Transmission, Host Range, and Serological Properties of the Viruses That Cause Lettuce Speckles Disease

B. W. Falk, J. E. Duffus, and T. J. Morris

Department of Plant Pathology, University of California, Riverside 92521; plant pathologist, Agricultural Research, Science and Education Administration, United States Department of Agriculture, U. S. Agriculture Research Station, Salinas, CA 93915; and Department of Plant Pathology, University of California, Berkeley 94720, respectively.
Accepted for publication 21 December 1978.

ABSTRACT

FALK, B. W., J. E. DUFFUS, and T. J. MORRIS. 1979. Transmission, host range, and serological properties of the viruses that cause lettuce speckles disease. Phytopathology 69: 612-617.

The speckles disease is caused by a virus complex that affects lettuce, sugar beets, and spinach in the Salinas and Pajaro Valleys of California. The complex consists of two viruses, beet western yellows virus (BWYV) and lettuce speckles mottle virus (LSMV). Both viruses are transmitted in a persistent manner by *Myzus persicae*, the green peach aphid; however, LSMV is aphid transmissible only when in a mixed infection with BWYV. LSMV is mechanically transmissible and when separated from BWYV by mechanical transmission its aphid transmissibility is lost. Aphid transmission from mixed infections extends the host range of LSMV compared to species infected following mechanical inoculation. Lettuce speckles mottle virus in mixed infections exhibits a serological relationship to BWYV and the in vitro stability of LSMV is greater in sap from plants infected with the speckles virus complex than from those infected with LSMV alone. These data suggest genomic masking of LSMV by BWYV coat protein in the mixed infection.

Additional key words: aphid, dependent transmission, genomic masking, virus.

Since the early 1960's, a previously unrecognized virus disease of lettuce, sugar beet, and spinach has been observed in the Salinas and Pajaro Valleys of California. The disease is named "speckles" for the characteristic small angular chlorotic spots (Fig. 1) induced in the outer leaves of infected lettuce plants. The disease is especially severe in early spring crops and in some years has caused significant losses in the early lettuce plantings. The disease disappears with the onset of summer.

Results of early studies revealed two unrelated viruses to be involved in the disease (Duffus, *unpublished*). One of the viruses is beet western yellows virus (BWYV), and the other was unidentified, but was called lettuce speckles mottle virus (LSMV). Both viruses are transmitted together by *Myzus persicae* (Sulzer), the green peach aphid. However, the aphid transmissibility of LSMV is lost when it is separated from BWYV by mechanical transmission. This article reports the transmission, host range, and serological characteristics of the two viruses.

MATERIALS AND METHODS

The speckles virus complex was isolated by transmission with green peach aphids from symptomatic (Fig. 1) field-infected lettuce (*Lactuca sativa* L. 'Calmar'). Throughout this article, the name speckles virus complex (SVC) will refer to mixed infection by BWYV and LSMV. The SVC was maintained in the greenhouse in *L. sativa* 'Monterey,' *Nicotiana clevelandii* Gray, and in *Physalis floridana* Rydb. The original isolate was used for all experiments. Nonviruliferous green peach aphids were reared on radish (*Raphanus sativus* L. 'White Icicle') in a growth chamber under light at 21 C. The green peach aphid was used for all aphid tranmission experiments unless otherwise stated. In most transmission experiments, nonviruliferous groups of aphids were given a 24-hr acquisition access to the virus source and a 48-hr inoculation access to individually caged test plants. The aphids were killed by fumigating the plants with nicotine sulfate. Preliminary experiments revealed that the symptoms showed by *N. clevelandii* plants differed when they were infected by LSMV, BWYV, or both viruses (Fig. 2). Therefore, plants were evaluated by symptoms for infection by LSMV, BWYV, or both viruses.

Host range of LSMV. Host range studies for LSMV were done using aphid- and mechanical transmission. Aphid transmission was done by giving aphids a 24-hr acquisition access period on SVC-infected lettuce or *Physalis floridana* and then an inoculation access to the desired host and to *N. clevelandii* control plants for 48 hr. Aphids were placed on the test plants in groups of 20–30 aphids per plant. Aphid and mechanical transmission recoveries back to *N. clevelandii* were made from test plants, regardless of whether symptoms developed, after 3–6 wk.

Mechanical transmission experiments were performed by grinding *N. clevelandii* infected only with LSMV in a mortar with 2% $K_2HPO_4 \cdot 3H_2O$) adjusted to pH 7.0 containing 0.25% sodium sulfite. A small amount of Celite was added and plants were inoculated with this suspension by means of a cotton swab.

Transmission characteristics of LSMV and BWYV. Experiments were done to determine the aphid and mechanical transmission characteristics of LSMV as compared to BWYV from plants infected with only LSMV, BWYV, or both viruses (the SVC). Aphid inoculations were done to *N. clevelandii* for LSMV and the

SVC and to *Capsella bursa-pastoris* L. for BWYV. Mechanical inoculations were made to *N. clevelandii*.

Because the association of BWYV and LSMV is necessary for aphid transmission of LSMV, another isolate of BWYV (RY-1) and a serologically related virus, turnip yellows virus (TuYV) (6) also were tested with LSMV to see if they also could effect the aphid transmission of LSMV. Eight *N. clevelandii* were mechanically inoculated with LSMV. One week later, when symptoms first appeared, two of the plants were inoculated by aphids wth the speckles BWYV isolate (SP-yell). Two plants each also were inoculated with RY-1 and TuYV. Two of the plants were left with only LSMV and two healthy *N. clevelandii* were inoculated by aphids with the SVC. One month later, aphid transmissions were attempted from each plant for LSMV to *N. clevelandii* and to *C. bursa-pastoris* for BWYV and TuYV.

Aphids were allowed sequential acquisition access to BWYV- and LSMV-infected leaves to see if BWYV-viruliferous aphids could acquire and transmit LSMV. *C. bursa-pastoris* leaves infected with BWYV and *Hyoscyamus niger* L. leaves infected with LSMV were used as acquisition sources. Aphids were allowed a 24-hr acquisition access on one source, followed immediately by 24 hr on the second source; then aphids were allowed 48-hr inoculation access to *N. clevelandii*.

Transmission of semi-purified preparations of BWYV and LSMV was attempted by membrane feeding (5). BWYV was purified by heat clarification of sap followed by differential centrifugation (7). LSMV- and SVC-infected plants were subjected to a slightly different purification procedure consisting only of bentonite clarification (16) and differential centrifugation. Fresh plant tissue was ground in a Waring Blendor with 2 volumes of 0.05 M phosphate 0.01 M glycine buffer, pH 7.0, and further disrupted with a VirTis 45 homogenizer. This was filtered through cheesecloth and 12 ml of a 1% bentonite solution was added per 100 ml of sap. One cycle of differential centrifugation first at 8,000 *g* for 15 min in a Sorvall centrifuge and SS-34 rotor, followed by 70,000 *g* for 2 hr in a Beckman Type 30 rotor and Spinco Model L centrifuge was used to concentrate the virus(es). LSMV, BWYV, LSMV plus BWYV (in vitro mixture of both viruses purified from separate plants), and the SVC (in vivo mixture purified from the same plant) were further purified by rate-zonal sucrose density gradient centrifugation on density gradient columns of 7, 7, 7, and 4 ml of 40, 30, 20, and 10% sucrose (w/v), respectively, for 4 hr at 64,000 *g* in the SW 25.1 rotor.

The gradients were fractionated and the infectious zone (12–15 ml deep) was removed, diluted to 20% sucrose with buffer and tested for infectivity by mechanical inoculation and for aphid inoculation by membrane feeding (4).

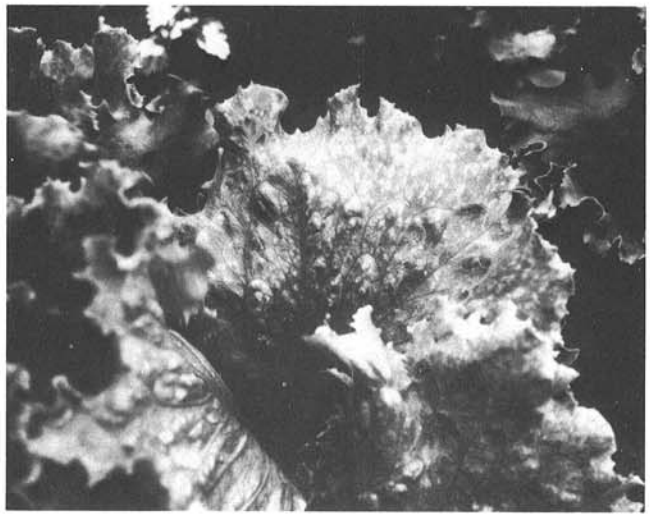

Fig. 1. Symptoms of the speckles disease in field lettuce; angular chlorotic spots in the outer leaves.

Serological and physical characteristics of LSMV. Serological infectivity neutralization (11) was used to determine the serological relationship of LSMV and BWYV when in infections of LSMV alone and in the mixed infection (SVC), both for aphid- and mechanically transmissible LSMV. Semipurified samples of LSMV, BWYV, and the combination of both viruses purified from a SVC-infected plant were incubated with an equal volume of antiserum diluted to one-fifth its original concentration with normal saline (0.9% NaCl) for 0.5 hr at 37 C. The sample was centrifuged at 8,000 *g* for 15 min and the supernatant was layered on sucrose gradients and centrifuged. Gradients were fractionated and assayed for infectivity by both mechanical and aphid inoculation.

Physical properties of LSMV. Longevity in vitro (17) experiments were performed on LSMV from singly and SVC-infected plants. Infected plants were ground in sterile mortars and the sap was squeezed through cheesecloth. One-milliliter samples were aged at 21 C and inoculated to *Chenopodium quinoa* Willd. and *N. clevelandii* at 0, 8, 24, 48, 72, 96, and 120 hr.

RESULTS

Host range of LSMV. In the summer, symptoms of LSMV and of the SVC in *N. clevelandii* were obtained only in plants maintained in cool, shady conditions in the greenhouse. Field observations of the speckles disease in lettuce indicated that symptoms disappeared with the onset of summer. Local lesion production by LSMV on *C. quinoa* was more consistent when test plants were

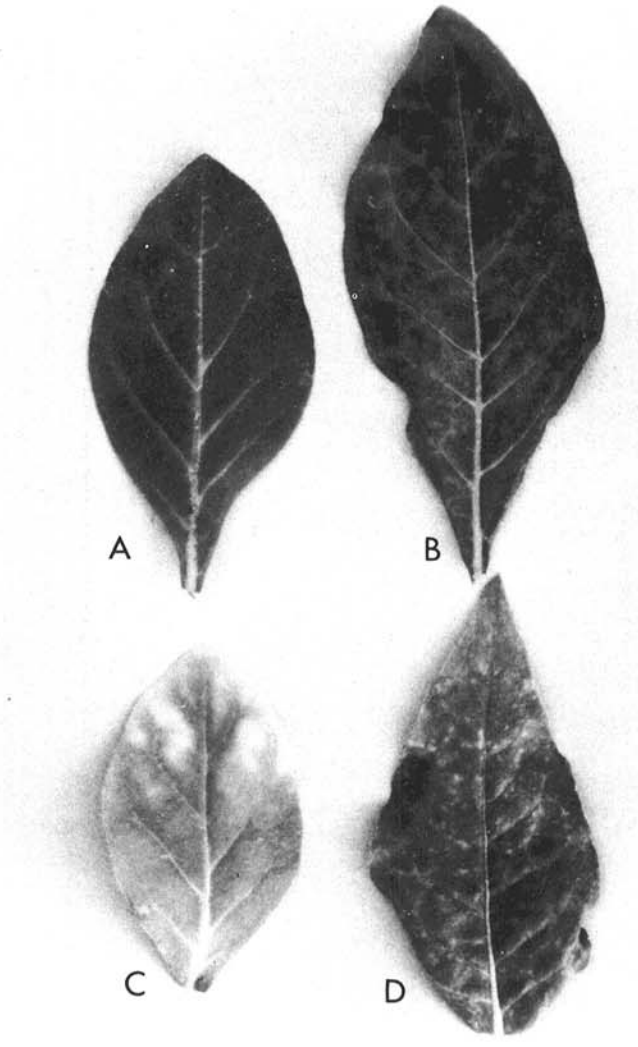

Fig. 2. Leaves of *Nicotiana clevelandii*. **A**, healthy; **B**, infected with lettuce speckles mottle virus (LSMV); **C**, infected with beet western yellows virus (BWYV); and **D**, infected with both LSMV and BWYV.

incubated for 24 hr in a dark chamber immediately prior to mechanical inoculation.

The host range of LSMV (Table 1) was greater for transmission by the green peach aphid than by mechanical transmission. Some host species were symptomless; therefore, all test plant species were tested for infection by aphid and mechanical inoculation of *N. clevelandii*. Mechanical transmission recoveries were performed from the plants that were inoculated by aphids with the SVC because many of the species were found to be hosts for LSMV and not BWYV; eg, *N. glutinosa, N. clevelandii* × *glutinosa, Erodium moschatum, Chenopodium capitatum,* and *C. murale*. Thus, after aphid inoculation of these plants, LSMV was not aphid-recoverable in the absence of BWYV, the helper. The LSMV was recoverable by mechanical transmission.

Of the species tested, the following were not hosts for LSMV by aphid or mechanical inoculation in these experiments: Amaranthaceae: *Gomphrena globosa* L.; Chenopodiaceae: *Beta macrocarpa* Guss., *Chenopodium amaranticolor* Coste & Reyn. Compositae: *Cichorium endiva* L., *Lactuca virosa* L., *Picris echioides* L., *Sonchus oleraceus* L., *Taraxacum officinale* Weber; Cruciferae: *Brassica arvensis* Wheeler, *B. juncea* (L.) Coss., *B. oleracea* L. var. *fruticosa* Metz., *B. pekinensis* (Lour.) Rupr., *Lepidium sativum* L., *Raphanus sativus* L. 'White Icicle', *Datura stramonium* L., *Lycopersicum esculentum* Mill., *Nicotiana tabacum* L., *Nicandra physalodes* (L.) Gaertn., *Petunia hybrida* Vilm.; Umbelliferae: *Apium graveolens* L. var. *dulce* DC., *Conium maculatum* L., *Coriandrum sativum* L., and *Daucus carota* L. var. *sativa* DC.

Transmission characteristics of LSMV and BWYV. Results of mechanical and aphid transmission of LSMV and BWYV from plants infected with either virus, and from SVC-infected plants are shown in Table 2. The LSMV was easily mechanically transmissible from both LSMV- and SVC-infected plants, but LSMV was only aphid transmissible from SVC-infected plants. *Aulacorthum solani* (Kalt.) and *Brevicoryne brassicae* (L.) also transmitted LSMV from SVC-infected plants, but not as efficiently as *M. persicae*. In the field, LSMV has been found only in mixed infections with BWYV in lettuce, spinach, and sugar beets. LSMV was not transmitted by aphids except from plants also infected with BWYV.

BWYV was not mechanically transmissible from plants containing only BWYV, but BWYV was sometimes mechanically transmitted from SVC-infected plants. Further experiments to examine the unexpected mechanical transmission of BWYV from SVC-infected plants were not done.

The helper activity of BWYV for aphid transmission of LSMV was not specific for the isolate of BWYV found with LSMV from the field. A BWYV isolate from radish (RY-1) and an isolate of turnip yellows virus (TuYV) also functioned as helper viruses for LSMV aphid transmission (Table 3). Furthermore, the two viruses of the SVC were separated, and then the SVC was recreated by separately inoculating LSMV and BWYV to a suitable host. The order of inoculation made no difference.

LSMV was not transmitted by aphids after sequential 24-hr acquisition access periods to BWYV- and LSMV-infected plants and then allowed inoculation access to 30 healthy *N. clevelandii*. The BWYV was aphid transmitted after sequential feeding experiments to 25 of 30 *N. clevelandii*, but LSMV was only aphid transmitted from SVC-infected controls.

Transmission of each virus was accomplished with semipurified

TABLE 1. Host range of lettuce speckles mottle virus (LSMV)

Plant species	Transmission	
	Mechanical[a]	Aphid[b]
Amaranthaceae:		
Amaranthus hybridus L.	−	+
Boraginaceae:		
Amsinckia douglasiana (A.) DC.	−	+
Chenopodiaceae:		
Chenopodium quinoa Willd.	+	−
C. capitatum L.	+	+
C. murale L.	−	+
Beta vulgaris L.	−	+
Spinacea oleracea L.	−	+
Compositae:		
Lactuca sativa L.	−	+
Cruciferae:		
Brassica napus L.	−	+
Capsella bursa-pastoris (L.) Medic.	−	+
Solanaceae:		
Nicotiana clevelandii Gray	+	+
N. glutinosa L.	−	+
N. glutinosa × *N. clevelandii*	+	+
Physalis floridana Rydb.	−	+
Hyoscyamus niger L.	+	+

[a]Mechanical inoculations were performed with freshly ground LSMV infected tissue to test plants using a cotton swab. At least 5 plants of each species were inoculated. All test plants were tested for LSMV at 3–6 wk by mechanical inoculation of *Nicotiana clevelandii*.

[b]Aphid transmission was done by allowing aphids acquisition access to detached speckles virus complex infected leaves in a petri dish for 24 hr. Aphids were then transferred to the test hosts for a 48 hr inoculation access period. Aphid and mechanical transmission recoveries from test hosts to *N. clevelandii* were made after 3–6 wk.

TABLE 2. Transmission of lettuce speckles mottle virus (LSMV) and beet western yellows virus (BWYV) from infected *Nicotiana clevelandii*

Mode of transmission	Component transmitted	Plants infected with indicated virus/viruses		
		LSMV	BWYV	LSMV + BWYV
Mechanical[a]	LSMV	20/20[c]	...	20/20
	BWYV	...	0/20	5/32
Aphid[b]	LSMV	0/20	...	20/20
	BWYV	...	30/30	20/20

[a]Mechanical transmission is to *N. clevelandii*. Source plants were ground in a mortar and plants inoculated with a cotton swab.

[b]*Myzus persicae* were given acquisition access to detached leaves in petri dishes infected with the indicated source. Twenty-five to thirty aphids were used to inoculate each plant. Inoculation was to *N. clevelandii* for LSMV and speckles virus complex infected plants, but was to *C. bursa-pastoris* for BWYV only.

[c]Ratio is the number of plants infected (numerator) over the number tested (denominator).

TABLE 3. Aphid transmission of lettuce speckles mottle virus (LSMV) from mixed infections with various beet western yellows virus isolates (RY-1 and SP-yell) and turnip yellows virus (TuYV)[a]

Component transmitted[b]	Plants infected with indicated virus/viruses				
	LSMV	LSMV + RY-1	LSMV + TuYV	LSMV + SP-yell	Speckles virus complex
LSMV	0/6[c]	3/6	6/6	6/6	6/6
Yellows		4/4	4/4	4/4	4/4

[a]*Nicotiana clevelandii* were first mechanically inoculated with LSMV and one week later with a BWYV isolate or TuYV.

[b]*Myzus persicae* were given acquisiton access to detached leaves in petri dishes infected with the indicated source. Twenty-five to thirty aphids were used to inoculate each plant. Inoculation was to *N. clevelandii* for LSMV, but was to *Capsella bursa-pastoris* for yellows only.

[c]Ratios represent aphid transmission from the source plants. Numerator is number of plants infected, denominator is the number tested.

preparations that were subjected to rate-zonal sucrose density gradient centrifugation (Table 4). Only LSMV purified from SVC-infected plants was aphid transmissible. Purified LSMV and BWYV from separate plants were mixed and centrifuged, both could be recovered from the same zone in the gradient; LSMV alone was mechanically transmissible but not aphid transmissible. The in vitro presence of BWYV with LSMV did not make LSMV aphid transmissible which suggests that the physical association of the viruses was not responsible for the dependent transmission and that simultaneous replication of both viruses in the same plant is necessary for LSMV to become aphid transmissible. Because LSMV was only aphid transmitted from plants infected with both BWYV and LSMV (the SVC), some virus/vector relationships for aphid-transmissible LSMV were investigated.

It was thought that LSMV perhaps could be acquired and inoculated in shorter periods than necessary for the transmission of the phloem-limited BWYV. However, the results (Table 5) show that the acquisition and inoculation thresholds for aphid transmission of LSMV are typical of those for persistent-circulative viruses and similar to those reported for BWYV (3). Although one of 10 plants was infected after an acquisition access period of 15 min, significant transmission occurred only after 24 and 48 hr of acquisition access. This suggests that feeding, not just probing, is necessary for the aphid to acquire LSMV from the mixed infection. The results of inoculation access experiments (Table 5) also showed that feeding is necessary for the aphid to induce infection, since no infection occurred after less than 2 hr of inoculation access time, and percent transmission increased with inoculation time.

TABLE 4. Transmission of lettuce speckles mottle virus (LSMV) and beet western yellows virus (BWYV) from purified preparations

Mode of transmission	Component transmitted	Purified preparation[a]			
		LSMV	BWYV	LSMV + BWYV	Speckles virus complex
Mechanical[b]	LSMV	20/20[d]	...	20/20	20/20
Aphid[c]	LSMV	0/20	...	0/20	20/20
	BWYV	...	20/20	20/20	16/20

[a]LSMV is a semipurified preparation from an LSMV infected plant. BWYV is a semipurified preparation from an BWYV infected plant. LSMV + BWYV is an in vitro mixture of these viruses, each semipurified from single infected plants and the speckles virus complex is LSMV and BWYV semipurified from a plant infected with both viruses.
[b]Mechanical inoculations were to *Nicotiana clevelandii* with material from density gradients. Material was mixed with Celite and the plants were inoculated with the mixture by means of a cotton swab.
[c]Aphid transmissions were from sucrose gradient purified preparations fed to aphids through membranes. Aphids were given a 24 hr acquisition access period. LSMV inoculations were to *N. clevelandii* and BWYV were to *Capsella bursa-pastoris* or *N. clevelandii*.
[d]Ratio is the number of plants infected (numerator) over the number tested (denominator).

TABLE 5. Transmission of lettuce speckles mottle virus from the speckles virus complex to *Nicotiana clevelandii* by *Myzus persicae*

Transmission aspect	Duration of access period (hr)						
	0	0.25	0.5	1.0	2.0	24	48
Acquisition[a]	0[c]	1	0	0	0	10	10
Inoculation[b]	0	0	0	0	4	5	10

[a]Aphids were given acquisition access to detached speckles virus complex infected lettuce leaves in petri dishes for the times given. Aphids were then transferred in groups of five to individual *Nicotiana clevelandii* and allowed an inoculation access period of 48 hr.
[b]Aphids were allowed access for 24 hr to detached speckles virus complex infected lettuce leaves in petri dishes, then transferred in groups of five to individual *Nicotiana clevelandii* plants and allowed inoculation access for the time given.
[c]The number of plants infected of 10 tested.

Retention of LSMV by aphids was determined by transferring single aphids to individual plants daily. Table 6 shows that some aphids transmitted LSMV for as long as 14 days, when the experiment was terminated. These retention data and the inoculation and acquisition access data indicate that LSMV-SP is transmitted in a persistent manner similar to BWYV, the other virus in the complex. *Chenopodium capitatum* was used as the assay host for this experiment because the aphids survived well on it and definite symptoms appeared in a few days. Aphids did not survive well on *N. clevelandii* or on lettuce; in addition, symptom expression on lettuce was poor in greenhouse-grown plants and developed slowly. It is interesting that *C. capitatum* is a host for LSMV but not BWYV, therefore, the transmitting aphids were initiating only infections of LSMV.

Serological characteristics of LSMV and BWYV. When investigating the serological properties of LSMV isolated from singly and SVC-infected plants, results indicated that genomic masking of LSMV by BWYV coat protein may have occurred. The infectivity of mechanically transmitted, antiserum-treated LSMV generally was less than that of untreated LSMV. This reduction occurred for semipurified mechanically transmitted LSMV from singly and SVC-infected plants when tested with antisera to BWYV or healthy shepherd's purse. The LSMV from singly infected plants was not affected differently by BWYV or healthy shepherd's purse antisera. LSMV and BWYV are not serologically related; in three experiments 3/28 plants were infected by LSMV incubated with BWYV antiserum and 5/28 were infected when incubated with healthy shepherd's purse antiserum, as compared to 17/28 infected untreated control plants.

Results of experiments with aphid-transmissible semipurified viruses from the SVC-infected plants showed that treatment with antiserum to healthy shepherd's purse did not reduce the infectivity of LSMV or BWYV, but BWYV antiserum destroyed the aphid transmissibility of both viruses (Table 7). Thus, aphid-transmissible LSMV in the mixed infection acquired the serological properties of BWYV.

Physical properties of LSMV. The in vitro longevity of LSMV

TABLE 6. Retention of lettuce speckles mottle virus (LSMV) by individual *Myzus persicae*[a]

Aphid	Transmission record (days)[b]													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	−	+	−	+	+	+	+	−	−	D				
2	−	+	−	−	+	+	−	+	−	−	−	−	+	+
3	−	−	+	+	+	+	+	−	−	+	D			
4	−	−	+	+	+	+	+	D						
5	−	−	−	−	−	−	+	+	D					
6	+	D												
7	−	+	+	+	+	+	+	+	−	−	+	D		
8	−	−	+	−	−	+	+	+	D					
9	−	−	D											
10	+	+	+	+	+	+	+	+	D					
11	−	−	−	+	−	−	−	+	−	−	−	−	−	−
12	−	−	+	+	−	−	+	+	+	−	−	+	+	+
13	−	−	+	−	+	+	−	−	−	+	−	−	−	−
14	+	+	−	+	+	+	+	+	+	−	D			
15	+	+	+	+	+	+	+	+	D					
16	+	−	+	+	+	+	+	+	+	−	−	−	+	+
17	−	+	+	+	−	+	+	+	+	−	D			
18	+	+	−	+	+	+	−	+	+	−	D			
19	+	+	+	−	+	+	−	−	−	D				
20	+	−	−	−	−	+	+	−	−	−	D			
21	−	−	+	+	+	+	−	−	D					
22	−	+	−	+	+	+	+	+	D					
23	−	−	D											
24	+	−	+	−	+	+	+	+	+	+	D			

[a]Aphids were given a 24-hr acquisition access period to detached, speckles virus complex-infected, lettuce leaves in petri dishes. Individual aphids were then transferred daily to virus-free *Chenopodium capitatum*. Plants were kept at 21 C and constant light.
[b]Symbols: plus (+) indicates infection and a minus (−) no infection; D is death of the aphid.

from singly infected plants was 24–48 hr by both systemic and local lesion assay (Table 8), whereas that of LSMV from SVC-infected plants was 24–48 hr as determined by local lesions, but by systemic assay on *N. clevelandii*, it was 96–120 hr. Thus, LSMV from SVC-infected plants was more stable than that from singly infected plants.

DISCUSSION

The aphid transmissibility of LSMV in a mixed infection with BWYV is most logically explained by genomic masking. The aphid transmission properties of LSMV from the SVC are like those of the helper virus, BWYV. Aphid-transmitted LSMV has a persistent-circulative relationship with the aphid, is serologically affected by BWYV antiserum, and LSMV from the mixed infection also is less rapidly affected by in vitro aging.

Those characteristics can be attributed to the coat protein of the virus particle. Some isolates of barley yellow dwarf virus (BYDV) and pea enation mosaic virus (PEMV) acquire aphid transmissibility because of their coat proteins (2,13,18). LSMV alone is not aphid transmissible, nor is it aphid transmissible when mixed with purified BWYV in vitro, or by sequential aphid feeding on LSMV and BWYV infected plants. It is only aphid transmissible as a result of the mixed infection and simultaneous replication of both viruses in the same plant. These conditions also exist for the genomic masking that occurs between isolates of BYDV (18).

Greater stability is exhibited for LSMV from the mixed infection when compared to LSMV alone, as determined by aging in vitro.

TABLE 7. Serological interactions of beet western yellows virus (BWYV) antiserum and aphid-transmitted lettuce speckles mottle virus (LSMV)

Antiserum	Component transmitted	Virus source[a]	
		LSMV	Speckles virus complex
HSP-AS[b]	LSMV	0/20[c]	20/21
	BWYV	...	20/20
BWYV-AS	LSMV	0/20	0/21
	BWYV	...	0/20

[a]Represents aphid transmission of LSMV and BWYV from semipurified antiserum treated preparations from LSMV infected plants and speckles virus complex infected plants. Samples were incubated with the desired antiserum for 0.5 hr at 37 C and then centrifuged in 10–40% sucrose gradients for 4 hr at 61,000 *g*.

[b]Antisera are to healthy shepherd's purse (HSP-AS), and beet western yellows virus (BWYV-AS).

[c]Numerator represents the number of plants infected and denominator represents the number tested.

TABLE 8. Longevity in vitro for lettuce speckles mottle virus (LSMV) from expressed sap of single or mixed infections

Source[a]	Assay host	Infectivity of virus or virus complex in sap aged:						
		0 hr	8 hr	24 hr	48 hr	72 hr	96 hr	120 hr
Speckles virus complex	*Nicotiana clevelandii*	+[b]	+	+	+	+	+	–
	Chenopodium quinoa	154[c]	168	110	0	0	0	0
LSMV	*Nicotiana clevelandii*	+	+	+	–	–	–	–
	Chenopodium quinoa	224	171	5	0	0	0	0

[a]The source is sap expressed from LSMV- or speckles virus complex-infected *Nicotiana clevelandii*. Samples were 1 ml, aged for the given time.

[b]Symbols: plus (+) indicates systemic infection in at least one of two *N. clevelandii* plants that were tested. A minus (–) indicates no infection.

[c]Numbers represent total local lessions from six *C. quinoa* leaves inoculated with sap aged for the given time.

Similar stabilization of an unstable animal virus (vesicular stomatitis virus [VSV]) occurs when in mixed infection with sindbis virus (SbV) (24). VSV alone has a thermolabile coat protein, but phenotypically mixed particles from the VSV-SbV mixed infections were thermostable and serologically related to SbV. Beet western yellows virus has been shown to have an in vitro longevity of at least 8 days (4), so the greater in vitro longevity for LSMV from SVC-infected plants could be explained by genomic masking of LSMV nucleic acid by BWYV coat protein.

The fact that semipurified, aphid-transmissible LSMV from speckles virus complex infected plants is neutralized by BWYV antiserum and not antiserum to healthy shepherd's purse is the strongest evidence suggesting genomic masking of LSMV by BWYV coat protein.

The effects of serum treatment on mechanically transmissible LSMV may be explained by the intrinsic nature of LSMV. LSMV is an unstable virus which lacks a functional coat protein (9). LSMV exhibits sensitivity to low concentrations of ribonuclease (9), and ribonuclease has been shown to be a common contaminant of serum (10). The nonspecific reduction in infectivity observed for mechanically transmitted LSMV could possibly be explained by ribonuclease in the sera.

As a direct result of the dependent transmission/geomic masking mechanism, aphid-transmissible LSMV has a wider host range than does mechanically transmitted LSMV. Therefore, LSMV can potentially cause disease in many plant species when transmitted with BWYV. The mixed infection may result in different disease syndromes: that in lettuce plants infected by LSMV and BWYV differs from that in plants infected by BWYV alone. The host range data show that LSMV also may infect plant species that are immune to BWYV. Thus, when the aphid acquires LSMV and BWYV from a plant with the mixed infection, it may initiate three types of infections: LSMV alone, LSMV and BWYV, or BWYV alone. In the field, when LSMV is inoculated to plants that are not hosts for BWYV, LSMV aphid transmissibility probably ceases because no helper virus is present. Therefore, inoculum from a source containing LSMV and BWYV would cause only primary LSMV infection in plants of such a species. A similar set of conditions governs transmission of the semipersistent, helper-dependent parsnip yellow fleck virus (PYFV). PYFV is dependent upon the presence of anthiscus yellows virus (AYV) for aphid transmission (8). In Britain, PYFV is the most common cause of viruslike symptoms in parsnip even though parsnip is resistant to AYV (15). Therefore, the primary inoculum that causes spread in the field probably comes from some other doubly infected host plants and secondary spread from parsnip to parsnip by aphids probably does not occur.

The LSMV may not exist alone in nature except in some species which are not hosts of BWYV. The virus in these hosts probably would not be transmitted except by vegetative propagation. In the mixed infection, however, LSMV could cause disease in many species, and travel distances (by the aphid vector) not attainable by mechanical transmission.

The mixed infection and dependent transmission of LSMV and BWYV resembles those of other virus complexes situations reported from various parts of the world such as carrot motley dwarf, tobacco rosette, tobacco yellow vein, and groundnut rosette (1,14,21,23). All are persistently transmitted by aphids. Adams and Hull (1), working with three of these virus complex-caused diseases, discovered an interesting characteristic of dependent transmission. By creating various combinations of mixed infections involving the different helper and dependent viruses, they were able to gain aphid transmission of the dependent viruses in some cases with helper viruses from different complexes and sometimes this resulted in a change in vector specificity for the dependent virus. Groundnut rosette assistor virus is transmitted by *Aphis craccivora*. Tobacco yellow vein virus (from the tobacco yellow vein disease complex) is transmitted by *M. persicae* when with the tobacco yellow vein assistor virus. In a mixed infection of groundnut rosette assistor virus and tobacco yellow vein virus, tobacco yellow vein virus became transmissible by *A. craccivora*. These results demonstrated that the activity of the helper viruses was not

always specific to the complex isolated from the field. Similar results were obtained in the present work with different isolates of BWYV and TuYV as helper viruses. Thus, if LSMV were to infect a host in nature that is not a host for BWYV, this may not necessarily result in a dead end. Another helper virus, possibly even one transmitted by a different vector could infect the plant and then make LSMV transmissible by the vector of the new helper virus.

The data of Adams and Hull (1) also showed a change in vector specificity corresponding to that of the introduced helper virus; this suggests that the genomic masking mechanism was operative and is in line with current theory that vector specificity for persistent-circulative viruses is determined by coat protein (2,12,13,19).

The disease potential of the SVC is great; often it causes a more severe disease reaction than by either virus alone. In nature, the helper virus (BWYV) is transmissible alone and often is found alone (3). BWYV causes a disease in lettuce, but does not appear to significantly affect the size and weight of crisphead lettuce at maturity (20). Field observations of the lettuce speckles disease suggest that when LSMV and BWYV infect the same lettuce plant, the stunting effect is much more severe than that caused by BWYV infection alone. The same effect was observed in greenhouse-grown *N. clevelandii*, especially in young plants. Similar results have been reported for motley dwarf in carrots (22). Mixed infection with both viruses is much more severe than infection only by carrot red leaf virus, the helper virus. Thus, it is apparent that genomic masking of LSMV, when in the mixed infection with BWYV, may significantly affect its transmission by aphids and the resulting plant disease.

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