The Influence of Inoculation Procedure on the Host Range of Pea Seed-Borne Mosaic Virus

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ABSTRACT

A total of 47 plant species representing 12 families became infected with pea seed-borne mosaic virus (PSbMV) when inoculated either by two mechanical inoculation methods or by use of two aphid vectors. No single inoculation procedure caused infection in all 47 species. The data indicated that infectable species can be classified into three general groups: (i) highly susceptible species in which most individual plants could be readily infected by either rub inoculation or by aphids; (ii) marginally susceptible species in which only one or two plants were infected regardless of the inoculation method; and (iii) species whose apparent susceptibility or immunity was dependent upon the inoculation technique.

Seventy-four plant species were not infected by any of the inoculation procedures.

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Additional key word: aphids.

Compared to other legume viruses pea seed-borne mosaic virus (PSbMV) has a limited host range (1, 2, 3). The only legume species reported as hosts are *Pisum sativum* L., *Lathyrus odoratus* L., *Medicago hispida* L., four species of *Vicia* and a few *Phaseolus vulgaris* L. cultivars. Outside the Leguminosae only four species of *Chenopodium* and *Tetragonia expansa* Thunb. have been infected by mechanical inoculation. However, a few plants of *Beta vulgaris* L. (sugar beet cultivar U.S. 75), *Capsella bursa-pastoris* (L.) Medik, and *Gomphrena globosa* L. were infected using viruliferous green peach aphids (*Myzus persicae* Sulz.). This indicated that the

TABLE 1. Plant species infected with pea seed-borne mosaic virus by aphids or mechanical inoculation

Family and Latin Name	Inoculation Method					
	Aphids[a]			Rub[b]		
	M.P.	A.P.	Symptom	Crude	Concn	Symptom
Aizoaceae						
Tetragonia expansa	0/8[c]	2/8	(S)[d]	1/10	1/10	(S)
Apocyanaceae						
Vinca rosea	2/10	2/10	(S)	2/15	1/15	(S)
Amaranthaceae						
Gomphrena globosa	1/5	2/5	(S)	1/10	1/10	(S)
Chenopodiaceae						
Beta vulgaris						
Sugar beet	3/45	1/45	(S)	2/55	3/55	(S)
Table beet	2/15	1/15	(S)	1/20	1/20	(S)
B. vulgaris var. *cicla*	1/15	1/15	(S)	1/15	1/15	(S)
Chenopodium album	0/5	0/5		3/5	4/5	CLL
C. amaranticolor	0/5	0/5	( )[e]	5/5	5/5	NLL
C. botrys	4/15	3/15	CM	1/20	1/20	CM
C. capitatum	1/15	1/15	(S)	15/15	15/15	CLL
C. foetidum	5/5	4/5	CM	4/5	5/5	CM
C. murale	0/5	0/5		3/5	4/5	CLL
C. quinoa	0/5	0/5		4/5	4/5	CLL
C. urbicum	1/5	2/5	CM	4/5	5/5	CLL + CM
Compositae						
Senecio vulgaris	0/10	0/10		0/15	1/15	(S)
Zinnia elegans	2/15	0/15	(S)	0/15	0/15	
Cruciferae						
Brassica pekinensis	3/15	2/15	(S)	0/20	0/20	
Capsella bursa-pastoris	1/15	0/15	(S	0/10	0/10	
Cucurbitaceae						
Cucurbita maxima	1/5	0/5	(S)	0/10	0/10	
Leguminoseae						
Lathyrus odoratus	4/5	4/4	LR,St	5/5	5/5	LR,St
Lens culinaris	2/5	4/5	St	4/5	5/5	St
Pisum sativum	5/5	5/5	LR,St,Vcl	+[f]	+[f]	LR,St,Vcl
Medicago lupulina	1/10	0/10	(S)	0/10	0/10	
Medicago sativa	3/65	2/65	(S)	0/15	0/15	
Vicia angustifolia	4/5	5/5	St	5/5	5/5	St
V. articulata	4/5	4/5	St	10/10	10/10	St
V. benghalensis	3/3	3/3	LR,St	5/5	5/5	St
V. cornigea	5/5	5/5	St	5/5	5/5	St
V. dasycarpa				5/5	5/5	St
V. ervilia	4/4	4/4	St	5/5	5/5	St
V. faba	5/5	5/5	VC	5/5	5/5	VC
V. globosa	4/4	4/4	St	5/5	5/5	St
V. hirsuta	5/5	5/5	St	5/5	5/5	St
V. hybrida	5/5	4/5	St	5/5	5/5	St
V. lutea	...	...		5/5	5/5	St
V. macrocarpa	4/5	5/5	St	5/5	5/5	St
V. monantha	5/5	5/5	St	5/5	5/5	St
V. narbonensis	5/5	4/5	St	5/5	5/5	St
V. onobrychoides	4/4	4/4	St	5/5	5/5	St
V. pannonica	4/4	4/4	LR,St	5/5	5/5	LR,St
V. persica	5/5	3/5	St	5/5	5/5	St
V. peregrina	...	...		5/5	5/5	St
V. sativa	5/5	5/5	St	5/5	5/5	St
V. villosa	2/3	2/3	St	5/5	5/5	St
Portulacaceae						
Portulaca grandiflora	2/15	1/15	St	0/20	0/20	
Ranunculaceae						
Aquilegia sp.	2/5	0/5	(S)	0/5	0/5	
Solanaceae						
Petunia hybrida	7/15	4/15	(S)	0/20	0/20	
Urticaceae						
Humulus lupulus	1/4	0/4	(S)	0/5	0/5	

[a]M.P. refers to *Myzus persicae*, A.P. to *Acrythosiphon pisum*.
[b]Crude = juice prepared by grinding 0.5 g PSbMV-infected pea leaf tissue in 2 ml neutral phosphate buffer.

Table 1. Continued

Concn = Ten g infected root tissue homogenized in 100 ml DIECA + cysteine and concd into 2 ml neutral phosphate buffer by differential ultracentrifugation.

[c]Number of plants infected/number of plants inoculated.

[d]Symptom abbreviations: CM = chlorotic mottle; CLL = chlorotic local lesions; LR = leaf roll; NLL = necrotic local lesions; St = stunted; Vcl = vein clearing; VC = vein chlorosis; and (S) = symptomless.

[e]An occasional necrotic lesion appeared on a few inoculated leaves (see text).

[f]Twenty-two varieties of peas were tested; all were infected.

inoculation procedures used affected the range of plants that appear susceptible to PSbMV. Such information would have a pronounced effect on epidemiological studies, particularly studies on possible overwintering hosts.

To establish more precisely the host range of PSbMV, we inoculated an extensive number of plant species, both mechanically and by aphids. The results presented below indicate that the host range of PSbMV is more extensive than previously reported and that the number of susceptible species is dependent upon the inoculation procedure.

MATERIALS AND METHODS.—A total of 121 plant species belonging to 21 families were tested for susceptibility to an isolate of PSbMV (ATCC isolate PV184) that was originally obtained from infected pea seed and maintained in *P. sativum* '447'. All test plants were grown from seed in a 22-26 C greenhouse using supplemental illumination of 1.5×10^4 lx and a 16-h photoperiod as needed. Five or more plants of each species were inoculated as described below. Similar numbers of noninoculated plants were included as controls for each species tested.

Two groups of plants (2-3 wk old) were rub inoculated, after a 24-48 h preinoculation dark treatment, with either (i) crude juice prepared by grinding 0.5 g of PSbMV-infected pea leaf tissue in 2 ml of 0.01 M neutral potassium phosphate buffer, or (ii) concd virus suspension prepared by homogenizing 10 g of PSbMV-infected pea root tissue in 100 ml of 0.01 M sodium diethyldithiocarbamate + 0.01 cystein·HCl in a blender followed by one cycle of differential ultracentrifugation and suspension of the pellet in 2 ml of neutral phosphate buffer. Inoculated plants were rinsed with water and held in 26 C growth chambers at 1.5×10^4lx with a 16-h photoperiod for 14-21 days. At this time, each inoculated plant was individually indexed for the presence of PSbMV.

Two additional groups of plants were inoculated by aphids; one group by *Acyrothosiphon pisum* Harris, the other by *Myzus persicae* Sulz. All aphids were starved 3 h at 28 C or 18 h at 10 C, then given a 3-min acquisition feeding on PSbMV-infected pea leaf tissue. Between 10 and 30 aphids were caged on each test plant. After an 18-h inoculation feeding period, test plants were fumigated in a closed chamber with dichloro-divinyl pyrophosphate (DDVP) and placed in growth chambers where they were sprayed twice weekly with nicotine sulfate.

The infectivity of each tissue preparation was tested on *Chenopodium amaranticolor* Coste & Reyn. In all trials, concd preparations produced 10-25 times more lesions than unconcd preparations. A representative sample from each group of viruliferous aphids was used to inoculate seedlings of the pea cultivar 447 using the conditions described above. In every case, 100% of the assay plants became infected.

All inoculated test plants were indexed 14-21 days after inoculation for the presence of PSbMV by rub inoculation on *C. amaranticolor*. Test plants inoculated by aphids were also indexed on *P. sativum* '447' plants using prestarved *M. persicae* given a 3-min acquisition feeding.

RESULTS.—A total of 47 plant species representing 12 families became infected with PSbMV (Table 1). However, no single inoculation procedure caused infection in all 47 species. Our data indicate that infected species can be classified into three general groups; (i) highly susceptible species, (ii) marginally susceptible species in which only one or two plants were infected regardless of the inoculation method, and (iii) species whose apparent susceptibility or immunity was dependent upon the inoculation technique.

Only a limited number of legume species and one *Chenopodium* species appeared to be highly susceptible to PSbMV; e.g., most plants of the species could be readily infected by either rub inoculation or by aphids. These species were *C. foetida* Schrad., *Lathyrus odoratus*, *Lens culinaris* Medik., *Pisum sativum* (all cultivars tested), and 20 species of *Vicia*. Infected plants of these species exhibited prominent symptoms which ranged from stunting and leaf rolling to chlorotic leaf mottle (Table 1).

Marginally susceptible species, those of which only one or two plants were infected regardless of the inoculation method, included *Vinca rosea* L., *Gomphrena globosa*, *Beta vulgaris* (sugar beet and table beet), *B. vulgaris* var. *cicla*, *C. botrys* L., and possibly *Tetragonia expansa* although this last species was not infected by *M. persicae*. All infected plants of these marginally susceptible species remained symptomless.

Six of eight *Chenopodium* species were readily infected by rub inoculation, but were either difficult to infect or were not infected at all by aphid inoculation. *Chenopodium album* L., *C. amaranticolor*, *C. murale* L. and *C. quinoa* Willd. produced chlorotic or necrotic lesions when rub inoculated, but only *C. amaranticolor* produced any evidence of infection when inoculated with aphids. A few necrotic lesions developed on *C. amaranticolor* leaves on which viruliferous aphids were allowed to feed. PSbMV was recovered from these lesions but not from the adjacent tissue, indicating that infection sometimes occurred, but virus multiplication (presumably) was limited by internal factors.

One or more plants of 10 species in eight familes were not infected by rub inoculation but were infected using one or both aphid species (Table 1). Of these *M. persicae* inoculations infected all 10 species whereas *A. pisum* inoculations infected only four; *Brassica pekinensis*

(Lour.) Rupr., *Medicago lupulina* L., *M. sativa* L. and *Portulaca grandiflora* Hook.

Grouped under their family names, the 74 plant species that were inoculated, but from which PSbMV could not be recovered include the following: **Amaranthaceae**– *Amaranthus retroflexus* L., *Celosia argentea* L. var. *cristata*; **Balsaminaceae**– *Impatiens balsamina* L.; **Boraginaceae**– *Anchusa* sp.; **Carophyllaceae**– *Dianthus caryophyllus* L., *Saponaria officinalis* L.; **Chenopodiaceae**– *Atriplex hortensis* L., *Beta vulgaris* L. (four varietal types), *Chenopodium ambrosioides* L., *C. bonus-henricus* L., *C. hybridum L.*; **Compositeae**– *Arctium minus* Bernh., *Aster* sp., *Centaurea maculosa* Lam., *Cirsium arvense* (L.) Scop., *Helianthus annuus* L., *Lactuca sativa* L. var. *longifolia* Lam. 'Dark Green Romaine', *L. scariola* L., *Spinacia oleracea* L., *Taraxacum officinale* Weber, *Tithonia rotundifolia* (Mill.) Blake; **Convolvulaceae**– *Convolvulus arvensis* L.; **Cruciferae**– *Brassica oleracea* var. *gemmifera* DC., *B. oleracea* var. *viridis* L., *B. nigra* L., *B. rapa* L., *Cardaria draba* L., *Raphanus sativus* L.; **Cucurbitaceae**– *Cucumis melo* L., *C. sativus* L.; **Dipsacaceae**– *Dipsacus sylvestris* Huds.; **Gramineae**– *Avena sativa* L., *Digitaria ischaemum* (Schreb) Muhl., *Hordeum vulgare* L., *Secale cereale* L., *Triticum aestivum* L., *Zea mays* L.; **Leguminoseae**– *Arachis hypogaea* L., *Coronilla varia* L., *Glycine max* (L.) *Merr.*, *Lupinus* sp., *Melilotus alba* Desr., *Phaseolus acutifolius* Gray var. *latifolius*, *P. vulgaris* L. (three varieties inoculated by aphids, 57 varieties rub inoculated), *Sesbania* sp. Scop., *Trifolium pratense* L., T. *repens* L., *Vigna sinensis* (Torner) Sav.; **Liliaceae**– *Asparagus officinalis* L.; **Malvaceae**– *Althaea rosea* Cab., *Malva parviflora* L., *M. neglecta* Wallr.; **Plantaginaceae**– *Plantago laneolata* L.; **Polygonaceae**– *Rumex crispus* L.; **Scrophulariaceae**– *Digitalis* sp.; **Solanaceae**– *Capsicum annuum* L., *Datura stramonium* L., *Hysocyamus niger* L., *Lycopersicon esculentum* Mill., *Nicandra physalodes* (L.) Pers., *Nicotiana alata* Link & Otto, *N. angustifolia* Ruiz & Pavon, *N. clevelandii* Grey, *N. debneyi* Domin, *N. glutinosa* L., *N. tabacum* L., *N. tabacum* L. var. 'Xanthi', *Physalis floridana* Ryb., *P. lanceifolia* Rugel ex Kuntze, *P. urightil* K., *Solanum melongena* L., *S. nigrum* L., *S. tuberosum* L.; **Umbelliferae**– *Daucus carota* L.

DISCUSSION.—The number of plant species and the number of plants within a species that were infected by rub inoculation was essentially the same whether the inoculum consisted of crude plant juice or virus suspensions concentrated into neutral phosphate buffer. This suggests that neither a limited virus concentration nor the presence of unidentified "inhibitors" in the inoculum were responsible for our failure to infect mechanically the 10 species which were infected only by aphids. For these species aphids appeared to deliver PSbMV in such a way as to bypass a host defense mechanism effective against virus applied to the leaf surface.

Although *M. persicae* and *A. pisum* appeared to be equally capable of transmitting PSbMV to pea cultivars and other highly susceptible hosts, several of the marginally susceptible species were infected by *M. persicae* inoculations but not by *A. pisum*. This may reflect the ability of *M. persicae* to feed on a wider range of plants than *A. pisum*.

Our results demonstrate that the number of plant species that can be infected with PSbMV depends in part on how the inoculations were made. This fact is not only important in comparing host ranges of virus isolates from different areas but also may be important in studies on the epidemiology of this disease. Widely distributed but marginally susceptible species such as *Capsella bursa-pastoris* may overwinter the virus even though only a low percentage of the plants can be infected. Since most of these species remain symptomless even though infected, large scale indexing surveys would be required to detect traces of inocula near pea fields. However, during periods of high vector activity, such as occur in Washington during the first 2 wk in May (Aapola, *unpublished*), PSbMV could be transmitted from these sources into commercial fields.

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