Isolation and Comparison of Two Strains of Soybean Mosaic Virus

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Contribution 427 of the Department of Plant Pathology and Physiology.

The authors gratefully acknowledge C. W. Roane for initially collecting the virus isolates and for advice and cooperation throughout the study; and G. W. Buss and R. M. Goodman for seeds of soybean cultivars and differentials.

Financial support from the Virginia Soybean Commission and from the College of Agriculture and Life Sciences (to P. L. Hunst) is gratefully acknowledged.

Accepted for publication 14 October 1981.

ABSTRACT

Hunst, P. L., and Tolin, S. A. 1982. Isolation and comparison of two strains of soybean mosaic virus. Phytopathology 72:710-713.

Two isolates of soybean mosaic virus (SMV) were collected in Virginia and characterized. One isolate, SMV-VA, was similar to a type strain and was classified into the G1 strain group on soybean differential cultivars. It infected only soybean cultivars known to be susceptible to SMV, and also infected cowpea and Alaska pea systemically, and Topcrop bean locally. The second isolate, SMV-OCM, induced mild symptoms on SMV-susceptible soybeans but caused severe systemic necrosis on SMV-resistant Marshall and Ogden soybeans, reactions typical of strains in the G3 strain group. It failed to induce necrotic lesions on Topcrop bean and to infect pea. The two strains reacted serologically with SMV-VA antiserum and to other known SMV antisera. SMV-VA induced typical scroll-type pinwheels in the cytoplasm of infected cells. Similar pinwheels were induced by SMV-OCM and were always associated with extensive cytoplasmic strands that extended into the vacuoles and contained virus particles.

Soybean mosaic virus (SMV) occurs in soybean (*Glycine max* (L.) Merr.) throughout the world. In Virginia, SMV can be isolated from commercial fields in all soybean-growing counties (19) and from a small percentage of commercial seed lots as a seedborne virus (C. W. Roane and S. A. Tolin, *unpublished*). Most isolates of SMV resemble the type strain in host range and symptomatology (2). Occasionally, isolates exhibit distinctively different properties that may or may not be similar to strains described by Ross (17) or by Cho and Goodman (3). This paper describes two SMV isolates and compares their host range, symptomatology, serology, and ultrastructure. A preliminary report has been presented (7).

MATERIALS AND METHODS

Source and maintenance of strains. The type-like isolate, designated SMV-VA, was isolated from Lee soybean in Montgomery County, VA, in 1968 and has been utilized in previous studies conducted in this laboratory (14,15). Another isolate, designated SMV-OCM, was collected in 1979 from a single plant of Mitchell soybean from breeder's plots growing in Orange County, Virginia. Both SMV-VA and SMV-OCM were mechanically transmitted and maintained in the greenhouse in Lee 68, Lee 74, or Essex soybean. Inoculum was prepared by homogenizing primary and trifoliolate leaves with a chilled mortar and pestle in 0.01 M sodium phosphate buffer (pH 7.0) and was rubbed onto Carborundum-dusted primary leaves with a pestle.

Host range. A host range study was conducted, under greenhouse conditions, for SMV-VA and SMV-OCM utilizing the species listed in Table 1. Both isolates were also inoculated to soybean differential cultivars, which included SMV-susceptible Rampage and SMV-resistant Marshall, Ogden, Buffalo, York, and Kwanggyo (3). Symptom development was observed over a 4-wk period. Plants not showing symptoms were back-inoculated to Essex soybean to detect symptomless virus infections.

Purification. SMV-OCM and SMV-VA were purified separately from Essex soybean using a modification of the SMV purification method described by Ross (16). SMV-VA was also purified from Lee 74 soybean. Freshly harvested SMV-infected leaves, 2–3 wk postinoculation, were homogenized in a Waring Blendor in cold 0.5 M sodium citrate with 1% 2-mercaptoethanol (1 g tissue in 2 ml). The homogenate was strained through cheesecloth. This extract was stirred vigorously in a cold room (4 C) while 7% *n*-butanol was added. After 20–30 min of additional stirring, the preparation was centrifuged at 10,000 rpm (10,000 *g*) for 10 min. The supernatant was strained through Miracloth (Calbiochem-Behring Corp., La Jolla, CA 92037) and allowed to stand in a cold room for 1.5 hr. The supernatant was again centrifuged (10,000 *g* for 10 min) and was then introduced onto a layer of 7–10 ml of 20% sucrose in 0.5 M sodium citrate and centrifuged for 3.0 hr at 30,000 rpm in a Beckman Type 30 rotor. The pellets were resuspended in 0.005 M sodium borate, pH 8.7 (0.5 ml/tube). After low-speed centrifugation (10,000 *g* for 10 min), the virus suspension was layered on 10–40% linear sucrose density gradients prepared in 0.005 M citrate buffer, pH 7.0, and then centrifuged for 1.5 hr at 27,000 rpm (Beckman Type 30 rotor). Virus zones were collected and concentrated by high-speed centrifugation (50,000 rpm, 1.5 hr, Beckman Type 65 rotor). Purified virus was resuspended in 0.005 M citrate, pH 7.0, at a concentration no greater than 3 mg/ml, as determined spectrophotometrically by using a value of $E_{260}^{0.1\%} = 2.4\ cm^2/mg$ (13).

Serology. The purified virus preparations, at approximately 1 mg/ml, were utilized in microprecipitin tests performed according to Ball (1). Crude sap, extracted in distilled water from SMV-OCM or from leaf tissue infected with SMV-VA, was used in Ouchterlony double-diffusion tests, which were done in 0.6% Ionagar No. 2 containing 0.2% sodium dodecyl sulfate, 0.1% sodium azide, and 0.55 or 0.70% sodium chloride (19). Serologically specific electron microscopy (SSEM) was performed by using crude sap from infected leaves. The modified Derrick system (12), with and without decoration, was employed in which dilutions of the antiserum at 1:10 and 1:100 were used.

Antiserum to SMV-VA was prepared at two different times. In 1973, SMV was purified as described above except that high-speed centrifugation did not utilize the layer of sucrose. Two rabbits were used; each received a total of 7 mg of virus. The initial injection of 3 mg was administered intravenously (i.v.). One week later, 1 mg of virus (emulsified in Freund's incomplete adjuvant) was injected intramuscularly (i.m.). Two weeks later, an additional 3 mg was administered both i.m. and i.v. Bleedings were made at 2 and 4 wk

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after the final injections. Homologous titer of resulting antisera in microprecipitin tests was 1:256–1:512.

Antiserum was prepared again in 1980 by injecting each of three rabbits initially with 6 mg of virus, administered both i.m. and i.v. Four weeks later, 0.1 mg of virus was injected i.v. Bleedings were made 2 wk after the final injection. Homologous microprecipitin titers of the antisera were 1:512–1:1024.

Sources of other antisera are given in the Results section of this article.

Electron microscopy. Particle size and morphology were determined from leaf dip or purified virus preparations negatively stained with 1% uranyl acetate.

Tissue samples (1–3 mm^2) of Essex soybean primary leaves, collected 4 wk following inoculation with SMV-OCM or SMV-VA, were fixed in Karnovsky's (6) formaldehyde-glutaraldehyde fixative, postfixed in 1% osmium tetroxide, dehydrated in a graded ethanol-acetone series, and embedded in ERL epoxy (18) resin (Polysciences, Inc., Warrenton, PA 18976). Thin sections were cut, stained with uranyl acetate and lead citrate, and examined in a JEM 100B transmission electron microscope (JEOL Co., Tokyo, Japan).

RESULTS

Host range. Results of the host range study are shown in Table 1. Both SMV-VA and SMV-OCM produced vein-clearing on the first expanding trifoliolate leaf at 5–7 days postinoculation, on Essex and Lee 74 soybean. However, in SMV-OCM infected plants, vein clearing was transient and subsequent trifoliolate leaves either developed a very mild mosaic and downward curling of the leaflets or had no symptoms at all. In contrast, plants inoculated with SMV-VA developed a distinct mosaic and rugosity on subsequent trifoliolate leaves. Also, the primary leaves inoculated with SMV-VA developed distinct chlorotic lesions, whereas those inoculated with SMV-OCM did not. Neither strain infected York soybean.

The two strains also differed in ability to infect other species. SMV-VA produced local necrotic lesions on bean (*Phaseolus vulgaris* L. 'Topcrop'), a mild systemic mottle on cowpea (*Vigna unguiculata* (L.) Walp. 'Blackeye'), and a mild mosaic on pea (*Pisum sativum* L. 'Alaska'). Even though SMV-OCM produced no visible symptoms on bean or cowpea, sap from these hosts was infective when inoculated to Essex soybean. Neither infected squash (*Cucurbita pepo* L. 'Caserta') or broadbean (*Vicia faba* L. 'Long Pod').

The results of inoculation to the soybean differential cultivars are shown in Table 2. According to the system of Cho and Goodman (3), SMV-VA can be classified in the G1 strain group since it infected only the cultivar Rampage systemically. Local necrotic lesions were produced on a few plants of Kwanggyo. SMV-OCM can be classified in the G3 strain group on the basis of production of local and systemic necrosis on cultivars Marshall and Ogden, and failure to infect cultivars other than Rampage.

Purification and serology. Both SMV-VA and SMV-OCM were easily purified and, when analyzed by density gradient centrifugation, sedimented as single zones. The yield of SMV-VA was generally higher, but precise comparisons were not performed.

SMV-VA reacted strongly in microprecipitin tests with SMV

TABLE 1. Host range study of two soybean mosaic virus (SMV) strains, VA and OCM

Host	Symptoms caused by SMV strains[a]	
	VA	OCM
Glycine max 'Essex'	SeMo	MMo
G. max 'Lee 74'	SeMo	MMo
G. max 'York'	NS	NS
Phaseolus vulgaris 'Topcrop'	LNL	NS-L
Vigna unguiculata 'Blackeye'	MSyMot	NS-L
Pisum sativum 'Alaska'	MMo	NS
Cucurbita pepo 'Caserta'	NS	NS
Vicia faba 'Long Pod'	NS	NS

[a]Symbols for symptoms: MMo = mild mosaic; SeMo = severe mosaic; NS = no symptoms; LNL = local necrotic lesions; MSyMot = mild systemic mottle; and L = latent infection.

TABLE 2. Reactions of soybean (*Glycine max*) differential cultivars to inoculation with two soybean mosaic virus (SMV) strains[a]

Soybean cultivar	Symptoms caused by SMV strains[b]	
	VA	OCM
Rampage	N/M[c]	N/M
York	–/–	–/–
Marshall	–/–	N/N
Ogden	–/–	N/N
Kwanggyo	LNL/–	–/–
Buffalo	–/–	–/–

[a]Differential cultivars as defined by Cho and Goodman (3).
[b]Symbols for symptoms: – = symptomless; N = necrosis; M = mosaic; and LNL = local necrotic lesions.
[c]Format for symptom symbols: (Reactions on inoculated primary leaves)/(Reactions on uninoculated trifoliolate leaves).

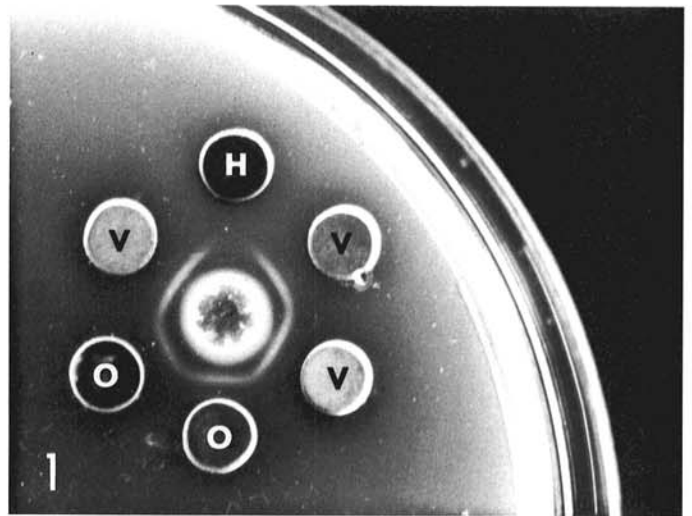


Fig. 1. Ouchterlony double-diffusion test of soybean mosaic virus (SMV) antiserum (center well) against crude sap extracts of healthy (H), SMV-VA-infected (V), or SMV-OCM-infected (O) Essex soybean in peripheral wells. The agar medium contains 0.6% Ionagar No. 2, 0.2% sodium dodecyl sulfate, 0.55% NaCl, and 0.1% NaN_3.

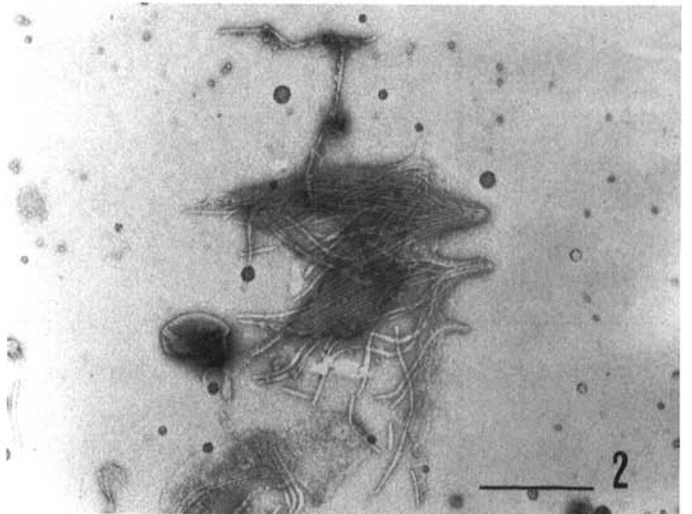
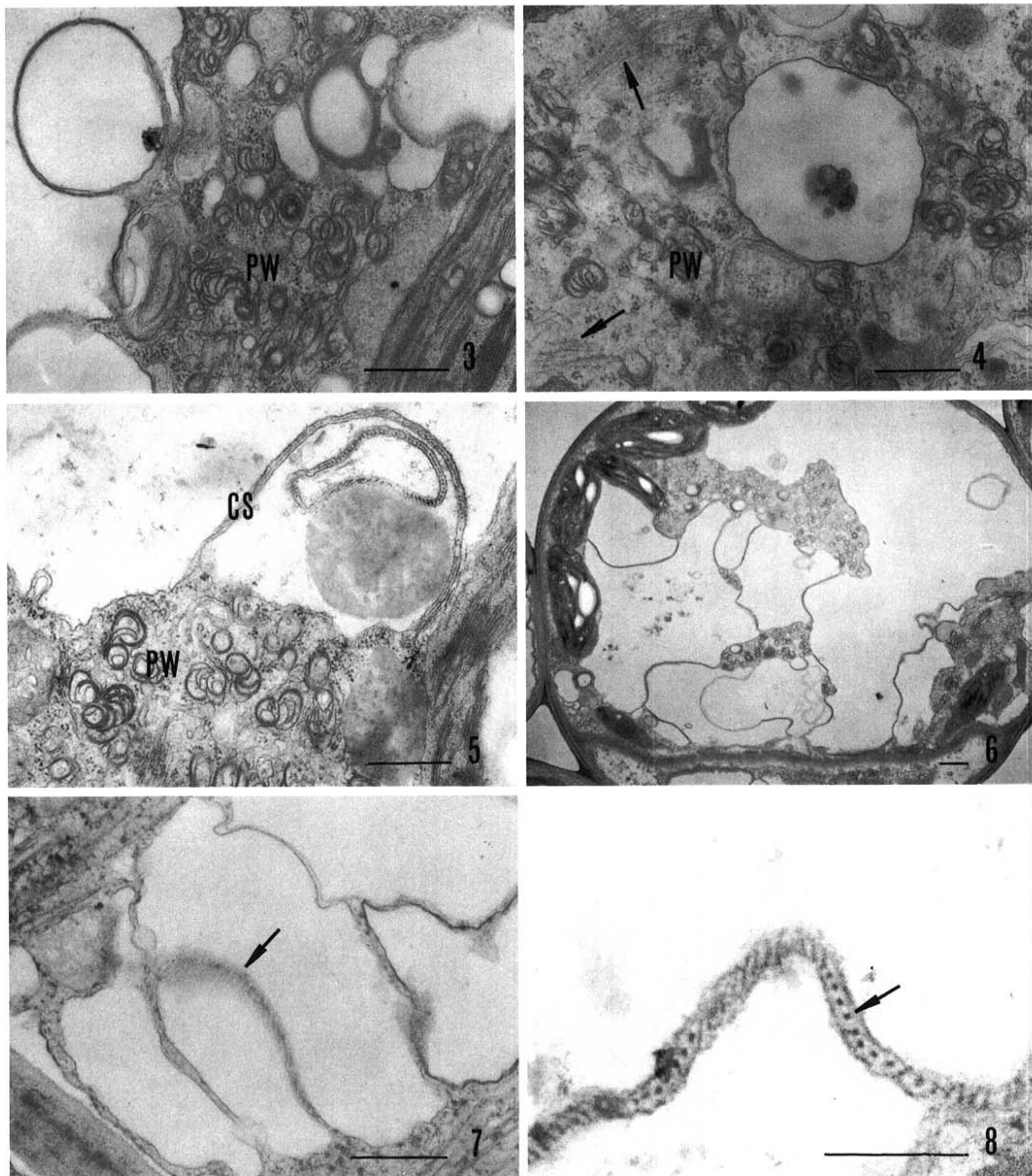


Fig. 2. Electron micrograph of a leaf dip from soybean infected with the OCM strain of soybean mosaic virus, negatively stained with 1% uranyl acetate. Bar = 0.5 μm.

antiserum prepared by Ross (16) and Dunleavy (5), which confirmed its identity as SMV. Purified SMV-VA and SMV-OCM could not be distinguished in microprecipitin tests with either the 1973 or 1980 antiserum, nor with another antiserum to SMV-VA prepared by H. M. Scott (Fayetteville, AR).

In Ouchterlony double-diffusion tests, precipitin lines formed with both SMV-VA and SMV-OCM. These lines were observed to fuse completely when either the old (with 0.70% NaCl) or new (with 0.55% NaCl) antiserum (Fig. 1) was used. In SSEM studies, particles of both strains adhered to grids coated with SMV-VA antiserum and also were decorated by the same antibodies. Particle counts were consistently three to four times higher with SMV-VA than with SMV-OCM.

The viruses were also tested against antisera to other legume



Figs. 3–8. Ultrastructure of Essex soybean leaf cells infected with two soybean mosaic virus (SMV) strains. (**3 and 4**) Pinwheel (PW) masses, produced by SMV-VA, in mesophyll cells. Virus particles can be seen near the pinwheels (arrows). (**5 and 6**) Pinwheels (PW) and cytoplasmic strands (CS) within mesophyll cells infected with SMV-OCM. (**7 and 8**) Close-up of cytoplasmic strands showing viruslike particles within strands (arrows). Bar lengths: Figs. 3–5, 0.5 μm; Fig. 6, 1.0 μm; Fig. 7, 0.5 μm; Fig. 8, 0.2 μm.

potyviruses in one-way microprecipitin tests. Antiserum to bean yellow mosaic virus (BYMV) strain OH-S (from R. T. Jones, Lexington, KY) and to clover yellow vein virus (CYVV) (from O. W. Barnett, Clemson, SC) both reacted with the SMV strains. In both cases, a stronger precipitin reaction was observed with SMV-VA than with SMV-OCM. The CYVV antiserum also reacted with SMV-VA in enzyme immunosorbent assays but the reciprocal reaction of CYVV with SMV antiserum was negative (*unpublished*). In gel diffusion tests, however, no reaction of the SMV strains with either CYVV or BYMV-OHS antiserum was observed.

Electron microscopy. Flexuous rods were observed in leaf dips and in purified preparations of both SMV-VA and SMV-OCM. The modal length was 752 nm and 745 nm for SMV-VA and SMV-OCM, respectively. In leaf dips of SMV-OCM rods appeared to be partially encased in membranes (Fig. 2). No comparable aggregations were observed in leaf dips of SMV-VA.

In thin sections of primary leaf tissue of the cultivar Essex infected with either SMV-VA or SMV-OCM, scroll-type pinwheels (4) were observed in all cell types. SMV-VA produced pinwheels in masses that were easily detected within the cytoplasm at low magnifications (Figs. 3 and 4). Virus particles were sometimes observed near the pinwheels (Fig. 4).

In tissue infected with SMV-OCM, pinwheels were also observed in the cytoplasm, sometimes in masses (Figs. 5 and 6). Cytoplasmic strands protruding into the vacuoles of the infected cells were always in close association with the pinwheels (Figs. 5 to 7). Rod-shaped, viruslike particles in transverse and longitudinal sections were observed within these membrane-bound strands (Figs. 7 and 8). These strands are believed to be the membrane-bound aggregates of particles observed in the leaf dips of SMV-OCM. Cytoplasmic strands were never observed in leaf tissue infected with SMV-VA.

DISCUSSION

According to the host range study, serological tests, and purification, the two isolates are distinct but closely related SMV strains. SMV-VA closely resembled the type strain of SMV in its biological and ultrastructural characteristics (2,5,10). Reaction of soybean differential cultivars to SMV-VA demonstrated it to belong in the G1 strain group, which is in agreement with other SMV isolates considered as type strains (3). This strain reacted strongly with SMV antisera (5,16). To our knowledge, this is the first report of infection of pea by SMV. Failure to infect broad bean and squash provided evidence that BYMV and CYVV were not present. The serological cross-reactions may indicate a distant relationship between SMV, BYMV-OHS, and CYVV.

Even though SMV-OCM is closely related serologically to SMV-VA, the former strain is unique in several biological properties. SMV-OCM produced extremely mild symptoms on susceptible soybean cultivars and did not produce local lesions on Topcrop bean. Detection of this strain by biological indexing would be difficult. If it were seedborne, SMV-OCM could easily go unnoticed in commercial seed lots. In fact, the source of our isolate was most likely infected seed.

The production of cytoplasmic strands within infected cells by SMV-OCM is not unique. Several potyviruses have been reported to produce cytoplasmic strands, but their function has never been elucidated. Martelli and Russo (11) interpreted the arrangement of viruslike particles into cytoplasmic strands within beet mosaic virus-infected tissue as accidental trapping. Kitajima and Lovisolo (9) suggested the phenomenon of cytoplasmic strands may be associated with a defense reaction of the cell. We believe the cytoplasmic strands produced by SMV-OCM to be related to the mild symptoms induced on susceptible soybean cultivars. This is supported by the fact that SMV-VA caused severe macroscopic symptoms, but induced no cytoplasmic strand development. In addition, two isolates of SMV from Illinois (from R. M. Goodman) induced mild symptoms and cytoplasmic strands within infected leaf cells (8). Since one isolate (019-2) was from the G1 strain group and the other (83-2) from the G3 strain group, this further suggests cytoplasmic strand production is the result of a specific virus/host interaction and is not related to Cho and Goodman's (3) strain classification. Examination of the effect of a single virus strain (SMV-OCM) which induces mild symptoms on one cultivar (Essex and severe symptoms on another cultivar (Ogden) should further elucidate this relationship.

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