Comparisons of Soil-Borne Wheat Mosaic Virus Isolates from Japan and the United States

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ABSTRACT

The host ranges of three isolates of soil-borne wheat mosaic virus (SBWMV) from Japan and three from U.S.A. were restricted mostly to Gramineae and Chenopodiaceae, and the isolates differed in symptoms they caused. Previously unreported host plants experimentally infected with SBWMV were maize, spinach, beet, Swiss chard, *Tetragonia expansa*, and tobacco. The three Japanese isolates tested induced necrotic local lesions or caused latent infection on tobacco, but the American isolates did not. The American isolates caused local latent infection in spinach. Infectivity of crude sap of leaves infected with any of six isolates was lost by heating at 50 - 60 C for 10 min and by aging at 15 C for 1 to 3 months. All isolates examined were associated with inclusion bodies, which were of three types. SBWMV had short particles, 110 - 160 nm, and long ones, about 300 nm in length. However, the isolates fell into three groups on the basis of lengths of their short particles and the shapes of the inclusion bodies. The six isolates examined had common antigens, but the Japanese isolates (serotype I) were partially differentiated serologically from the American isolates (serotype II). Serological relationships were not correlated with the lengths of the short particles.

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Soil-borne wheat mosaic virus (SBWMV) is transmitted by the plasmodiophoraceous fungus, *Polymyxa graminis* Led., is 20 nm in diam, and has peaks in length distribution at 160 nm and 300 nm. Recently, Gumpf (5) reported purification and properties of SBWMV, with particular reference to particle length. However, the information on the nature of the virus was limited.

SBWMV occurs in several countries (3, 7, 9), and it is suspected that SBWMV has strains like other plant viruses. However, a comparison of characters of various isolates of SBWMV collected from different parts of the world has not been reported, so we compared the host range, particle length, shapes of inclusion bodies, and serological relationships of isolates from Japan and U.S.A.

MATERIALS AND METHODS.—Eight virus isolates collected from Japan and America were examined (Table 1). They were maintained by successive mechanical transfers to young rye plants. It was observed that the Japanese isolate (J-C) which caused many vacuolate inclusion bodies sometimes changed during successive propagations to variants which caused very few and small inclusion bodies (J-C-V). During the successive transfers of the isolate from T. T. Hebert (US-A-O), which originated from rye plants grown on infested soil, inclusion bodies changed in shape from oval with vacuoles to irregular without vacuoles. This newly selected isolate, designated US-A, was used in this study. Rye plants were inoculated with SBWMV obtained from M. K. Brakke, using desiccated leaves of infected wheat as inoculum. Microscopic examination of epidermal strips of the inoculated rye leaves revealed the presence of two kinds of inclusion bodies. One was oval or elongated in shape with many vacuoles (US-B) (Fig. 1-C); the other was irregular in shape with no vacuole (US-C) (Fig. 1-D). Two isolates characterized by the different types of inclusions were separated by serial mechanical transfers to young rye plants. J-B, J-C, J-D, J-E, and US-A-O are original isolates obtained from plants grown on infested soil; J-A, US-B, and US-C are isolates obtained from plants mechanically inoculated with extracts of desiccated leaves of infected plants; and US-A and J-C-V are isolates obtained during successive propagations by mechanical inoculation of US-A-O and J-C, respectively. The characteristics of the isolates, including those of the new isolates, remained unchanged after many successive transfers, owing to careful selection made at each transfer.

Manual inoculations were made with crude sap obtained by grinding infected plant tissues with two to five volumes of 0.1 M phosphate buffer (pH 7.0) containing 0.001 M potassium cyanide, and rubbing the preparation with a cotton swab or the finger on Carborundum-dusted leaves. Leaves then were rinsed with water and the plants were placed in growth cabinets controlled at 10 - 15 C. Recovery of the

TABLE 1. Source of soil-borne wheat mosaic virus isolates

Isolate	Locality	Supplied by
J-A	Saitama, Japan	
J-B	Iwate, Japan	Tohoku Nat. Agr. Exp. Sta.
J-C	Chiba, Japan	
J-D	Saitama, Japan	Central Agr. Exp. Sta.
J-E	Okayama, Japan	T. Inouye
US-A	U.S.A.	T. T. Hebert
US-B	U.S.A.	M. K. Brakke
US-C	U.S.A.	M. K. Brakke

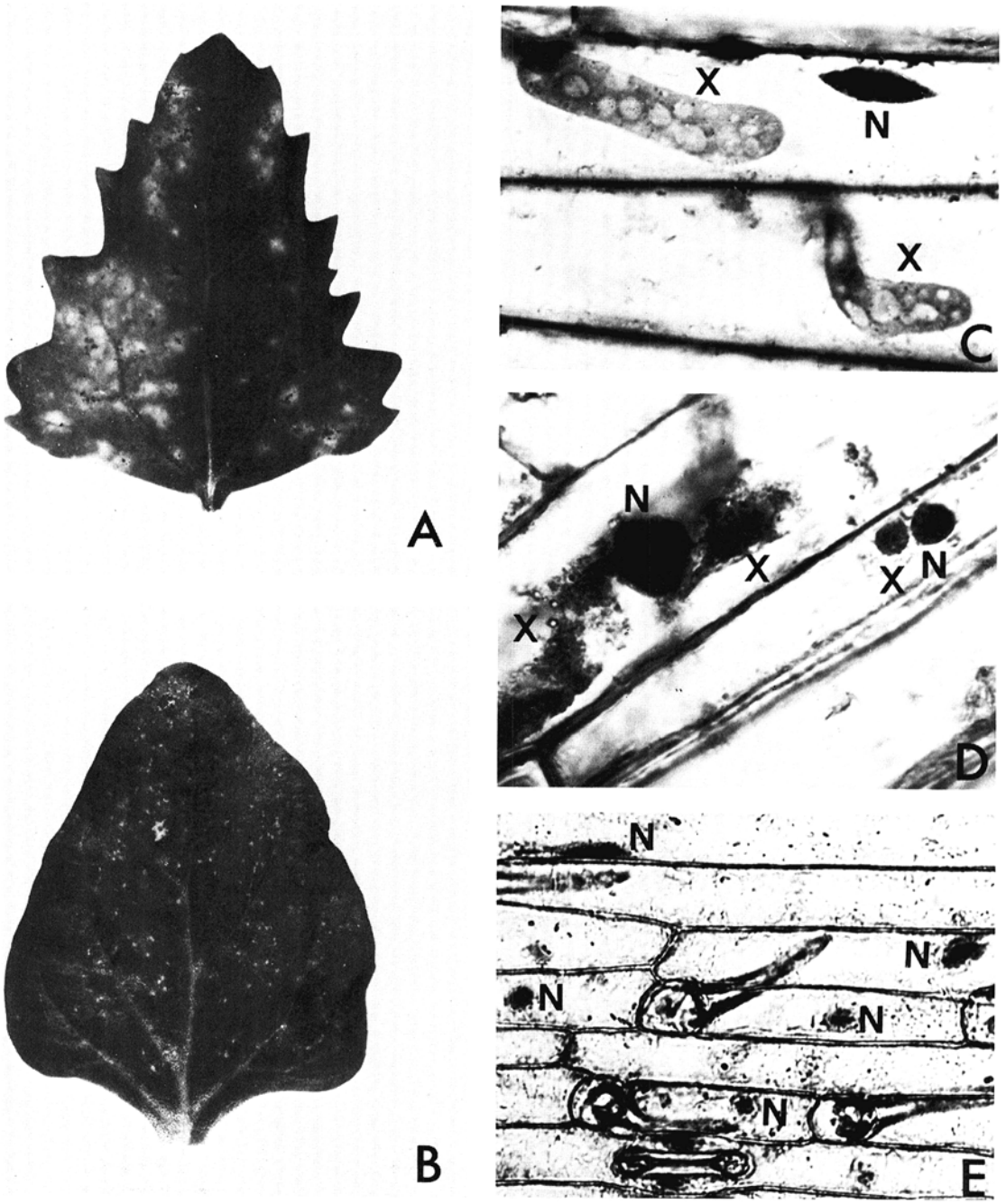

Fig. 1. A, B) Symptoms induced by J-A isolate of soil-borne wheat mosaic virus: **A)** chlorotic and necrotic local lesions on *Chenopodium quinoa*; **B)** necrotic local lesions on *Tetragonia expansa*. **C,D,E)** Inclusion bodies (X) in epidermal cells of rye plants infected with various isolates of soil-borne wheat mosaic virus (N = nucleus); **C)** US-B isolate (group I, × 1,500); **D)** US-C isolate (group II, × 1,500); **E)** J-A isolate (group III, × 850).

viruses was made from inoculated leaves or systemically infected leaves by inoculations made on *Chenopodium quinoa*. *C. quinoa* is highly sensitive to SBWMV and develops distinct local lesions suitable for assay studies. SBWMV particles were found by electron microscopy in "dip" preparations made from local lesions on *C. quinoa*.

Leaf-dip preparations from infected leaves were shadowed with chromium. Crude juice from leaves of SBWMV-infected rye was used for studying the

TABLE 2. Host reactions to several isolates of soil-borne wheat mosaic virus

Plant species	Isolate					
	J-A	J-B	J-C	US-A	US-B	US-C
Hordeum vulgare 'Goseshikoku'	S[a]	S	–	S	S	S
'Golden Melon'	S	S	–	S	S	S
Secale cereale 'Petkuser'	S	S	S	S	S	S
Triticum aestivum 'Hatakeda'	S	S	SL	S	S	S
'Harvest Queen'	S	S	S	S	S	S
Zea mays	L	L	L	L	L	L
Nicotiana tabacum 'Ky 57'	L	LL	LL	–	–	–
'Bright Yellow'	L	L	L	–	–	–
Beta vulgaris	LL	LL	LL	LL	LL	LL
B. vulgaris var. *flavescens*	L	L	L	L	L	L
Chenopodium album	L			L	L	L
C. amaranticolor	L	L	L	L		
C. murale	L			L		
C. quinoa	L	L	L	L	L	L
Spinacia oleracea 'Minster Land'	–	–	–	LL	–	–
'Nippon'	–	–	–	LL	LL	LL
Tetragonia expansa	L,S	L,S	L,S	L,S	L,S	L,S

[a]S = systemic infection; L = local infection; SL = the virus was recovered from young leaves; LL = the virus was recovered from only inoculated leaves; – = no infection.

thermal inactivation point and longevity in vitro. Infectivity was assayed on *C. quinoa*.

Epidermal strips of infected leaves were used for studies of inclusion bodies. Unfixed strips were stained in Giemsa solution for 2 to 3 min, washed in 50% alcohol solution, rinsed in water, and finally mounted in water for examination by light microscopy.

The virus was purified by the method described by Saito et al. (13).

Antisera against five isolates were prepared in rabbits given two intravenous and two intramuscular injections of purified virus preparations. The antisera titers ranged from 1/800 to 1/1,600 in complement fixation tests. The serological relationship among various isolates of SBWMV was examined by complement fixation and absorption tests.

RESULTS.—*Host reactions.*—Table 2 shows the host ranges of six isolates of SBWMV. Five isolates except J-C induced mosaic on wheat. Systemically infected leaves of wheat inoculated with J-C were symptomless, but the virus was recovered from them. Barley plants infected with US-A or US-C showed mosaic and necrosis, but necrotic symptoms did not appear on barley infected with J-A, J-B, or US-B. The virus was not recovered from barley inoculated with J-C. Rye plants inoculated with six isolates of SBWMV showed three types of symptoms: (i) severe mosaic and rosetting, and plant stunting (J-A, J-B); (ii) mosaic and plant stunting (US-A); and (iii) mild mosaic (J-C, US-B, and US-C). All six isolates caused necrotic local lesions on maize and chlorotic or necrotic local lesions on four species of *Chenopodium* (Fig. 1-A). *Tetragonia expansa* inoculated with three Japanese isolates developed necrotic or chlorotic spots on inoculated leaves and other young leaves. Necrotic local lesions induced by J-A were especially clear (Fig. 1-B). On the other hand, *T. expansa* inoculated with three American isolates had indistinct local and systemic chlorotic ringspots. Five Japanese isolates, including J-D and J-E, differed from the three American isolates in that the Japanese isolates caused necrotic or chlorotic local lesions, whereas tobacco was not susceptible to the American isolates. SBWMV particles could be found by electron microscopy in dip preparations from local lesions in tobacco, and was recovered from them by inoculations made on *C. quinoa* or rye plants. The virus was recovered from symptomless inoculated leaves of spinach inoculated with American isolates, but was not recovered when Japanese isolates were similarly tested.

No infection occurred in *Avena sativa, Dactylis glomerata, Lolium scabrum, Phleum pratense, Oryza sativa, Nicotiana glutinosa, Lycopersicon esculentum, Pisum sativum, Trifolium incarnatum, Phaseolus vulgaris, Vicia faba, Glycine max, Cucumis sativus, Zinnia elegans,* and *Gomphrena globosa.*

Physical properties.—Infectivity of crude sap for six isolates (J-A, J-B, J-C, US-A, US-B, and US-C) was last at 50-60 C for 10 min and by aging at 15 C for 1 to 3 months.

Inclusion bodies.—Light microscopy of epidermal strips of rye, wheat, and barley plants infected with isolates of SBWMV revealed the presence of inclusion bodies (Fig. 1-C, D, E). They could be grouped in three categories: (i) inclusion bodies oval or elongate, with smooth margins, and many large or small vacuoles (J-B, J-C, J-D, J-E, US-B, US-A-O) (group I); (ii) inclusion bodies present as mixture of two kinds, one irregular in form and rough margins, and other, oval-shaped and with smooth margins, both without vacuoles and tending to disappear in old leaves (US-A, US-C) (group II); and (iii) inclusion bodies small and occurring rarely (J-A, J-C-V) (group III).

Particle-length distributions.—Each of the isolates had a different particle-length distribution (Fig. 2). All eight isolates had long particles about 300 nm in length, together with short particles that were characteristic for each isolate. The ratio of short and long particles was different for certain of the isolates, although most isolates had more short particles than long ones. Eight isolates of SBWMV were grouped into three types on the basis of lengths of short particles (Fig. 2, Table 3).

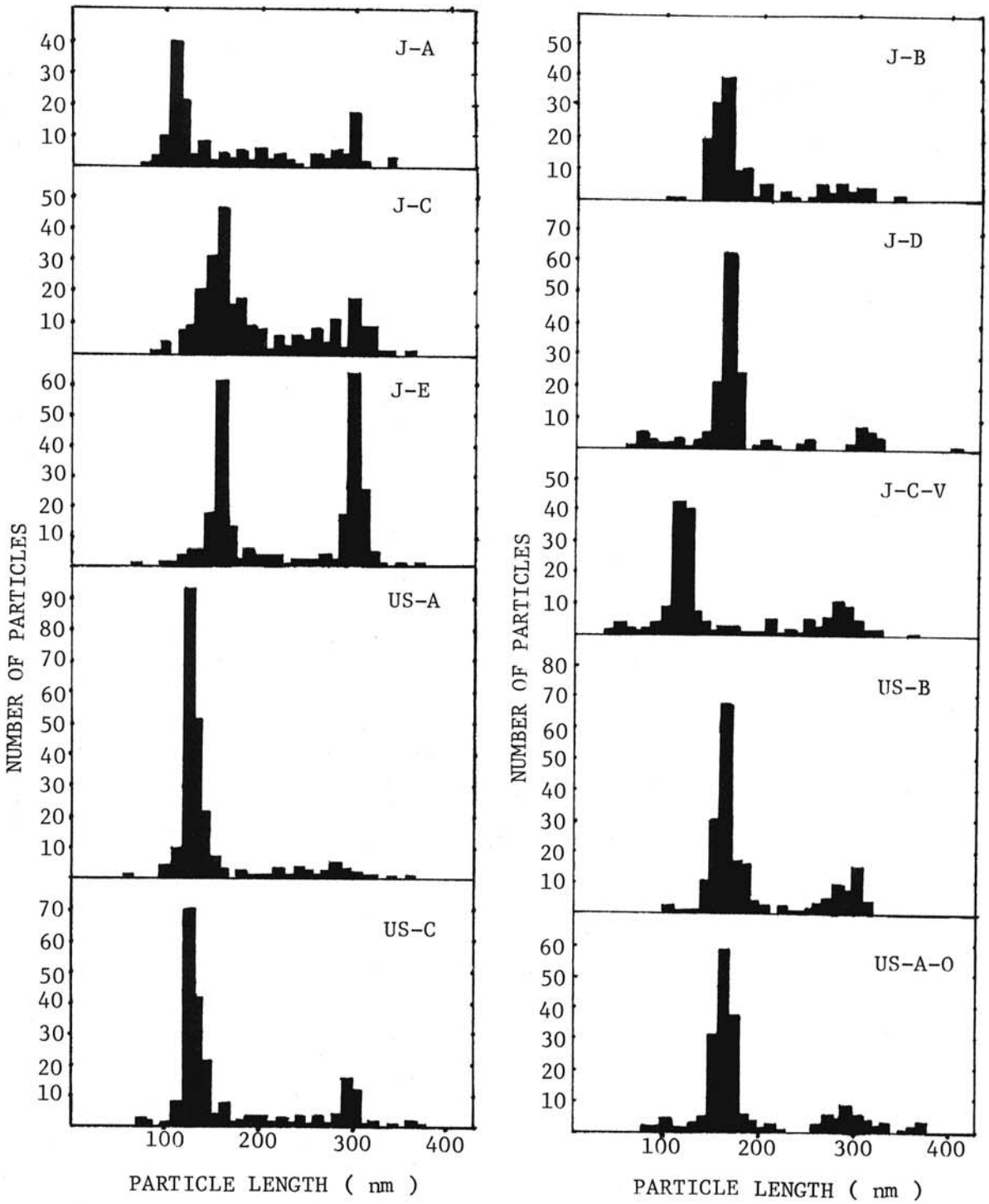

Fig. 2. Particle-length distributions of soil-borne wheat mosaic virus isolates.

TABLE 3. Lengths of short and long particles of soil-borne wheat mosaic virus

Group	Virus isolate	Lengths (nm)	
		Short particle	Long particle
I	J-B	160	300
	J-C	160	300
	J-D	160	300
	J-E	160	300
	US-B	160	300
	US-A-O	160	290
II	US-A	120	280
	US-C	120	290
III	J-A	110	300
	J-C-V	110	280

The US-A isolate, which originally belonged in group I (US-A-O), when examined after several successive propagations, had a particle-length distribution pattern like that of isolates of group II. Similarly, sometimes during successive propagations a Japanese isolate (J-C) that belonged in group I changed to one that belonged in group III. The variant (J-C-V) from J-C induced formation of small inclusion bodies like those associated with isolates of group III. Furthermore, the same changes as were observed for J-C; i.e., in lengths of short particles and the type of inclusion bodies, were observed also for J-B. Thus, sometimes the short particles of SBWMV changed in length to shorter ones, but they did not change from short to longer particles.

There was a correlation between the type of inclusion bodies and particle-length distribution. For instance, J-A and J-C-V, which induced small inclusion bodies, had short particles about 110 nm in length. US-A and US-C which induced inclusion bodies that were irregular in form, had short particles about 120 nm in length. Furthermore, J-B, J-C, J-D, J-E, US-B, and US-A-O which induced inclusion bodies that were bounded with a clear margin and contained vacuoles, had short particles of about 160 nm in length. It should be noted that all the isolates obtained directly from plants infected via soil (J-B, J-C, J-D, J-E, US-A-O) had inclusion bodies belonging to group I and short particles of about 160 nm in length.

TABLE 4. Serological relationship among isolates of soil-borne wheat mosaic virus

Serotype	Virus isolate	Titre[a] obtained with antisera for isolate indicated				
		J-A	J-B	US-A	US-B	US-C
I	J-A	800	1,600	800	200	100
	J-B	800	1,600	400	200	100
	J-C	800	1,600	400	200	100
II	US-A	800	1,600	1,600	800	800
	US-B	800	1,600	1,600	800	400
	US-C	800	1,600	1,600	800	400

[a]Figures are reciprocals of the dilution end points of the antisera in complement fixation tests.

Serological tests.—Serological reactions with antisera against five isolates indicated that the six isolates of SBWMV fell into two serotypes (Table 4). Serotype I consisted of three Japanese isolates (J-A, J-B, and J-C): these had higher dilution end points in reactions with Japanese antisera than with American antisera. Serotype II consisted of three American isolates (US-A, US-B, and US-C) which reacted equally with the Japanese and the American antisera. In absorption tests, the homologous titers of antisera after absorption with heterologous antigens, were higher with American than with Japanese antisera (Table 5). These results indicated that Japanese and American isolates had both common and different antigens.

Thus, grouping of the six isolates of SBWMV by their geographical origin and by serological relationship gave the same results. However, groupings based on serotype and particle-length distribution patterns differed in the complement of isolates that they contained.

DISCUSSION.—Although SBWMV is transmissible through soil to many kinds of grasses, the frequency of sap transmission of the virus was low. The host range of SBWMV was restricted to several genera of Gramineae and *Chenopodium* species (3, 8, 10, 12). We found that maize, beet, Swiss chard, spinach, *Tetragonia expansa*, and tobacco inoculated with SBWMV developed local lesions or latent symptoms. However, American isolates could be distinguished from Japanese isolates on the basis of the reactions of inoculated leaves of spinach and tobacco. Differences in infectivities of these isolates in spinach and tobacco were correlated with differences in serotypes and geographical origins of isolates. Therefore, spinach and tobacco could prove useful as indicator plants for distinguishing strains of SBWMV.

Cell inclusions previously observed by light microscopy in SBWMV-affected plant cells (7, 11, 14) apparently belong to group I according to our classification. On the other hand, McKinney (10) reported the occurrence of SBWMV without cell inclusions or with cell inclusions which differed in certain characters from those usually found. However, attempts to group the isolates of SBWMV on the basis of types of inclusions apparently have not previously been made.

Gold et al. (4) reported a modal length of 128 nm for SBWMV, and Saito et al. (13) reported rods of SBWMV were usually about 170 nm in length. Brandes et al. (2), Canova (3), and Brakke et al. (1) showed that lengths of rods of SBWMV frequently were about 160 nm and about 300 nm. Recently, Gumpf (5) confirmed that particle-length distribution patterns for SBWMV preparations exhibited two peaks (at 148 nm and 295 nm), and found that preparations consisting principally of long particles with some short particles were infectious but

TABLE 5. Serological relationship among various isolates of soil-borne wheat mosaic virus using the antiserum after absorption with isolates

		Titre of antiserum[a]							
	Antiserum	J-A		J-B		US-A	US-B		US-C
Virus isolate	Absorbed isolate	J-C	US-A	J-C	US-B	J-A	J-B	US-A	US-A
J-A		0	40			0			0
J-B				20	80		0	0	
J-C		0		20					
US-A		0	0		0	640	640	0	0
US-B			0	0	20	640	320	20	
US-C									20

[a]Figures are reciprocals of the dilution end points of the antisera in complement fixation tests. 0 means no reaction with antisera at twenty times dilution.

preparations consisting only of short particles were noninfective. We also found that all isolates of SBWMV examined had particles of two different lengths, although some isolates had relatively few long particles, and we have confirmed Gumpf's finding that infection occurs only with preparations containing both short and long particles (Tsuchizaki et al., *unpublished data*).

SBWMV fell into three groups on the basis of lengths of their short particles. All isolates collected from plants that became infected via transmission in soil had short particles about 160 nm in length. However, some isolates underwent a change in the length of the short particles from 160 nm to about 110-120 nm when they were propagated repeatedly by sap-inoculation of plants grown in a growth cabinet. Harrison & Woods (6) observed that two British isolates of tobacco rattle virus (TRV), which had two peaks of particle-length distribution, underwent a change in length of the short particles when the isolates were subcultured repeatedly in a greenhouse. Whether these TRV isolates initially existed as mixtures, or whether variants were produced and these were selected during propagation, is unknown. It is clear, however, that successive mechanical transmission of SBWMV effected a change in length of the shorter particles; their length changed from about 160 nm to about 110 or 120 nm. That the grouping derived on the basis of types of inclusion bodies and those derived on the basis of length of short particles were comparable suggests that the short particles may play some important role in the formation of inclusion bodies.

It was shown by serological tests that isolates from the same countries were closely related, whereas isolates from different countries had different serotypes. Although serotype I and serotype II had many properties in common, they differed in their infectivities to tobacco and spinach. Moreover, such serological relationship and length of short particles was not correlated; apparently antigenic properties and length of short particles are independent characteristics.

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