Host Range, Aphid Transmission, and Properties of Muskmelon Vein Necrosis Virus

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ABSTRACT

A slightly flexible, rod-shaped virus found in muskmelons in California was partially purified from broadbean (*Vicia faba*) by differential centrifugation and zone electrophoresis. The virus, named muskmelon vein necrosis virus (MVNV) is apparently restricted in the cucurbitaceae to the genus *Cucumis*. MVNV induced a distinct veinal necrosis in varieties of *Cucumis melo* in all but the terminal leaves. It infected numerous legumes systemically, and induced local lesions on several hosts, including *Chenopodium amaranticolor* and *Gomphrena globosa*.

A serological relationship with red clover vein mosaic virus (RCVMV) was revealed in gel diffusion tests against RCVMV antiserum with electrophoretically purified MVNV treated with detergent. MVNV had a normal length of 674 mμ and a diam of approximately 15 mμ. MVNV had a thermal inactivation point between 50 and 55°C, 2-7 days of longevity in vitro, and a 10^{-3}-10^{-4} dilution end point, depending on the source plant. The green peach aphid *Myzus persicae* transmitted MVNV very efficiently in a nonpersistent manner from *Vicia faba* to *C. melo* var. *reticulatus* 'Persian', but less efficiently when reciprocal transmissions were attempted. The aphid acquired virus during probes as short as 10 sec. Virus transmission increased with increased probe duration up to 30 sec, but declined following longer probes. Phytopathology 60:166-170.

Muskmelon vein necrosis virus (MVNV) was first reported from California by Freitag in 1952 (3) as a distinct cucurbit-infecting virus. Symptoms of the characteristic disease caused by this virus in muskmelons have been observed since that report, and Milne et al. (6) isolated MVNV in 1968 in California from some early-planted *Cucumis melo* L. var. *inodorus* Naud. 'Casaba', 'Crenshaw', and *C. melo* L. var. *reticulatus* Naud. 'Persian' melons. At that time, the virus was recognized as being rod-shaped, but distinct from watermelon mosaic virus (WMV) both in particle morphology and in the type of disease it induced in muskmelons. The present study was done to characterize the virus and establish its identity.

MATERIALS AND METHODS.—*Purification of MVNV.*—Virus for purification was increased in *Vicia faba* L. 'Long Pod Fava' (broadbean). Three to 4 weeks after inoculation, freshly harvested bean leaves and stems (500-1000 g) were cooled overnight at 4°C, then homogenized in a Waring Blendor with an equal volume of 0.3 M potassium phosphate buffer (pH 7.6) containing 0.05 M sodium diethyldithiocarbonate and 0.2% mercaptoacetic acid. The pulp was removed by straining the cold extract through cheesecloth, emulsified in a blender in one-fifth volume of chloroform, and left to stand for 45 min at 4°C. The emulsion was broken by centrifugation (11,700 *g* for 20 min). The supernatant was decanted, filtered through glass wool, and centrifuged at 78,000 *g* for 1.5 hr, and the resulting pellets were each resuspended in 2-3 ml of 0.037 M potassium phosphate buffer (pH 7.6) containing 0.1% mercaptoacetic acid. The virus was purified further by centrifugation and zone-electrophoresis similar to the procedure (5) used in purifying watermelon mosaic virus (WMV).

Serology.—To produce antiserum to MVNV, rabbits were injected intravenously with 2 ml of purified virus, followed at weekly intervals with four injections each of 4 ml of virus emulsified with an equal volume of Freund's incomplete adjuvant. Intramuscular and subcutaneous injections were alternated. Finally, 1 week prior to bleeding, 1 ml of virus was injected intravenously. Microprecipitin and Ouchterlony agar double-diffusion serological tests were carried out as previously described (5).

Electron microscopy.—Leaf dips (2) or epidermal strips (4) were used to obtain preparations of MVNV for examination in the electron microscope. Specimen contrast was obtained either by staining with 2% potassium phosphotungstate (KPT) or by shadowing with palladium. Particle length was determined on samples prepared from leaflets of *Vicia faba* 3 weeks after inoculation. The magnification of the electron microscope was calculated for each series of plates from photographs of a grating replica (28,800 lines/inch).

Insect transmission.—For transmission tests, noninoculative green peach aphids, *Myzus persicae* (Sulz.), were reared on sugar beets, radish, and Chinese cabbage; the potato aphid, *Macrosiphum euphorbiae* (Thos.), and the melon aphid, *Aphis gossypii* Glov., were reared on squash. All aphid species were kept at greenhouse temperatures of about 21°C. The beetles used were collected in the field.

RESULTS AND DISCUSSION.—*Host range of MVNV.*—A number of species from different plant families was tested to determine their susceptibility to MVNV. Inoculum was taken from Persian plants showing typical symptoms of the disease, and recoveries were attempted back to Persian from most plants.

Susceptible plants included:

Cucurbitaceae.—*Cucumis melo* L. var. *reticulatus* Naud. 'Persian', 'PMR-45', 'Golden Gate', 'King Henry',

'Honeyrock', 'SR-91', 'Top Mark', B633-3 (7); *C. melo* L. var. *inodorus* Naud. 'Green-fleshed' (Honeydew), 'Crenshaw', 'Casaba'; *C. melo* L. var. *chito* Naud. 'Mango'; *C. sativus* L. 'National Pickling', 'SMR-58'.

Leguminosae.—Vicia faba L. (broadbean) 'Long Pod Fava', 'Bell Bean'; *V. sativa* L. (common vetch); *V. villosa* Roth. (hairy vetch); *Cyamopsis tetragonoloba* (L.) Taub. (guar); *Sesbania macrocarpa* Muhl.; *Lupinus nanus* Dougl.; *L. densiflorus* Benth.; *Melilotus indica* (L.) All. (sour clover); *M. alba* Desr. (white sweet clover); *Astragalus oxyphysus* Gray; *Pisum sativum* L. 'Laxton's Progress', 'Dwarf Telephone' (garden pea); *Lathyrus odoratus* L. (sweetpea); *Trifolium hybridum* L. (Alsike clover); *T. hirsutum* All. (rose clover); *T. incarnatum* L. (crimson clover); *T. pratense* L. (red clover); *T. repens* L. 'Ladino Clover' (white clover); *T. repens* L. (common white clover); *Medicago hispida* Gaertn. (burr clover).

Chenopodiaceae.—Chenopodium amaranticolor Coste & Reyn.; *C. quinoa* Willd.

Amaranthaceae.—Gomphrena globosa L.

Plants which did not prove susceptible to MVNV in greenhouse mechanical inoculation tests included the following species:

Cucurbitaceae.—Cucurbita pepo L. 'Small Sugar', 'Yellow Crookneck', 'Early White Bush Scallop', 'Table Queen'; *C. maxima* Duch. 'Warted Hubbard', *C. mixta* Pangalo 'Green Striped Cushaw'; *C. moschata* Poir 'Kentucky Field'; *Citrullis vulgaris* Schrad. 'Striped Klondike' (watermelon); *Momordica balsamina* L.; *Luffa acutangula* Roxb.

Leguminosae.—Vigna sinensis Savi 'Blackeye' (cowpea); *Phaseolus vulgaris* L. 'Pinto', 'Refugee', 'Medal', 'Blue Lake', 'Bountiful', 'Sutter Pink', 'Red Kidney'; *P. aureus* Roxb.; *Lotus uligninosus* L. (big trefoil); *Medicago polymorpha* L. (California burr clover); *M. sativa* L. (alfalfa); *Glycine max* (L.) Merr. (soybean).

Solanaceae.—Nicotiana clevelandii Gray; *N. glutinosa* L.; *N. tabacum* L. 'Turkish' (tobacco); *Datura stramonium* L. (Jimson-weed).

Compositae.—Lactuca sativa L. (lettuce); *Callistephus chinensis* Nees. (aster).

Chenopodiaceae.—Beta vulgaris L. (sugar beet).

Caryophyllaceae.—Dianthus caryophyllus L. (carnation); *D. barbatus* L. (sweet william).

Malvaceae.—Lavatera trimestris L.

Symptoms.—Symptoms were similar in all horticultural varieties of *C. melo* L. var. *reticulatus, C. melo* var. *inodorus,* and *C. melo* var. *chito* mechanically inoculated in the greenhouse. The initial symptoms, local necrotic reddish-brown lesions, appeared in about 10 days on inoculated cotyledons and leaves of muskmelon plants. The lesions were usually surrounded by a halo of light-green tissue. The second or third leaf from the terminal developed a chlorotic stippling associated with the smaller veins, followed by vein clearing, cupping, and chlorosis. The interveinal areas became raised, and the young leaves assumed a rugose-like appearance. The small veins developed a rusty brown cast, becoming progressively necrotic with aging (Fig. 1-A). The first to become affected were the smaller veins along the margins and bases of the leaves. These areas then enlarged and wilted, and the leaves eventually become senescent, dry, and brittle. The petiole also became necrotic, together with a necrotic, cork-like streaking of the stem (Fig. 1-C). Severe necrosis usually resulted in death of the inoculated muskmelon plants.

Naturally infected melon plants were stunted, and had chlorotic, rugose, downward-cupped leaves with vein yellowing and light-green mottled areas. The chlorotic and necrotic spotting (Fig. 1-B) suggested the type of damage associated with *Empoasca* leafhopper feeding or spider mite infestations. The necrotic veinal tissue developed into enlarged necrotic areas usually first evident along leaf margins, but later spreading over the entire leaf surface. Also characteristic of the disease symptoms was a brown, necrotic, cork-like streaking along the stems of infected plants.

Muskmelon vein necrosis virus has been recovered from naturally infected cantaloupe, honeydew, casaba, and crenshaw melons collected in the field. The virus has also been recovered by sap inoculation from naturally infected field peas. The virus could overwinter in such legume crops, which would then be a source of aphids as well as inoculum for the muskmelon crop in the spring. Observations made in Stanislaus County, California, indicated that MVNV was always more evident early in the season, and often difficult to find on later-planted melon crops. This should be expected if the legume crop is a good virus source for aphids and muskmelons are a poor source.

The virus was recovered from cucumber, but inoculated plants showed no obvious symptoms, apart from a slight stunting. Many of the leguminous species from which MVNV was reisolated showed no symptoms. Although broadbean was a good source of MVNV for studies of aphid transmission, purification, and electron microscopy, it was unsatisfactory as an assay host because the mild mottling on younger leaves and necrotic streaking of stems were often not evident or easily recognized. However, MVNV did induce local lesions on white sweet clover and common vetch, and it produced vein chlorosis (Fig. 2-A) and vein banding (Fig 2-B) on red clover. Local lesions were also induced in *C. amaranticolor, C. quinoa,* and *G. globosa.*

Insect transmission.—Three species of aphids were tested as vectors in preliminary trials: green peach aphid, *Myzus persicae* (Sulz.); potato aphid, *Macrosiphum euphorbiae* (Thos.); and melon aphid, *Aphis gossypii* Glov. Groups of 10 aphids were given 1 day of test feeding/test plant following 1 day of acquisition. Only the green peach aphid transmitted the virus from melon to melon, but it was an inefficient and erratic vector, infecting only 13 of 150 (8.7%) Persian melon plants. The potato aphid and melon aphid each failed to infect 50 melon plants, though transmission might be achieved by fasting and short feeding periods in additional tests with other source-test plant combinations.

Persian melon and *Vicia faba* were then compared as virus source plants with groups of 10 green peach aphids given 1-day acquisition and 1-day test feeding

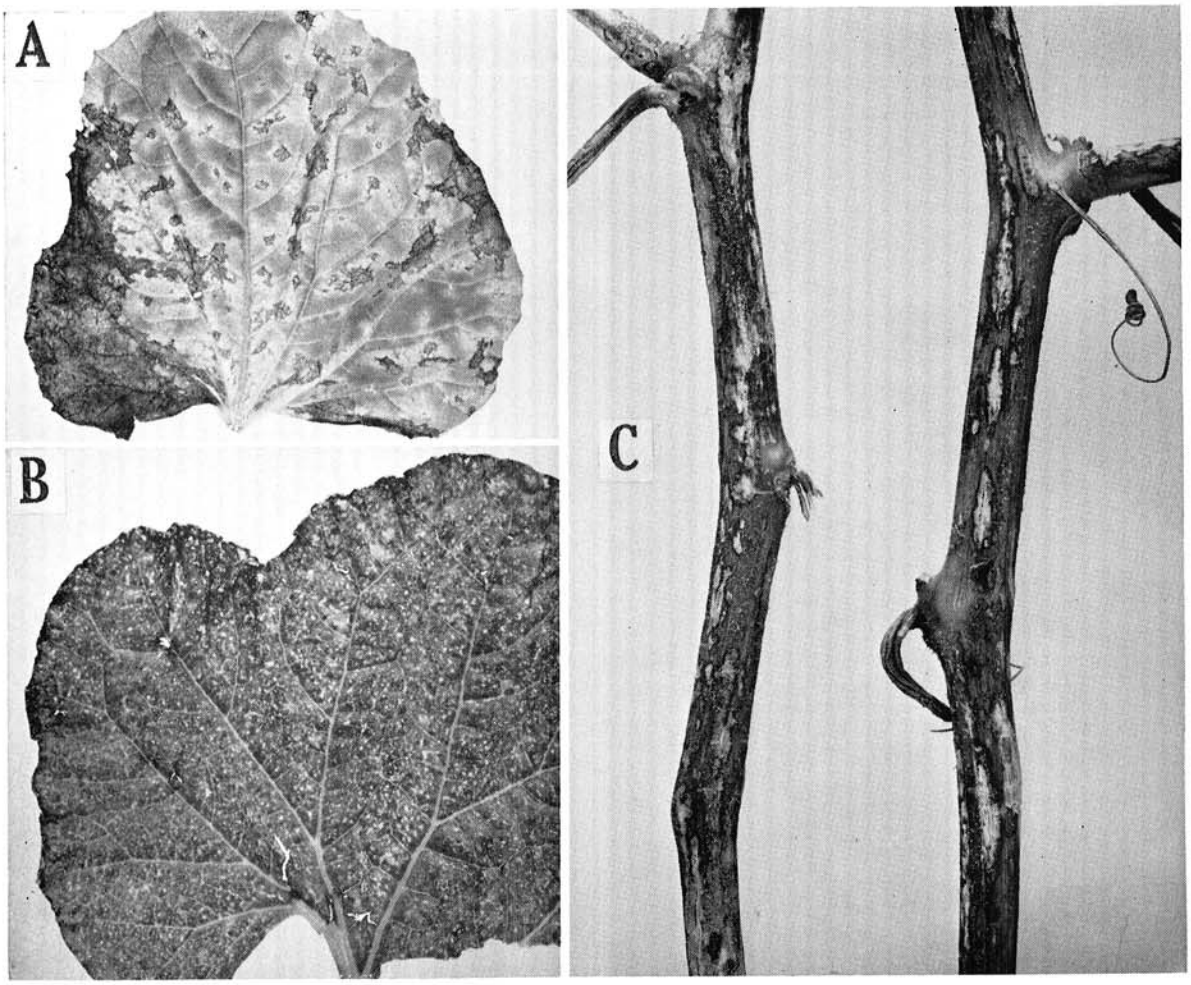

Fig. 1. Symptoms of muskmelon vein necrosis virus; **A)** casaba melon leaf with necrotic veins and marginal necrotic areas; **B)** chlorotic and necrotic spotting of leaf from a naturally infected Persian melon; and **C)** same as B, but showing irregular necrotic corklike streaking of stems.

periods. The test plant, again, was Persian melon, because *Vicia faba* often develops mild symptoms that are difficult to identify. *Vicia faba* was found to be a better source plant for the aphids, with 103 of 137 plants (74.5%) developing infection, in contrast to only 22 of 162 (13.5%) when aphids were transferred from melons.

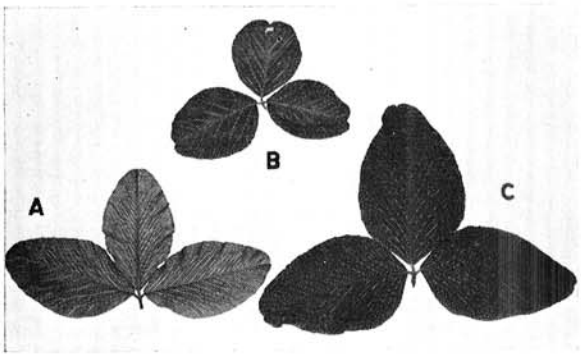

Fig. 2. Symptoms produced in red clover leaves after inoculation with an isolate of MVNV. **A)** Vein yellowing with interveinal chlorosis; **B)** mild chlorotic vein banding; **C)** healthy.

When groups of 20 green peach aphids were given a 1-day acquisition feeding period on garden pea, burr clover, or melon, and then transferred to Persian melon test plants, burr clover was the best virus source plant. Infection of melon test plants was 1 of 15 from garden pea, 4 of 25 from melon, and 10 of 15 from burr clover.

Single green peach aphids fasted for 1 hr, and then, given one short-timed, naturally interrupted acquisition feeding on *Vicia faba* infected with MVNV, transmitted virus to healthy Persian melon test plants during a 1- to 3-hr test access period as follows: 10 sec, 2/8; 11-20 sec, 12/93; 21-30 sec, 11/48; 31-40 sec, 1/20; 41-50 sec, 0/8; 51-60 sec, 0/3; and 61-120 sec, 3/20. The results indicate an acquisition threshold period of 10 sec, which is consistent with a nonpersistent virus-vector relationship.

MVNV was not transmitted by western spotted cucumber beetle, *Diabrotica undecimpunctata undecim-*

TABLE 1. Physical properties of muskmelon vein necrosis virus (MVNV)

				Longevity in vitro				Source plant
Control/days	1	2	3	4	5	6	7	
20/20[a]	1/20	1/20	0/15	0/10	0/15	0/10	0/10	Persian[b]
19/20	19/20	15/20	8/20	7/15	3/20	1/20	1/15	Broadbean[c]
				Thermal inactivation				
Control/ Temp.	40°C	45°C	50°C	55°C	60°C	65°C	70°C	
30/30	19/20	20/20	27/30	0/30	0/30	0/10	0/10	Persian
				Dilution tolerance				
Control/dilution	10^{-1}	10^{-2}	10^{-3}	5×10^{-3}	10^{-4}	5×10^{-4}	10^{-5}	
23/25	21/25	18/25	2/25	0/25	0/25	0/25	0/25	Persian
25/25	24/25	20/25	15/25	14/25	12/25	3/25	2/25	Broadbean

[a] Numerator = no. plants infected; denominator = no. plants inoculated.
[b] Persian melon, *Cucumis melo* L. *reticulatus* Hand.
[c] Broadbean, *Vicia faba* L.

punctata Mannerheim, or the western striped cucumber beetle, *Acalymma trivittata* (Mannerheim). When two insects were transferred to each melon test plant in the cotyledon stage, the spotted cucumber beetle failed to infect 37 plants, and the striped cucumber beetle, 52 plants.

Physical properties and particle morphology.—Source plants for MVNV in these tests were Persian melons and broadbean, and assays were on Persian melon. All tests were conducted with crude plant sap; the results are presented in Table 1. Crude plant sap extracted from Persian melons infected with MVNV remained active for 8 months when frozen and stored in test tubes.

Virus particles typical of viruses in the Potato Virus S Group (1) were quite common in negatively stained dip preparations from Persian melon leaves showing symptoms of MVNV in the field or greenhouse. Virus particles were more abundant, however, in leaflets of *Vicia faba* plants inoculated with MVNV. Consequently, this host was used to prepare specimens for particle measurements. From 500 particles of MVNV measured, a normal length of 674 mμ was calculated based on 78% of the particles. A particle diameter of approximately 15 mμ was determined. The particles, although comparatively rigid, were often slightly curved to one side and very consistent in length compared with the threadlike particles of WMV (Fig. 3).

Purification and serology.—Zone-electrophoresis revealed a slow moving opalescent band exhibiting birefringence and containing rod-shaped particles. This band was infectious to *Vicia faba* and Persian melon plants. As in the purification of WMV (5), no such properties were associated with an opalescent band moving more rapidly toward the cathode. Serological tests revealed that this latter band comprised the host material.

An antiserum of low titer was obtained by injecting the virus band from electrophoresis purification. A homologous titer of 64 was determined in micro-precipitin tests with virus adjusted to 0.5 absorbance at 260 mμ. Gel diffusion tests with this serum against homologous virus treated with 2% sodium dibutylnaphthalene sulfonate (Leonil SA) (5) were not satisfactory, probably because of the low titer of the serum.

Because MVNV infected legumes readily and was similar in particle morphology to red clover vein mosaic virus (RCVMV) (8), preliminary serological tests were carried out. Antiserum to RCVMV supplied by C. Wetter (Saarbrucken, Germany), was used in gel diffusion tests. An isolate of RCVMV, obtained from R. J. Shepherd, was purified and used as the homologous test antigen. The results, illustrated in Fig. 4, demonstrate that MVNV is serologically related to RCVMV. No conclusions can be reached, however, as to the closeness of the relationship between RCVMV and MVNV in advance of reciprocal tests with antiserum to MVNV, cross protection tests, and inoculation of cucurbits with several isolates of MVNV and RCVMV.

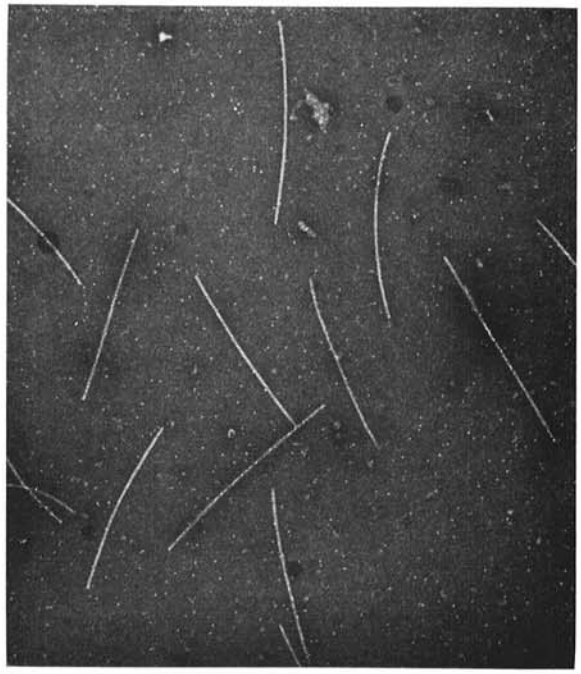

Fig. 3. Negatively stained, rod-shaped virus particles found in leaf dips prepared from *Vicia faba* systemically infected with MVNV (about × 39,500).

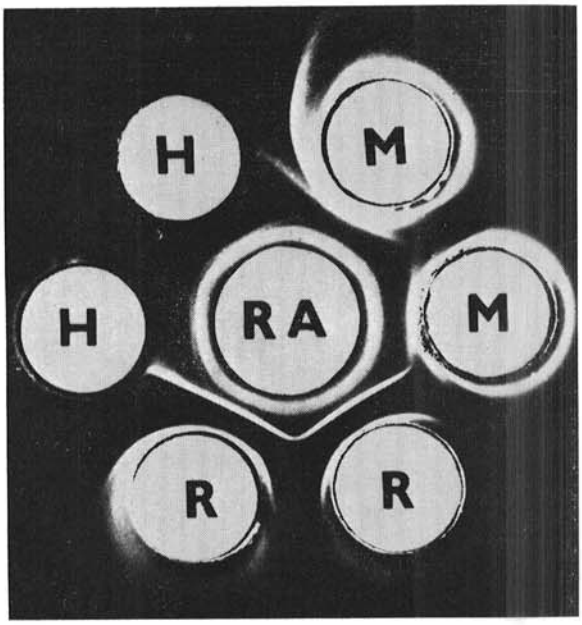

Fig. 4. Agar-gel diffusion with detergent-treated viruses and healthy sap. The center well contains RCVMV antiserum (RA). The outer wells contain MVNV (M), RCVMV (R), healthy sap (H).

DISCUSSION.—The failure to demonstrate infection of cucurbits other than those belonging to the genus *Cucumis* is in marked contrast to findings with other common cucurbit-infecting viruses such as WMV and cucumber mosaic virus (CMV). Useful for identifying MVNV was this apparent lack of infection of non-*Cucumis* cucurbits, together with the distinctive symptoms produced in Persian and the local-lesion reaction on *C. amaranticolor*.

The number of leguminous hosts demonstrated in greenhouse mechanical inoculation tests, together with the indicated serological relationship to RCVMV, suggests that leguminous plants in nature may be the main source of MVNV inoculum for cucurbit hosts. So far, MVNV has been found only in Sutter and Stanislaus counties in California, and only a small percentage of the plants usually exhibit symptoms in these areas. This apparent lack of secondary spread is in marked contrast to observations in fields infected with WMV in California, where secondary spread can result in almost 100% infection with WMV by the end of the growing season. An explanation for the relative infrequency of MVNV may lie in the inefficiency with which *M. persicae* transmitted MVNV from Persian compared with *V. faba* as the source plant.

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