The Transmission of Pea Seed-Borne Mosaic Virus by Three Aphid Species

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Published with approval of the Director, Wisconsin Agricultural Experiment Station. Supported in part by funds supplied for Project 120 by the Green Giant Company.

The helpful suggestions of R. K. Chapman are gratefully acknowledged.

Accepted for publication 16 February 1971.

ABSTRACT

Pea seed-borne mosaic virus was transmitted by *Acyrthosiphon pisum, Myzus persicae,* and *Macrosiphum euphorbiae* in a typical stylet-borne manner; i.e., after single-probe acquisition and with very short retention periods. Colonies of *M. euphorbiae* tended to be more efficient vectors than those of *M. persicae,* which in turn were often more efficient than *A. pisum,* but there was some variation among colonies of each species. Alatae were generally more efficient than apterae. When the virus was maintained exclusively by mechanical inoculations, there was a decrease in aphid transmissibility, but the process was reversed after several consecutive aphid inoculations. *Pisum sativum, Vicia faba, Vicia villosa,* and *Lathyrus cicera* were equally adequate as inoculum sources for aphid uptake. Phytopathology 61:825-828.

A new virus disease of peas (*Pisum sativum* L.), referred to as pea seed-borne mosaic or pea fizzletop, has appeared recently in the USA and possibly in other countries (2, 8, 9, 10, 11). It has caused special concern because of its seed-borne nature. Preliminary information is available on insect transmission of the USA isolates. In Wisconsin, Stevenson & Hagedorn (11) found that the virus was transmitted by *Myzus persicae* (Sulzer) (green peach aphid) and *Acyrthosiphon pisum* (Harris) (pea aphid) after acquisition feedings of a few minutes, which suggests that it is stylet-borne. Mink et al. (10), however, indicated later that their Washington isolate of the virus was transmitted by *M. persicae,* but only with acquisition feeding of no less than 1 and no more than 4 hr. This would appear to constitute a manner of transmission which coincides neither with the stylet-borne mechanism nor with the circulative one, and not even with the so-called semipersistent type (3, 13). The purpose of the present research was to elucidate these apparent contradictions, and also to obtain additional information on transmission potential by the two aphids as well as *Macrosiphum euphorbiae* (Thomas), the potato aphid. The latter species was included because it is often prevalent in the early part of the pea-growing season in Wisconsin and, thus, may play an important role in dissemination of the virus.

MATERIALS AND METHODS.—*The virus.*—The isolate of pea seed-borne mosaic virus (PSbMV) used was the same as described by Stevenson & Hagedorn (11). It had been collected in 1968, and maintained since by serial mechanical inoculations of pea. During most of this investigation, it was also maintained by consecutive aphid inoculations. The mechanically transmitted virus was designated "subisolate M", and the aphid-transmitted virus, "subisolate A". Pea source plants of either subisolate were generally used for transmission 14 to 21 days after inoculation, when symptoms were distinct in four to six expanded leaves. The second or third leaf from the apex was generally used for feeding aphids. When broad bean (*Vicia faba* L.), hairy vetch (*Vicia villosa* Roth.), and *Lathyrus cicera* L. were used as source plants, they were inoculated by aphids when 7-9 days old, and used 14 to 21 days after inoculation.

Test plants.—Infectivity was tested on pea, cultivar Dark Skin Perfection. Careful examination of over 800 noninoculated plants from the seed lot indicated no evidence of seed-borne infection by PSbMV. Seed was planted directly in 2-inch plastic pots filled with steamed field soil and maintained at 16-20 C in an aphid- and virus-free greenhouse. These plants were inoculated by aphids when 6-9 days old, and mechanically when 10-14 days old.

Insect colonies.—Colonies of the three aphid species under investigation were kept inside glass and screen cages in a greenhouse at 16-24 C. Aphids were used only when enough vigorous individuals were available in a noncrowded colony, provided by weekly transfers to fresh host plants.

The colony of *Acyrthosiphon pisum* used in the early part of this work was supplied by the Wisconsin Alumni Research Foundation (WARF); other colonies were collected from alfalfa fields near Baraboo, Racine, and Prairie du Sac and were maintained on pea. The *Myzus persicae* colony used in most tests was supplied by R. W. Fulton and termed "greenhouse"; another colony, collected near New London, was supplied by the Department of Entomology, and the rest were collected near Madison from seed cabbage (Walnut Street), pepper (Verona Road), and field cabbage (Arlington); they were all maintained on Chinese cabbage. All four colonies of *Macrosiphum euphorbiae* (from Wild Rose, New London, Arlington, and Madison) were kindly supplied by A. Aziz, Research Assistant in the Department of Entomology, and were maintained on squash.

Transmission.—Generally, vigorous adult apterae or alatae were collected with a moist brush from source plants and kept fasting in large glass vials for 1 to 3 hr before each test. Each individual aphid was allowed to probe only once on the source. For this, aphids were placed as often on the petiole as on the adaxial leaf surface of the source plant, though some moved from one to the other before probing. Each aphid was carefully watched with a ×4 binocular magnifier, and the time during which the labium touched the epidermis at a perpendicular angle was recorded as "probing time", although there is no assurance that actual probing oc-

curred throughout that period (3). A single aphid was transferred to each test plant immediately after it ended the probe; alatae were confined to the test plants with a plastic beaker. After 1 to 3 hr, the aphids were killed by spraying with a thiodan-malathion mixture. Any variations from this standard procedure are indicated where pertinent.

Inoculated test plants were kept in a third greenhouse at 20 to 28 C for at least 15 days. Most infected plants showed symptoms after 4 to 8 days (depending on temperature), but a longer period was allowed in order to confirm questionable cases, as well as to ascertain the health of control plants.

RESULTS.—*Acquisition access time.*—Aphids of the three species were allowed to feed on infected leaves for varying periods of time, ranging from single probes to several hr, then were placed on the test plants in groups of four/plant. The virus sources used were pea plants inoculated with subisolate M. Between 7 and 10% of the aphids transmitted the virus after being allowed one single, naturally terminated probe on the source. On the other hand, only one out of 120 *M. persicae,* and no *A. pisum* or *M. euphorbiae,* transmitted after periods of 10 min to 18 hr on the source plant. These results are similar to those generally reported for stylet-borne viruses (3, 13).

Virus retention.—The ability of vectors to retain PSbMV after acquiring it from infected plants was tested at an early stage of this work with a poor vector-source combination and, toward the end, with a much better one. In the first test, apterae of the WARF colony of *A. pisum* and subisolate M-infected plants were used. In the second, alatae of the greenhouse colony of *M. persicae* were the vectors, and the source plants were infected with subisolate A. The results indicated a short retention period, its actual length somewhat influenced by the relative efficiency of the vector-source combination (Table 1). The *A. pisum* colony retained only a fraction of its low infectivity for a few min after uptake, and did so only when the aphids were kept from feeding. Aphids of the *M. persicae* colony retained some infectivity after 30 min if they were not feeding, and after 5 min if feeding.

Effect of method of inoculum maintenance.—Early tests were made using inoculum maintained in peas by consecutive mechanical inoculation (subisolate M). However, it appeared later that aphid-inoculated plants might be better sources for aphid uptake, and thus, subisolate A was started. After at least three successive passages of this subisolate by means of aphids, aphid-transmissibilities of both subisolates were directly compared. Colonies of the three aphid species were used; each species was tested on different days, so that this was not a strict comparison among species. The aphids of each colony were allowed to probe alternatively on pea plants infected with one or the other subisolate. In each replication of each test, plants were chosen that had been inoculated for the same length of time (usually 14-20 days) and showed similar symptoms on the top leaves, particularly on the leaflet that was used for aphid probing. (The source plants of subisolate M, however, often showed a more advanced degree of wilting of the inoculated leaves.) A total of eight source plants of each subisolate was used. The data were analyzed by the paired t-test.

TABLE 1. Retention of pea seed-borne mosaic virus by *Acyrthosiphon pisum* apterae and *Myzus persicae* alatae when feeding or fasting after acquisition[a]

Acquisition-inoculation interval		Transmission frequency[b] by	
Time, min	Place	*A. pisum*[c] (WARF colony)	*M. persicae*[d] (Greenhouse colony)
0		6/30	11/40
5	Vial	3/30	11/40
	Plant	0/30	2/40
30	Vial	0/30	2/40
	Plant	0/30	0/40
120	Vial	0/30	0/40
	Plant	0/30	0/40

[a] Pea used as source and as test plant.
[b] Plants infected/plants inoculated.
[c] Four aphids/test plant; source plants infected with subisolate M.
[d] One aphid/test plant; source plants infected with subisolate A.

The frequency of aphid transmission was generally higher with subisolate A than with subisolate M (Table 2). The difference was not significant for the poorly transmitting WARF pea aphid colony, but was significant for the more efficient Greenhouse and Arlington colonies of the green peach and potato aphids, respectively. Mechanical inoculations of dilute juice to peas repeatedly failed to indicate any higher titer of subisolate A than of M in the source leaflets; in fact, the titer was almost always higher in leaflets infected with subisolate M.

Effect of source plant species.—One of the objectives of this work was to determine whether the known systemic hosts of PSbMV differed as sources of virus for aphid transmission. This knowledge would not only

TABLE 2. Differences in transmissibility of two subisolates of pea seed-borne mosaic virus[a]

Method of transmission	Transmission frequency[b]		
	Subisolate A[c]	Subisolate M[d]	Significance of difference[e]
Aphids (one aphid/plant)			
Acyrthosiphon pisum (WARF)	4/40	0/40	N.S.
Myzus persicae (Greenhouse)	13/40	4/40	*
M. euphorbiae (Arlington)	21/40	4/40	**
Mechanical (1:50 dilution)	23/60	42/60	*

[a] Pea used as source and as test plant.
[b] Plants infected/plants inoculated.
[c] Maintained by serial aphid transmissions.
[d] Maintained by serial mechanical transmissions.
[e] Comparisons between both subisolates analyzed by the paired t-test. *, ** = significant at 5 and 1% levels, respectively.

TABLE 3. Effect of vector and source species on the transmission of pea seed-borne mosaic virus by apterous and alate aphids to pea

Vector (colony)	Source	Transmission[a] by Apterae	Alatae
Myzus persicae (Greenhouse)	*Vicia faba*	3/20	7/20
	Vicia villosa	4/20	9/20
	Lathyrus cicera	7/20	7/20
	Pisum sativum	5/20	9/20
	All sources	19/80	32/80
Acyrthosiphon pisum (Prairie du Sac)	*Vicia faba*	3/20	5/20
	Vicia villosa	4/20	5/20
	Lathyrus cicera	6/20	6/20
	Pisum sativum	4/20	6/20
	All sources	17/80	22/80
Macrosiphum euphorbiae (New London)	*Vicia faba*	10/20	15/20
	Vicia villosa	7/20	11/20
	Lathyrus cicera	8/20	9/20
	Pisum sativum	7/20	10/20
	All sources	32/80	45/80

[a] Plants infected/plants inoculated.

facilitate further aphid transmission research, but also would help in estimations of inoculum potential in the field. An early test indicated that the WARF colony of *A. pisum* transmitted equally poorly when probing on broad bean (*Vicia faba*) or on pea infected with subisolate M. A similar comparison was made with subisolate A, this time including *M. persicae* as well as *A. pisum*; again, no clear differences between the sources were observed. Finally, a more extensive comparison of four source plants was made, including *Vicia villosa* (vetch) and *Lathyrus cicera* in addition to pea and broad bean. In this test, apterae and alatae of selected colonies of the three vector species were also compared, and were all used the same day in each replication of each source. Due to time limitations, testing on different source plants had to be done on different days. In order to minimize the effect of variation among individual plants, five different plants of each source species were used, one for each replication. Again, the four host plant species tested were, on the average, remarkably similar as virus sources (Table 3); combined transmission frequencies by all three vectors were 43, 40, 43, and 41 (out of 120) from *V. faba, V. villosa, L. cicera,* and *P. sativum,* respectively.

Vectors.—1) *Variations within aphid species.*—Comparisons were made among several colonies of each of the three vector species. Of the four *A. pisum* colonies tested, one (Prairie du Sac) appeared to be more efficient than the others and was maintained for further tests. With *M. persicae,* the differences among the five colonies tested were less marked; the Greenhouse colony was maintained, mainly because it had been used in previous tests. Three of the *M. euphorbiae* colonies transmitted with fairly good frequency, but one (Madison) failed to transmit in five trials of eight aphids each; the New London colony was maintained for further tests.

2) *Interspecific aphid comparisons.*—The selected colonies of the three vector species were directly compared, as already mentioned, by simultaneous testing of apterae and alatae on four source plant species; a $3 \times 2 \times 4$ factorial arrangement was used. The results (Table 3) were consistent with those obtained in previous tests. The Prairie du Sac colony of *A. pisum* transmitted about twice as often as did the WARF colony; it remained somewhat less efficient than the *M. persicae* colony, but the difference was not significant. Both were, in turn, significantly less efficient than the *M. euphorbiae* colony. These differences among the three aphid species were consistent, regardless of what plant species was used as a virus source. All aphids appeared to probe equally readily on the four sources, except for *M. euphorbiae,* which was always reluctant to probe on *Vicia villosa* and sometimes on *Lathyrus cicera*; however, the data indicate that this did not affect the transmission probabilities of those potato aphids that did probe on these two sources. Alatae were significantly better vectors than apterae, regardless of aphid species or source; in 10 of the 12 vector-source combinations used, the frequency of transmission by alatae was higher than that of apterae, and in the remaining two it was the same.

Of those characteristics of the aphid species possibly affecting the transmission process, only the duration of the probes could be measured. The pooled data of eight experiments, in which aphids were individually timed and tested, is presented in Table 4. It suggests that probing duration was not the reason for the observed differences in transmission frequency among species. The pea and the green peach aphids transmitted with consistent frequency (about 12 and 25%, respectively) whether their probes were short, medium, or long within the allowed 10- to 90-sec range. The potato aphid transmitted somewhat less frequently when its probes were long or very long, but this happened with only a very small fraction of the aphids; it would have been meaningful only if the other two species had shown a similar decrease in efficiency, as well as many probes, in a given probing period.

DISCUSSION.—The data on acquisition and retention periods clearly indicate that PSbMV is transmitted by aphids in a manner typical of stylet-borne viruses (3, 4, 13). This is further substantiated throughout the other tests. Kvicala & Musil's (9) pea leaf-rolling virus, which may be identical to PSbMV, is also transmitted in this way. The reason for the conflicting report from Mink et al. (10) is unclear. There is a possibility that

TABLE 4. Frequency of transmission of pea seed-borne mosaic virus by three aphid species, as related to the duration of their probes (combined data from eight experiments, all with subisolate A as source)

Probe duration, sec	Transmission frequency[a] *Acyrthosiphon pisum*	*Myzus persicae*	*Macrosiphum euphorbiae*[b]
10-15	34/275	36/132	66/137
16-30	26/208	87/365	68/134
31-60	14/116	43/140	8/30
61-90	6/57	10/41	1/9

[a] Plants infected/plants inoculated.
[b] Nontransmitting Madison colony not considered.

they worked with a different virus, although most characteristics of the virus itself and of the disease it caused, as described by them in Washington, coincide with those observed and described in Wisconsin (11). Furthermore, an isolate of the virus, obtained from infected seed collected in Washington, has been readily transmitted in this laboratory by *M. euphorbiae* after single probes on the source (Lim & Hagedorn, *unpublished data*). Thus, there is reason to believe that the Washington virus may also be stylet-borne.

The known readily infected, systemic host species of PSbMV proved to be remarkably similar as sources for uptake by those aphids that probed on them, whatever the aphid species. However, it is possible that the reluctance of *M. euphorbiae* to probe on *Vicia villosa* and *Lathyrus cicera* might render these hosts less favorable, in nature, as sources for this virus. *Vicia villosa,* a biennial common weed, could act as a reservoir of the virus.

One of the original purposes of this investigation was to estimate the relative potential of the three aphid species as vectors of PSbMV in nature. As a group, the colonies of *A. pisum* that were used appeared to be less efficient vectors than those of *M. persicae,* and these in turn less so than those of *M. euphorbiae* (except for the nontransmitting Madison colony of *M. euphorbiae*). This information is useful for further work with PSbMV and even for studies on the nature of stylet-borne transmission. However, it is impossible to predict, on the basis of these results, the relative vector efficiency under natural conditions, because it became evident that two colonies of the same aphid could differ almost as much as two aphid species in transmission efficiency. An example of this is the difference between the WARF and Prairie du Sac colonies of *A. pisum,* which is apparent if Tables 2 and 3 are compared. Also significant is the marked contrast between the high transmission (about 50%) of the New London colony of *M. euphorbiae* on one hand, and the lack of transmission of the Madison colony on the other. Thus, the possibility of finding greater intraspecific variation, if a larger sample of each species were tested, cannot be ruled out. But even if the present limited differences in transmitting efficiency among species were confirmed, this might have little relevance to the potential of such species as vectors in nature. Other factors, such as the relative populations of a given aphid species, the time of its peak incidence in pea fields, and its relative mobility, might be equally important or more important in vector efficiency of a given aphid than laboratory tests could predict (5, 7, 14).

It would appear that "subisolate A" of PSbMV used in this work corresponds to the one that exists in nature, and that "subisolate M" represents a partial variant that arose through maintenance by successive mechanical transmissions. The "production" of subisolate A, which was achieved about midway in this study by serial aphid transmissions, could be considered a reversal of the process. Further investigations are in progress on the nature and the extent of this variation. A similar situation, except that loss of aphid transmissibility was total, has been reported for bean yellow mosaic virus (6) and for cucumber mosaic virus (1); the process appears different from the loss of aphid transmissibility by one-step mutation, as reported by Swenson et al. (12). This phenomenon should be taken into consideration when studying the properties of PSbMV in the laboratory. Something similar can be said of the greater efficiency of alatae in transmitting the virus; while this difference is irrelevant in the field, because the apterae's restricted mobility makes them negligible vectors in any case (7, 14), it nevertheless should be considered whenever efficient aphid transmission is required for laboratory work with this virus.

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