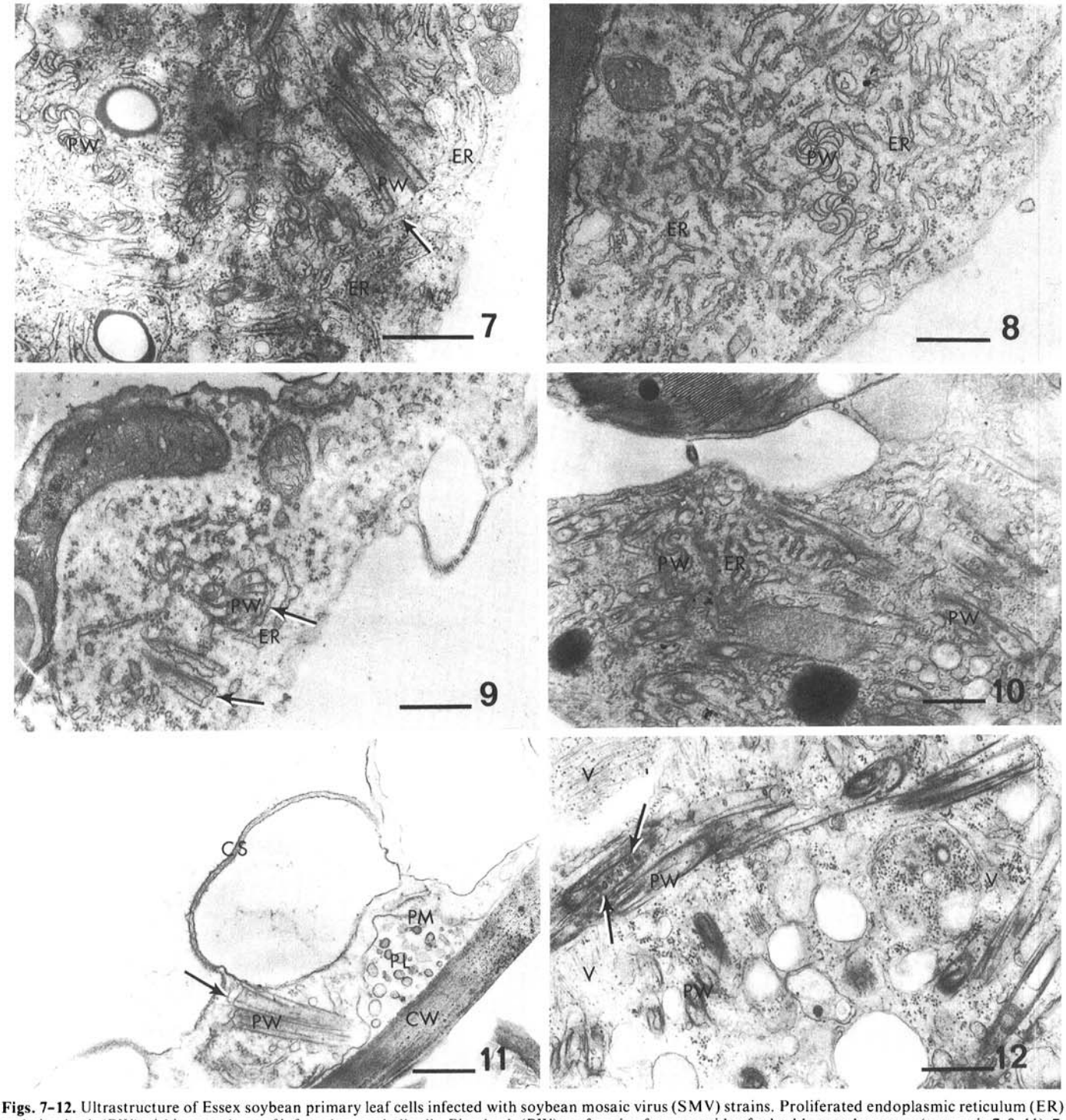
on Essex soybean plants. Initial vein-clearing on the first expanding trifoliolate leaf appeared consistently at 6–7 days after inoculation of Essex plants with each of the virus strains. However, VA/G1-infected plants developed severe symptoms on the trifoliolate leaves 8–9 days after inoculation, but plants infected with any of the other three strains developed only mild mosaic symptoms both at this time and later. No serological differences were detected between the four strains in double diffusion tests with antiserum to SMV-VA. A close relationship of the mild strains to the severe strain was also demonstrated by the inability of VA/G1 to induce severe systemic symptoms in Essex soybean plots previously infected with either OCM/G3, IL/G3, or IL/G1 (*unpublished*).

Symptom severity in susceptible Essex soybean plants was associated with cytoplasmic strand development. Cytoplasmic strand production with these strains is strain specific and not strain group specific, since only the severe strain, VA/G1, failed to induce strands, and strands were induced by members of both G1 and G3 strain groups.

Even though cytoplasmic strands have been reported for several potyviruses, their function has never been determined (1,9,16). Kitajima and Lovisolo (9) suggested that production of cytoplasmic strands within cells of *Datura* infected with henbane mosaic virus is a defense reaction of the infected cells. Weintraub and Ragetli (16) offered a similar suggestion that there may be fewer virions of carnation vein mottle virus in infected cells of *Dianthus* because virions were packaged into cytoplasmic strands rather than being formed into aggregates within the cytoplasm. However, they also suggested that reduction in yield upon purification might be due to a failure to disrupt the strands and release the virions.

Formation of cytoplasmic strands is hypothesized to be an intracellular virus localization mechanism leading to the tolerant reaction of Essex soybean plants to the three mild SMV strains. The results of the time sequence study with these four SMV strains suggest that severity of symptoms is not due to a difference in initial virus replication rate, since pinwheel inclusions were detected in cells infected with any of the strains at 9 days and initial systemic symptoms appeared at 7 days. Further support for this conclusion comes from the results of Ouchterlony double diffusion tests, in which all four strains produced precipitin bands of comparable intensity and equivalent dilution end-points. However, by using serologically specific electron microscopy (SSEM) with the two Virginia strains, greater numbers of particles were observed from VA/G1-infected tissue than from OCM/G3-infected tissues (8). The difference in the results between the gel diffusion and SSEM tests might be explained by the release of viral antigens from the cytoplasmic strands in the SDS agar and the failure to disrupt the strands upon grinding the tissue with water for SSEM.

The areas of proliferated endoplasmic reticulum (ER) seen in the cytoplasm of cells infected with these SMV strains appear to be pinwheel-forming areas (Figs. 7–10). Similar areas have been reported in wheat cells infected with wheat spindle streak mosaic virus (WSSMV) (5), and in morning glory cells infected with sweet potato russet crack virus (RCV) (10). Lawson et al (10) also observed vesiculated areas containing loop and circle configurations, in association with pinwheels induced by RCV. They interpreted the appearance of these areas with a dissociation



Figs. 7–12. Ultrastructure of Essex soybean primary leaf cells infected with soybean mosaic virus (SMV) strains. Proliferated endoplasmic reticulum (ER) and pinwheels (PW) within cytoplasm of infected mesophyll cells. Pinwheels (PW) are forming from one side of a double-membrane sac (arrows in 7, 9, 11). **7,** Mesophyll cell infected with SMV-OCM/G3, 14 days after inoculation. **8** and **9,** Mesophyll cells infected with SMV-IL/G3 10 days after inoculation. Cytoplasmic strand protruding into vacuole. **10** and **11,** Cells infected with SMV-IL/G1, 11 days after inoculation, with pinwheels (PW) and cytoplasmic strands (CS) near area containing plasmalemmasomes (PL) between cell wall (CW) and plasma membrane (PM). **12,** Mesophyll cell infected with SMV-VA/G1, 28 days after inoculation, with pinwheels (PW) in cytoplasm with small vesicles (arrows) between pinwheel arms. Virus particle masses (V) evident in cytoplasm. Bars = 0.5 μm.

of pinwheels, rather than as part of a pinwheel-forming process. Lawson et al (10) suggested that pinwheels are formed at the plasmalemma and then migrate into the cytoplasm. Hooper and Weise (5) suggested that pinwheels of WSSMV are formed abutted to the surfaces of membranes and are originally closed and have a definite number of arms. Based on our electron microscopic investigations, we suggest that the vesiculated areas are pinwheel-forming areas and not degradation areas. This suggestion would account for the presence of pinwheel masses observed in the cytoplasm later in the infection process (28 days). The appearance of pinwheel inclusion-forming areas was similar for all the strains, even though the ultimate location of the virus particles within the cells was different.

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