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Title Effects of Intrinsic Factors in the Transmission of Bean Yellow Mosaic Virus by Aphids

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The effects of the inherent transmissibility of the virus and of the inherent transmitting ability of aphids on the transmission of bean yellow mosaic virus (BYMV) were studied along with four virus-vector relationships of BYMV and the green peach aphid, Myzus persicae (Sulz.). In addition, investigations were made on the transmission of clover yellow mosaic virus (CYMV) by aphids and on the effects of temperature on the susceptibility of Lincoln pea to inoculation with bean yellow mosaic virus (BYMV) by M. persicae.

All eight aphid species included in these tests transmitted BYMV. The aphids ranked in the order of descending efficiency of BYMV transmission as follows: Macrosiphum euphorbiae (Thos.), Benton Co. (Oregon) clone of Acyrthosiphon pisum (Harris), Myzus persicae

(Sulz.), Aphis fabae Scop., Columbia Co. (Washington) clone of A. pisum, Macrosiphum rosae (L.), Therioaphis riehmi (Borner), Brachycaudus helichrysi (Kltb.), and Cavariella aegopodii (Scop.). Efficiency of transmission varied from 62 percent to 7 percent. B. helichrysi, C. aegopodii and T. riehmi have not previously been reported to transmit BYMV.

Collections of the pea aphid, Acyrthosiphon pisum (Harris), from Oregon and Washington included biotypes differing in BYMV transmission, fecundity, body size and host preference. No differences were found among M. persicae clones.

BYMV isolates differed in symptom expression and in the ease with which they were transmitted by aphids. Aphid transmissibility of BYMV was lost or greatly reduced following a single mechanical transfer. The vectorless isolate multiplied to the virtual exclusion of the aphid transmissible isolate when broad bean plants were inoculated simultaneously with both these isolates.

Different areas of broad bean leaves were not equal as sources of BYMV for M. persicae. More aphids transmitted the virus from the interveinal chlorotic area than from the green areas along the veins.

Post-inoculation temperature for 48-56 hours had a considerable influence on Lincoln pea susceptibility to BYMV infection by M. persicae inoculation. More plants were infected at 27 and 30°C than at 15, 18 or 24°C. Post-inoculation temperature treatment for 24 hours or less did not have any appreciable effect. Pre-inoculation temperature for 47-56 hours also considerably influenced plant susceptibility to BYMV infection by aphid inoculation. Twice as many plants were infected at 15°C as at 30°C. The effects of pre- and post-inoculation temperatures were not additive. The number of plants infected depended entirely on post-inoculation temperature.

Artificial termination of acquisition probes did not have any appreciable effect on BYMV transmission by M. persicae. No significant differences in virus transmission were found for aphids with acquisition probes in the 11- to 45-second range. Virus transmission increased with an increase in the number of test probes. Loss of BYMV by feeding M. persicae could be expressed exponentially. Half-life of the retention of virus by feeding aphids was about three minutes.

Clover yellow mosaic virus could be easily confused with BYMV on the basis of symptom expression in Dwarf

Horticultural and Bountiful cultivars of the bean, *Phaseolus vulgaris* L., *Pisum sativum* L. cv. Lincoln, *Vicia faba* L. (secondary symptoms, especially on new sprouts), and in *Chenopodium amaranticolor* Coste and Reyn. (primary reaction). It was not transmitted by *A. pisum*, *A. fabae*, *C. aegopodii*, *M. euphorbiae*, *M. rosae* and *M. persicae*.

EFFECTS OF INTRINSIC FACTORS IN THE TRANSMISSION OF BEAN YELLOW MOSAIC VIRUS BY APHIDS

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INTRODUCTION

Intrinsic factors in insect transmission of a virus are the inherent transmitting ability of the vector and the inherent transmissibility of the virus. Other factors affecting transmission are considered extrinsic. Vector specificity in transmission of plant viruses is well known. No insect species has transmitted all the known viruses and no virus has been transmitted by all the insect species. Even among the vectors of a virus, there are large differences in transmission efficiency, and viruses differ in the ease with which they are transmitted by the same insect species. These differences among insect vectors and among viruses are due to the inherent transmitting ability of the vector and the inherent transmissibility of the virus.

Smith (32, p. 325-326) recognized the possibility that the biological races of an insect vector species may differ in virus transmitting ability and that the strains of a virus may vary in insect transmissibility. Existence of strains of leafhopper species differing in virus

transmission has been demonstrated by several workers (33, p. 58-59)(18, p.88)(2, p. 50)(6, p. 208). Intra-specific variation in the transmission of circulatory viruses by aphids has also been well established (34, p. 68)(45, p. 538)(4, p. 1)(27, p.881). Though some studies have been made, the influence of inherent variation within an aphid species on the transmission of stylet-borne viruses has not yet received much investigation. No such information is available about the aphid species transmitting bean yellow mosaic virus (BYMV).

Differences have been found in the transmission of strains of an aphid-borne circulatory virus (26, p.746-747), and in that of the strains of leafhopper-borne viruses (29, p. 310)(5, p. 231). The relative transmission of bean yellow mosaic virus (BYMV) strains by aphids has not been studied so far.

The work reported in this paper was carried out to study the effects of the inherent transmissibility of the virus and of the inherent transmitting ability of aphids on the transmission of bean yellow mosaic, a stylet-borne virus. In addition, information was sought on the effect of one extrinsic factor (viz. temperature) on the susceptibility of plants to BYMV infection by aphid

inoculation because recent findings (41)(43, p. 70-99) indicated that it would have a large effect. A few experiments were made to study the vector-virus relationships in the transmission of BYMV by Myzus persicae (Sulzer).

REVIEW OF LITERATURE

Inherent Transmitting Ability of Aphids

Aphid species differed in their ability to transmit bean yellow mosaic virus (BYMV)(36, p. 728). Differences were found among aphid species in cucumber mosaic virus transmission (40, p. 423)(14, p. 520). Myzus persicae (Sulz.) transmitted watermelon mosaic virus to 95 percent of the plants inoculated, whereas transmission by 6 other aphid species varied from 32 to 0 percent (14, p. 520). Also variation among aphid species was found in potato virus Y transmission (10, p.335-339).

Intraspecific variation in virus transmission by aphids is an extension of the variation among species. Rochow (28, p. 714-716) recently reviewed the literature on the intraspecific variation in the virus transmitting ability of aphids. Only a few species of aphids transmitting stylet-borne viruses have shown such variation. Simons (31, p. 612) found differences between clones of Aphis gossypii Glover in the transmission of cucumber mosaic virus. Clones of Myzus persicae (Sulz.) and of Aphis fabae Scop. varied in beet yellows virus transmission (4, p. 1). Frazier (15, p. 307; 16, p. 436) reported

colonies of Chaetosiphon (Pentatrichopus) fragaefolii (Cock.) differing in strawberry vein-banding virus transmission.

Inherent Transmissibility of Viruses

Strains of a single stylet-borne virus have been found to differ in aphid transmissibility. Bhargava (3, p. 386) reported that four cucumber mosaic virus (CMV) strains were transmitted by Myzus persicae with varying efficiency. The difference in aphid transmissibility could not be explained on the basis of titre of virus in plants. Three CMV strains were found to differ in aphid transmissibility by Simons (30, p. 148).

Swenson and Nelson (40, p. 423) compared transmission of three CMV strains using several aphid species. The isolate from Daphne odora Thunbg. was transmitted by aphids much less frequently than the type strain or the isolate from gladiolus. Also the type strain and the isolate from gladiolus differed in aphid transmissibility. Frazier (16, p. 436) found that a clone of Chaetosiphon (Pentatrichopus) fragaefolii (Cock.) transmitted three strains of strawberry vein-banding virus but failed to transmit the fourth, the type strain.

Several workers have experienced the partial or complete loss of insect transmissibility of viruses following the maintenance of virus cultures by mechanical transfer. Jensen (21, p. 67-70) and Hitchborn and Thomson (20, p. 175-176) have reviewed this literature.

MATERIALS AND METHODS

A. Definitions

Colony plant was a plant on which aphids were reared. The aphids on a single colony plant are referred to as a colony.

Source plant was a diseased plant used as a virus source for aphids or for mechanical inoculation.

Acquisition probes refers to probes on the source plant. These were measured from the time the tip of the rostrum touched the leaf surface until it was removed.

Preliminary starvation denotes the period of starvation after the aphid was removed from a colony plant but before the acquisition probe.

Test plant was a healthy plant on which an aphid was placed following the acquisition probe.

Test probe denotes the time which elapsed from the moment the tip of the aphid rostrum touched the leaf surface of a test plant until it was removed.

Test feeding was the total time spent by the aphid on a test plant.

Aphid inoculation refers to the exposure of a test plant to an aphid which has probed a source plant.

Single-probe method refers to the method of aphid inoculation in which the aphid was allowed to probe a test plant only once for 8-60 seconds.

Conventional method was the method of aphid inoculation in which the aphid was allowed to feed on one test plant throughout its entire infective period.

Mechanical inoculation refers to rubbing healthy plant leaves with juice, diluted with a buffer, from a diseased plant. Inoculated leaves were dusted with carborundum to facilitate infection.

Transmission refers to inoculation resulting in an infected plant.

B. Materials

Several isolates of bean yellow mosaic virus (BYMV) were used in these investigations. The first, designated as isolate 1, was described by Swenson from red clover near Geneva, New York, in 1954 (35, p. 1121). This isolate has been maintained in broad bean, Vicia faba L., or Dwarf Horticultural cultivar of the bean, Phaseolus vulgaris L. by transfer with the aphid Myzus persicae (Sulz.) since 1956. The second, designated as isolate 11, was isolated by Dr. W. A. Frazier of the Department of

Horticulture, Oregon State University, from Blue Lake bean from the Willamette Valley, Oregon, in 1951. Since then, it has been maintained in Blue Lake or Dwarf Horticultural bean by mechanical transfer.

The other five isolates were collected by Dr. R. E. Ford, Agricultural Research Service, United States Department of Agriculture, Corvallis, Oregon, in the summer of 1961. These five isolates were maintained in broad bean by mechanical transfer. Isolate 65 was maintained, however, by aphid transfer after December 1962. Other information about these isolates follows:

Designation of Isolate	Collection Data	
	Host Plant	Place
65	Pisum sativum L.	Mt. Vernon, Washington
66	Trifolium hybridum L.	Mt. Vernon, Washington
68	P. sativum L.	Moses Lake, Washington
88	P. sativum L.	Dayton, Washington
105	P. sativum L.	Mt. Vernon, Washington

Several aphid species were included in these transmission tests. An alphabetical listing of these species along with the plant used for rearing each follows:

Aphid	Colony Plant
Acyrthosiphon pisum (Harris)	Pisum sativum L. cv. Early Perfection
Aphis fabae Scop.	Vicia faba L.
Brachycaudus helichrysi (Kltb.)	Apium graveolens L.
Cavariella aegopodii (Scop.)	A. graveolens L.
Macrosiphum euphorbiae (Thos.)	Cucurbita maxima Duch. cv. Butter Cup
M. rosae (L.)	Rosa hybrid
Myzus persicae (Sulz.)	Brassica pekinensis Rupr.
Therioaphis riehmi (Borner)	Melilotus alba Desv.

In the case of A. pisum (Harris) and M. persicae (Sulz), more than one clone was included. Additional information about the clones is included under Results. M. rosae (L.) was not reared in greenhouse. Apterous aphids were collected directly from roses and used in virus transmission tests. Other aphids were collected locally in late spring and early summer of 1962 and were colonized on the plants listed. The aphid colonies were caged individually and generally were kept in a greenhouse room separate from rooms used for raising plants.

Broad bean, V. faba L., was the source plant for the virus in all experiments. Unless otherwise specified, Pisum sativum L. cv. Lincoln (Greenfeast or Homesteader) was used as test plant.

All the seeds were treated with phygon to reduce root rot and damping off. In the case of peas, broad bean and garden bean, Phaseolus vulgaris L., copper A fungicide was added to soil at the time of planting to further prevent damping off.

The soil used in all experiments was a three to one mixture of river-bottom loam and peat moss. It was supplemented with one cup each of ammonium nitrate and 6-10-4, and one-half cup of lime per 22-25 gallons.

C. Methods

Aphid Inoculation

Apterous aphids, other than very young nymphs, were used in all trials. Unless otherwise specified, a different colony was used in each replicate for each aphid clone in the trials comparing aphid clones for BYMV transmission. Similarly a different source plant was used in each replicate for each BYMV isolate in the tests comparing the aphid transmissibility of BYMV isolates.

Aphid clones and BYMV isolates would, therefore, represent the experimental variables and not merely reflect the peculiarities of a single colony plant, or of a single source plant, as might have happened if all aphids for a clone in an experiment were obtained from a single colony, or if only one source plant were used for a BYMV isolate in an experiment.

Aphids were removed from the colony plants and placed in 50 ml. Erlenmeyer flasks for a preliminary starvation period of one hour or longer. Preliminary starvation increases transmission of stylet-borne viruses. Swenson (37, p. 523) reported that maximum effect of preliminary starvation on BYMV transmission was obtained in 15 minutes. The starved aphids were placed on the last fully opened leaf of the source plant and allowed an acquisition probe of 11-45 seconds before they were transferred to test plants. Maximum transmission of BYMV by aphids occurs with acquisition probes of 11 to 45 seconds (37, p. 522). In all trials, except the one where natural termination and artificial termination of acquisition probes were the experimental variables, only aphids terminating acquisition probes naturally within 11-45 seconds were transferred to test plants. Others were

discarded. Transmission of some stylet-borne viruses is reduced if acquisition feeding is forcibly terminated (8, p. 81)(10, p. 337)(42, p. 54).

Only one aphid was transferred to each test plant. In the case of conventional method each aphid was allowed a test feeding of 1 to 24 hours except in those experiments testing the effect of post-inoculation temperature. At the end of the test feeding, aphids were removed by fumigating the test plants with nicotine. In post-inoculation temperature experiments, each aphid was allowed a 15-17 minutes test feeding,after which it was removed with a camel's-hair brush and killed. In the case of single-probe method, each aphid was allowed a single test probe of 8-60 seconds on the first test plant. Test probes shorter than eight seconds were disregarded. The aphid was removed from the test plant at the end of 60 seconds feeding, if it had not already ceased probing. The conventional method of inoculation by aphids was used unless otherwise specified. Acquisition probes and test probes were watched with a 10X hand lens and timed with a stop watch.

Statistical Methods

A randomized block design was used in all virus transmission experiments. Replicates differed in time of inoculation. The order of inoculation of plants for the various treatments was randomized within replicates. The chi square test is theoretically more appropriate to virus transmission data than is the F test. In practice little difference results, however, from the test selected (22, p. 419). Analysis of variance and F test were used in all experiments because of greater simplicity. The probability values, P, represent the probability of obtaining a particular variance ratio if the null hypothesis were true. P-values greater than 0.05 were arbitrarily considered to be non-significant (NS). Individual degree of freedom test was used to make specific comparisons. Regression analysis was applied in one case where the treatments were quantitative.

RESULTS

A. Variation in Transmission Among Aphid Species

A preliminary experiment was made in August 1962, to transmit bean yellow mosaic virus (BYMV) isolate 1 with Cavariella aegopodii (Scop.). Five of 50 aphids transmitted the virus. C. aegopodii has not previously been reported as a vector of BYMV.

Six aphid species were tested for transmission of two BYMV isolates in a factorial experiment (Table 1). There were 12 aphid-virus combinations. All six aphid species transmitted both the BYMV isolates. Transmission efficiency of aphids varied, however, considerably. Myzus persicae, Acyrthosiphon pisum (clone B), and Macrosiphum euphorbiae were quite efficient vectors with a transmission of 56, 60 and 62 percent, respectively. Cavariella aegopodii and Macrosiphum rosae were inefficient, with 4 and 18 percent, respectively, transmitting the virus. Transmission (42 percent) by Aphis fabae was intermediate. All aphid species, except M. persicae, transmitted the two BYMV isolates alike. M. persicae transmitted isolate 65 about twice as often as isolate 1.

Transmission of BYMV isolate 1 by Therioaphis

riehmi and Brachycaudus helichrysi was tried in one experiment. Myzus persicae was included in this experiment as a standard. Both T. riehmi and B. helichrysi were quite inefficient. Five of 50 B. helichrysi, and 7 of 50 T. riehmi transmitted the virus. M. persicae transmitted BYMV to 34 of 50 plants (Table 2). B. helichrysi and T. riehmi have not previously been recorded as vectors of this virus.

BYMV transmission by M. persicae and Acyrthosiphon pisum was compared in three other experiments. Results of the experiments are given in Tables 5, 8 and 10. A summary of the transmission (percent) follows:

BYMV Isolate	Aphids			Reference to Table
	M. persicae	A. pisum Clone B	A. pisum Clone C	
1	34.0	34.0	-	8
1	37.5	35.0	20.0	10
65	67.0	76.0	33.0	5
65	50.0	54.0	-	8

Clone B of A. pisum was as efficient a vector of BYMV as was M. persicae. Clone C of A. pisum was, however, a relatively poor vector of this virus.

Collections of Myzus persicae were made from widely separated areas in the state of Oregon in the summer of

1962. The aphids so collected were established on Chinese cabbage, Brassica pekinensis Rupr. These colonies were maintained by transfers of single aphids through several generations. This procedure gave clones of M. persicae obtained from single parthenogenetically reproducing females.

The clones were designated according to the place of collection as follows:

Place of Collection	Designation
Benton Co., Oregon	B
Clackamas Co., Oregon	C
Jefferson Co., Oregon	J
Linn Co., Oregon	L

A clone, designated as clone B-d, was initiated from a single apterous aphid from clone B. Also another clone, designated as clone L-d, was started by a single apterous aphid from clone L. These duplicate clones were started to determine experimental variation in the BYMV transmission experiment described in the following paragraph.

Transmission of BYMV isolate 1 by six clones of M. persicae, including two duplicate clones, was compared in one experiment, comprising ten replicates, and 300 aphids (Table 3). Aphids were obtained from two sets of colonies.

Those for replicates 1-6 were from one set of colonies, and the ones for replicates 7-10 from a second set. Each aphid was fed on two test plants. Only a single probe of 8-60 seconds was allowed on the first plant. After an aphid completed its first probe, it was then transferred to a second plant, on which it was left for the remainder of its infective period.

There was a great variation in the BYMV transmitting ability of the M. persicae clones. There was, however, as much variation between identical clones as among different clones. There were thus no differences in the inherent transmitting ability of the different clones. About twice as many second plants as first plants were infected by all clones.

Table 1. Transmission of two BYMV isolates by six aphid species.

Replicates	Aphid Species											
	Myzus persicae		Acyrthosiphon pisum (Clone B)		Macrosiphum euphorbiae		Aphis fabae		Macrosiphum rosae		Cavariella aegopodii	
	1[a]	65	1	65	1	65	1	65	1	65	1	65
1	2[b]	4	2	4	3	1	2	1	0	0	0	0
2	1	3	3	1	3	2	2	2	0	0	0	0
3	2	4	5	2	3	4	2	1	2	1	0	0
4	2	3	3	3	2	5	2	3	1	2	1	0
5	3	4	3	4	4	4	2	4	2	1	0	1
Total	10	18	16	14	15	16	10	11	5	4	1	1

[a] 1 and 65 = BYMV isolates.

[b] Each number = plants infected out of five inoculated.

Table 2. Transmission of BYMV isolate 1 by three aphid species.

Replicates	Aphid Species		
	Myzus persicae	*Therioaphis riehmi*	*Brachycaudus helichrysi*
1	4[a]	1	1
2	2	0	2
3	4	1	0
4	2	2	0
5	4	0	0
6	3	0	0
7	4	1	0
8	4	0	2
9	3	0	0
10	4	2	0
Total	34	7	5

[a]Each number = plants infected out of five inoculated.

Table 3. Transmission of BYMV Isolate 1 by six clones of *Myzus persicae*.

A. Data

Replicates	*M. persicae* Clones												
	B		B-d		L		L-d		C		J		
	F[a]	S[b]	F	S	F	S	F	S	F	S	F	S	Total
1	3	2	1	1	1	3	1	2	2	5	2	4	27
2	1	2	2	1	2	3	3	1	0	1	2	4	22
3	2	2	1	2	3	3	3	5	2	2	2	3	30
4	2	1	2	0	2	4	2	2	1	2	0	5	23
5	3	5	1	4	2	4	2	5	3	3	3	3	38
6	1	2	0	3	2	3	1	2	1	2	0	2	19
7	1	3	0	1	0	3	3	2	0	3	3	3	22
8	0	2	3	5	2	4	2	5	2	4	2	4	35
9	2	4	0	0	2	3	2	5	0	3	3	4	28
10	4	3	0	2	1	5	4	5	2	4	1	2	33
Total	19	26	10	19	17	35	23	34	13	29	18	34	277

[a]Pairs of plants, out of five pairs inoculated, with the first plant infected.

[b]Pairs of plants, out of five pairs inoculated, with the second plant infected.

Table 3. Continued.

B. Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Total	227.59	119			
Replicates	29.67	9	3.30	2.75	0.01
Treatments	79.29	11	7.21	6.01	0.0000 002
Aphid Clones	24.94	5	4.99	4.16	0.002
Clone B vs. Clone B-d	6.40	1	6.40	5.33	0.025
Clone L vs. Clone L-d	0.62	1	0.62	0.52	NS
Methods of Inoculation	49.41	1	49.41	41.18	0.0000 001
Aphid Clones X Methods of Inoculation	4.94	5	0.99	0.82	NS
Error	118.63	99	1.20		

B. Intraspecific Variation in the Pea Aphid

i. Differences in BYMV Transmission

Pea aphids, Acyrthosiphon pisum (Harris), were collected from widely separated areas in Oregon and Washington in the summer of 1962. These aphids were established on pea, Pisum sativum L. cv. Early Perfection in the greenhouse. Colonies from these collections were maintained by transfers of single aphids through several generations. At the end of this period, these collections were represented by clones of A. pisum obtained from single, parthenogenetically reproducing females. Unless otherwise indicated, pea aphids were reared on Early Perfection pea.

The various clones were designated according to the original collection from which they were obtained, as follows:

Origin	Designation
Benton Co., Oregon	B
Columbia Co., Washington	C
Jackson Co., Oregon	Ja
Jefferson Co., Oregon	Je
Klamath Co., Oregon	K
Linn Co., Oregon	L

Duplicates of clones B and L were derived by initiating colonies with a single apterous aphid taken from each of these two clones. These duplicate clones were designated by the letter "d" following the designation of the parent clone. The duplicate clones were used to determine experimental variation in the BYMV transmission experiments.

In August 1962, a preliminary experiment (Table 4) compared the transmission of two BYMV isolates by eight pea aphid clones including the two duplicate clones. Each aphid was fed on two test plants. Only a single probe of 8-60 seconds was allowed on the first plant. After an aphid completed its first probe, it was then transferred to a second plant, on which it was left for the remainder of its infective period. The experiment was replicated five times using 400 aphids.

Results and variance analysis of the experiment are given in Table 4. A summary of the data (percent transmission on the basis of one or both plants of a pair infected) listing the pea aphid clones in the order of descending efficiency of BYMV transmission, follows:

Clones	Percent Transmission
Je	68
K	62
Ja	58
B-d	58
B	56
L-d	50
L	44
C	12

Thus the clones differed considerably in their BYMV transmitting ability. There were almost no differences between identical clones (i.e. between clones B and B-d, and between clones L and L-d). Two hundred and four of 400 aphids infected one or both plants in a pair. Of these 204 aphids, 90 infected the first plants, and 179 infected the second plants. BYMV isolate 11 was transmitted as frequently as isolate 65.

There was no interaction between pea aphid clones and BYMV isolates, and between pea aphid clones and inoculation methods. A highly significant ($P = 0.005$) interaction was present, however, between the virus isolates and inoculation methods. A summary of the data (plants

infected out of 200 inoculated for each BYMV isolate-inoculation method combination) follows:

BYMV Isolate	First Plant	Second Plant	Total
11	37	100	137
65	53	79	132
Total	90	179	269

Isolate 65 was transmitted more frequently than isolate 11 to the first plants. Isolate 11 was transmitted, however, more frequently than isolate 65 to the second plants. This virus isolates X inoculation methods interaction will be discussed further in the section on variation in transmissibility of BYMV isolates. The virus isolates X aphid clones X inoculation methods interaction was absent.

Only clones B and C were selected for further comparisons. Clone C was selected as atypically poor in BYMV transmission among pea aphid clones. Clone B represented the more efficient clones. Also, it has been included in some other experiments.

Transmission of BYMV isolate 65 by clones B and C was compared again in October 1962. The experiment was replicated 20 times and involved 200 aphids. Clone C was a much poorer vector than clone B. Seventy-one of 100 aphids of clone B transmitted the virus as compared to 14

of 100 aphids in the case of clone C. This experiment, therefore, confirmed the previous results (Table 4).

A final experiment (Table 5) of the series compared the ability of the pea aphid clones to transmit BYMV isolate 65 with that of M. persicae in June 1963. The materials used in this experiment were identical with those used in 1962 experiments, except that M. persicae was included. The results of this experiment followed the same pattern as in earlier experiments. Clone B was a much better vector (76 percent transmission) than clone C (33 percent transmission). M. persicae transmitted the virus to 67 of 100 plants.

ii. Other Differences

After it was established that clones of the pea aphid differed in their ability to transmit BYMV, experiments were made to find out if these clones varied in other respects. Biological races of the pea aphid differed in body size, body weight, fecundity and feeding injury (19, p. 22)(13).

Fecundity

It was observed that clone C reproduced at a rate much faster than clone B. An experiment was made to

verify this observation. Aphids, born during the same 24-hour period, were reared to early reproductive maturity on Perfection pea. Six of these aphids of each clone were confined individually to Perfection pea plants. The progeny of each aphid was removed on the third, sixth, and eighth day. The progeny counts are shown in Table 6. Clone B produced an average of 6.0 $\pm$ 3.3 nymphs per female as compared to 37.3 $\pm$ 13.7 nymphs per female by clone C.

Body Size

Aphids, born during the same 24-hour period, were reared to productive maturity on broad bean. Fifth aphids from each clone were measured by means of an ocular micrometer mounted on the eye piece of a binocular microscope. The aphids were killed in 70 percent ethyl alcohol, placed in a drop of glycerine, and measured without a coverglass. Measurements were completed within five hours after the aphids were placed in alcohol.

Body length was the distance from the tip of the head to the base of the ovipositor and width was the distance across the insect at the widest portion of the abdomen. Body length/body width ratio was calculated for

all the 100 aphids measured because such ratios are usually more stable than the linear measurements (23, p. 136-137). A summary of the mean body measurements and of variance analysis (P-values) follows:

Body Measurements	Aphid Clones		Variance Analysis
	B	C	
	Millimeters	Millimeters	P
Length	3.63±0.24	4.02±0.15	0.0000 001
Width	1.69±0.20	1.64±0.06	0.001
Length/width	2.14±0.06	2.45±0.02	0.0000 001

The two aphid clones thus considerably differed in body size.

Host Preference

No experiments were made to compare the host preference of the two clones. The comments in this paragraph are based on general observations made during the investigations reported above. Clone C colonized pea cv. Pride though its reproduction and development were much reduced. This cultivar of pea was completely resistant to clone B.

The experiments in this section indicated that pea aphids collected from different localities in Oregon and Washington differed considerably in BYMV transmitting ability, fecundity, body size and in host preference.

Table 4. Transmission of two BYMV isolates by eight A. pisum clones.

A. Data

BYMV Isolates		Pea Aphid Clones: B F[a]	B S[b]	B-d F	B-d S	C F	C S	Ja F	Ja S	Je F	Je S	K F	K S	L F	L S	L-d F	L-d S	Total
11	1[c]	1	3	1	3	0	2	2	3	0	1	0	4	2	1	2	1	26
	2	0	4	1	3	0	1	2	3	2	4	2	2	0	2	0	2	28
	3	0	4	2	4	0	0	1	4	2	4	0	3	1	3	1	4	33
	4	2	2	0	2	0	0	1	2	1	4	1	3	2	3	1	0	24
	5	0	2	0	2	0	0	1	2	0	3	1	4	3	3	2	3	26
	Sum	3	15	4	14	0	3	7	14	5	16	4	16	8	12	6	10	137
65	1	0	1	3	3	0	1	1	3	3	3	2	2	0	1	1	1	25
	2	1	1	1	3	0	1	1	1	1	1	1	1	1	0	1	1	16
	3	1	3	3	1	0	0	1	3	3	5	3	5	1	0	3	1	33
	4	1	2	1	3	0	0	2	2	0	4	1	2	2	1	2	1	24
	5	2	4	1	2	0	1	3	3	2	2	2	4	1	2	1	4	34
	Sum	5	11	9	12	0	3	8	12	9	15	9	14	5	4	8	8	132
Total		8	26	13	26	0	6	15	26	14	31	13	30	13	16	14	18	269

[a]Pairs of plants, out of five pairs inoculated, with the first plant infected.

[b]Pairs of plants, out of five pairs inoculated, with the second plant infected.

[c]Replicates.

Table 4. Continued.

B. Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Total	226.74	159			
Replicates	10.15	4	2.54	2.67	0.05
Treatments	135.14	31	4.36	4.59	0.0000 01
BYMV Isolates (V)	0.15	1	0.15	0.16	NS
Aphid Clones (A)	54.39	7	7.77	8.18	0.0000 02
Clone B vs. Clone B-d	0.62	1	0.62	0.65	NS
Clone L vs. Clone L-d	0.22	1	0.22	0.23	NS
Inoculation Methods (M)	49.50	1	49.50	52.10	0.0000 001
VXA	7.50	7	1.07	1.13	NS
VXM	8.57	1	8.57	9.02	0.005
AXM	13.15	7	1.88	0.20	NS
VXAXM	1.88	7	0.27	0.28	NS
Error	117.45	124	0.95		

Table 5. Transmission of BYMV isolate 65 by *M. persicae* and by two clones of *A. pisum*.

Replicates	*M. persicae*	Pea Aphid Clones B	C
1	5[a]	4	1
2	3	3	1
3	4	4	0
4	3	2	1
5	5	3	0
6	4	4	0
7	3	4	3
8	5	4	3
9	3	5	3
10	3	3	2
11	5	4	4
12	2	5	2
13	4	3	3
14	3	2	2
15	4	5	2
16	4	5	1
17	1	4	2
18	1	5	1
19	2	4	1
20	3	3	1
Total	67	76	33

[a]Each number = plants infected out of five inoculated.

Table 6. Fecundity of two A. pisum clones.

Replicates	Clone B	Clone C
1	1[a]	47
2	4	50
3	9	18
4	10	36
5	7	24
6	5	49
Mean	6.0	37.3
Standard Deviation	± 3.3	± 13.7

[a]Each number = progeny of one aphid.

C. Variation in Transmissibility of BYMV Isolates

A preliminary experiment compared the transmission of six BYMV isolates by M. persicae (Table 7). There were very large differences among the virus isolates. Isolates 105 and 65 ranked high, with a transmission of 56 and 52 percent, respectively. Isolate 88 was transmitted least frequently (16 percent). Isolates 1, 66, and 68 were intermediate, with 24, 34, and 38 percent transmission, respectively.

Transmission of isolates 1 and 65 was compared in another experiment (Table 1). Six aphid species were included in this test. The experiment has been described earlier under the section on variation in transmission among aphid species. The two virus isolates were transmitted alike by all the aphid species except M. persicae. Transmission of isolate 65 by M. persicae was about twice as frequently as of isolate 1.

A. pisum (clone B) transmitted isolates 1 and 65 alike in the previous experiment (Table 1). M. persicae transmitted, however, isolate 65 more frequently than isolate 1. A 2 x 2 factorial experiment was made to verify this interaction between virus isolates and aphid

species (Table 8). The two virus isolates were 1 and 65, and the two aphid species M. persicae and A. pisum (clone B). There was a total of four virus-aphid combinations. The experiment was replicated ten times using 200 aphids. Both the aphids transmitted isolate 65 more frequently than isolate 1. There was no difference in the transmission efficiency of the two aphids. Analysis of variance showed no interaction between virus isolates and aphids.

Transmission of isolates 11 and 65 by the pea aphid was compared in one experiment (Table 4), described in the section on intraspecific variation in the pea aphid. Isolate 11 was transmitted as frequently as isolate 65. There was, however, a highly significant interaction between virus isolates and inoculation methods. A summary of the data showing this interaction is given on page 26. Isolate 65 was transmitted more frequently than isolate 11 to the first plants. Isolate 11 was transmitted, however, more frequently than isolate 65 to the second plants.

A similar interaction between virus isolates and inoculation methods was found by Welton (44) when he compared the transmission of BYMV isolates 1 and 65 by M. persicae. A summary of Welton's data follows:

BYMV Isolate	First Plant	Second Plant	Total
1	54	92	146
65	46	43	89
Total	100	135	235

Isolate 1 was transmitted more frequently than isolate 65. There was not much difference in the transmission of the two isolates to the first plants. Transmission of isolate 65 to the second plants was, however, much less frequent than that of isolate 1.

The virus isolates X methods of inoculation interaction indicated a reduced transmission of isolate 65 by the subsequent test probes. Comparatively less stability of isolate 65 on the stylets of the aphid was a possible explanation of this reduced transmission. A 2 x 4 factorial experiment was conducted to verify this possibility. Isolates of BYMV used were 1 and 65. Aphids, Myzus persicae, were given a post-acquisition starvation of 0, 5, 15, and 30 minutes, by placing in small flasks, before transferring them to the test plants. There were thus 8 virus X post-acquisition starvation combinations. The experiment was replicated 16 times using 384 aphids. Only one source plant of isolate 65 and two source plants of

isolate 1 were used for the entire experiment. The source plants were inoculated by aphid transfer from stock cultures, which were maintained by aphid inoculation.

Results of the experiment and statistical analysis of data are shown in Table 9. Isolate 65 was transmitted significantly more often than isolate 1. Transmission of both the isolates decreased considerably with an increase in post-acquisition starvation time. The decrease after 30 minutes was significant at the 2 percent level. Virus isolates X post-acquisition starvation interaction was absent. The results of this experiment further verified the results of previous experiments (Tables 1, 7, and 8) regarding the relative transmission of BYMV isolates 1 and 65 by M. persicae. This did not solve, however, the problem of virus isolate X inoculation method interaction.

Besides aphid transmissibility, BYMV isolates varied in symptom expression. On Lincoln pea, isolate 1 gave a simple, nondiscrete mottle of dark and light green. At times, especially during summer, these symptoms faded away. Some of the distinctly infected plants looked apparently healthy with the passage of time. On the same host, isolates 65 and 105 started with a discrete mosaic pattern of green and chlorotic spots. Later, especially

on the new growth, a distinct mosaic pattern of green and yellow developed. The symptoms of isolate 65 did not fade away with the passage of time.

On broad bean, isolate 1 gave a mosaic of small green and chlorotic spots. Isolates 65 and 105 gave similar symptoms on this host in the early stages of infection. On the new growth, isolates 65 and 105 produced large chlorotic areas separated by continuous green areas. The green areas were in the proximal region of the leaflets and along the veins. The chlorotic areas were more frequent in the terminal region and in the interveinal areas.

The green and chlorotic areas of broad bean leaves were compared for availability of BYMV isolate 65 to M. persicae in one experiment comprising 300 aphids. The experiment was replicated 30 times. Ninety of 150 aphids transmitted the virus from the chlorotic areas and 70 of 150 aphids transmitted from the green ones. Variance analysis, not included in the thesis, showed this difference to be significant at the 2 percent level. Bradley (9, p. 366) found that epidermis of tobacco leaves was not uniform as a source of potato virus Y for aphids. Interveinal epidermis was better than that of adjacent

veins. Variation within the leaves as source of virus for aphids may be one of the sources of variation in virus transmission.

Welton[1] inoculated two broad bean plants mechanically with BYMV isolate 1 on February 23, 1963, for use as source plants in some of his experiments. On March 22, 1963, he fed 24 aphids, Myzus persicae, on each of these plants and transferred them to Lincoln pea, one aphid to a plant. Ten of 24 aphids transmitted the virus from one of these source plants, but none of 24 transmitted from the other source plant. In my experiments described in the subsequent paragraphs in this section, the first source plant and the virus obtained from it would be referred to as isolate 1 (the regular laboratory isolate of Dr. K. G. Swenson), and the second source plant and the virus obtained from it would be designated as isolate 1-W.

An experiment was made to find out if the failure of the aphids to transmit BYMV in the above case was due to variation in aphid population coupled with a small

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[1]Personal communication with Dr. R. E. Welton, Southern College of Education, Ashland, Oregon.

sample size or if it was a case of loss of aphid transmissibility. One hundred M. persicae were used in ten replicates. On April 15, 1963, 50 aphids were fed on each of the two source plants used by Welton. Thus there were five aphids for each source plant in each replicate. The number of aphids, which transmitted virus, follows:

	Source Plant	
Replicate	Isolate 1	Isolate 1-W
1	2	0
2	3	0
3	1	0
4	2	0
5	2	0
6	0	0
7	1	0
8	2	0
9	2	0
10	2	0
Total	17	0

The above two experiments indicated that the aphid transmissibility of BYMV from source plant 1-W was evidently lost or greatly reduced. A 3 x 3 x 2 factorial experiment (Table 10) was made to ascertain if this

reduction in aphid transmissibility involved a change in the virus or if it was inherent with the single source plant used in these two experiments. The three factors were: (1) BYMV isolates, (2) aphids, and (3) test plants. There was a total of 18 virus-aphid-test plant combinations. The experiment was replicated 10 times.

In this experiment 34 source plants were inoculated with isolate 1, 36 with isolate 1-W, and 34 with both 1 and 1-W. Source plants were inoculated mechanically. In the case of a single isolate, two leaflets of each plant were rubbed with inoculum. In the case of the combination of the two virus isolates, one leaflet of each plant was rubbed with isolate 1 and the other leaflet with isolate 1-W. The order of inoculation of source plants with the two isolates in the composite group was randomized. Each set of source plants was inoculated within 15 minutes. Ten typical plants were used from each of the three sets of source plants. Isolate 1-W produced more severe symptoms in these plants than isolate 1. The source plants of the composite group had symptoms more like the symptoms of isolate 1-W than those of isolate 1.

The results of the experiment are given in Table 10. None of 120 aphids of the two species transmitted isolate

1-W, but 37 of 120 aphids transmitted isolate 1. Transmission from the composite source (1 + 1-W) was very low. Only one aphid, pea aphid (clone C), of 120 aphids of the two species transmitted the virus from this group of source plants. Pea aphid (clone B) transmitted the virus as frequently as M. persicae. Pea aphid (clone C) was not, however, as efficient a vector as M. persicae. There was no difference in the susceptibility of broad bean and Lincoln pea. Also, there were no interactions.

The lack of aphid transmissibility of BYMV in the last two experiments could not have been due to the single source plant used, since isolate 1-W was not transmitted from any of the 10 source plants in this experiment as well. This indicated a change in the virus itself. The very low transmission of virus from the composite source would indicate that isolate 1-W in some way inhibited the transmission of isolate 1. Badami and Kassanis (1) reported that undescribed viruses in potato decreased the multiplication and aphid transmissibility of potato virus Y. Swenson (39) and Carpenter (12, p. 84-85) found, however, that an unrelated vectorless virus, white clover mosaic, did not affect BYMV transmission by aphids.

In another experiment, two broad bean plants were inoculated with BYMV in August 1963, from a virus culture which had been maintained by aphid transfer since 1956. One of the plants was inoculated mechanically, and the other by aphid transfer. These two plants were used as virus source for M. persicae in September, 1963. Only single-probe method of aphid inoculation was used. M. persicae transmitted BYMV to 28 of 288 plants from the source plant inoculated by aphid transfer, but to none of 288 plants from the source plant that was inoculated mechanically. Thus BYMV transmissibility by aphids was lost or greatly reduced once again just after a single mechanical transfer. Only six weeks elapsed between the date of inoculation of the source plant from the aphid maintained culture until the time it was used as source of BYMV for aphids.

Tests reported herein indicated that BYMV isolates differed in symptom expression and in the ease with which they were transmitted by aphids. Aphid transmissibility of BYMV was lost at two occasions following a single mechanical transfer. There is, however, always the possibility that transmission might occur if a sufficiently large number of aphids were used.

Table 7. Transmission of six BYMV isolates by M. persicae.

Replicates	BYMV Isolates					
	1	65	66	68	88	105
1	2[a]	4	1	2	2	3
2	0	4	3	2	0	4
3	1	3	3	3	0	3
4	1	2	1	2	0	3
5	2	3	2	1	0	2
6	0	3	2	2	0	2
7	2	1	1	3	0	1
8	2	2	1	1	1	3
9	0	2	3	2	2	4
10	2	2	0	1	3	3
Total	12	26	17	19	8	28

[a]Each number = plants infected out of five inoculated.

Table 8. Transmission of two BYMV isolates by two aphid species.

A. Data

Replicates	Aphid Species: *M. persicae*		*A. pisum* (Clone B)		Total
	1[a]	65	1	65	
1	1[b]	3	5	1	10
2	2	3	1	3	9
3	1	2	0	5	8
4	2	1	3	2	8
5	3	3	2	0	8
6	2	1	0	3	6
7	2	3	0	2	7
8	1	4	3	5	13
9	2	3	0	4	9
10	1	2	3	2	8
Total	17	25	17	27	86

B. Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Total	73.1	39			
Replicates	8.1	9	0.90	0.43	NS
Treatments	8.3	3	2.77	1.32	NS
Virus Isolates (V)	8.1	1	8.10	3.86	0.05
Aphids (A)	0.1	1	0.10	0.05	NS
VXA	0.1	1	0.10	0.05	NS
Error	56.7	27	2.10		

[a] 1 and 65 = BYMV Isolates.

[b] Each number = plants infected out of five inoculated.

Table 9. Retention of two BYMV isolates by non-feeding *M. persicae*.

A. Data

Replicates	BYMV Isolate 1				BYMV Isolate 65				Total
	0[a]	5	15	30	0	5	15	30	
1	0[b]	0	0	1	2	2	1	0	6
2	1	0	0	1	2	1	2	0	7
3	0	0	1	0	1	1	1	1	5
4	0	0	1	1	1	0	1	1	5
5	0	0	0	0	0	0	2	2	4
6	1	0	1	0	1	0	0	0	3
7	2	1	0	0	2	1	1	1	8
8	0	0	1	0	2	0	1	2	6
9	1	0	2	0	1	2	1	0	7
10	2	2	2	0	2	0	2	2	12
11	1	1	0	0	2	1	2	0	7
12	1	1	0	3	3	1	0	1	10
13	0	3	0	1	0	1	1	1	7
14	0	1	1	0	3	2	0	0	7
15	2	1	1	0	1	1	0	1	7
16	1	2	1	1	1	2	1	0	9
Total	12	12	11	8	24	15	16	12	110

B. Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Total	87.47	127			
Replicates	9.22	15	0.61	0.94	NS
Treatments	10.09	7	1.44	2.22	0.05
Virus Isolates(V)	4.50	1	4.50	6.92	0.01
Retention Time(T)	4.03	3	1.34	2.06	NS
Zero min. vs. 30 min.	4.00	1	4.00	6.15	0.02
VXT	1.56	3	0.52	0.80	NS
Error	68.16	105	0.65		

[a]Retention time in minutes.

[b]Each number = plants infected out of three inoculated.

Table 10. Transmission of two BYMV isolates by M. persicae and by two clones of A. pisum.

Replicates	Treatments																	
	M. persicae						A. pisum (Clone B)						A. pisum (Clone C)					
	1[a]		1-W		1+1-W		1		1-W		1+1-W		1		1-W		1+1-W	
	P[b]	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B
1	1[c]	2	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0
2	1	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0
3	2	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
5	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0
6	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
7	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0
8	1	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
9	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0
10	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0
Total	8	7	0	0	0	0	5	9	0	0	0	0	5	3	0	0	0	1

[a] 1 and 1-W = BYMV isolates.

[b] Test plants; P = Lincoln Pea, B = Broad bean.

[c] Each number = plants infected out of two inoculated.

D. Temperature Effects on Host Susceptibility

A series of experiments was made to study the effects of pre- and post-inoculation temperature on the susceptibility of Lincoln pea to BYMV infection by M. persicae inoculation. The plants were exposed to different temperatures in growth chambers unless otherwise specified. The growth chambers maintained temperature within $\pm 1^{\circ}C$ of the desired temperature. The photoperiod was 12 hours and the source of light was Sylvania Gro-Lux fluorescent lamps.

Welton (43, p. 83-84) found that susceptibility of Lincoln pea to infection with BYMV by aphid inoculation increased with an increase in post-inoculation temperature in the 12 to $30^{\circ}C$ range for 48 hours. I made an experiment to find out the minimum time required to obtain the effect of post-inoculation temperature. It was a two-factor factorial experiment, the two factors being (a) post-inoculation temperature, and (b) duration of exposure of plants to the post-inoculation temperature. Lincoln peas were grown on a greenhouse bench for nine days. The plants were inoculated with BYMV isolate 1 on the tenth day. The aphids were allowed a test feeding of 15-17 minutes, after which the aphids were removed and the test

plants were moved into two growth chambers at 18 and 30°C, respectively. During test feeding the plants were kept on the greenhouse bench. The plants were moved from the growth chambers to the greenhouse bench after 1 hour, 4 hours, and 24 hours from the time they were placed therein. The experiment was replicated 32 times. Two plants were inoculated for each treatment within each replicate.

The results of the experiment are given in Table 11. All the plants were equally susceptible. Post-inoculation temperature treatment up to 24 hours did not affect the susceptibility of Lincoln pea to BYMV isolate 1. Analysis of variance did not show any significant differences between the two temperatures or the durations of temperature exposures.

Welton (43, p. 91-99) reported that the effects of pre-inoculation and post-inoculation temperatures were not additive. Only the post-inoculation temperature affected the susceptibility of Lincoln pea to BYMV when pre-inoculation temperatures of 15 and 30°C were combined with post-inoculation temperatures of 15 and 30°C in a factorial experiment. In his earlier experiments (43, p. 70-81) it was found, however, that a decrease in the pre-inoculation temperature in the range of 15 to 36°C

resulted in a significant increase in the susceptibility of Lincoln pea to BYMV when the post-inoculation temperature was maintained at about 24°C.

A series of three factorial experiments was conducted to further investigate the effect of pre- and post-inoculation temperature on the susceptibility of Lincoln pea to BYMV isolate 1 infection by aphid inoculation. In the first experiment the susceptibility of plants was compared at 18 and 30°C in a pre- and post-inoculation combination. Plant susceptibility was evaluated by two methods of aphid inoculation. Each aphid was fed on a pair of test plants. Only a single probe of 8-60 seconds was allowed on the first plant of a pair. After an aphid completed its first probe, it was then transferred to the second plant. The aphid was left on the second plant for 15-17 minutes after which it was removed and killed. One aphid was used for each pre- and post-inoculation temperature combination within each replicate. There were 42 replicates.

Lincoln peas were grown on a greenhouse bench for nine days and were moved into growth chambers at 18 and 30°C, respectively, on the tenth day at 9:00 a.m. These were inoculated on the twelfth day from 8:00 a.m. to

4:00 p.m. The plants were removed from the growth chambers just at inoculation time and were returned to the chambers after the aphids were removed. Half of the plants exposed to the pre-inoculation temperature of 18°C were returned to the chamber maintained at 18°C and the other half to the chamber at 30°C. Similarly the plants kept at the pre-inoculation temperature of 30°C were divided equally between the two chambers maintained at 18 and 30°C, respectively. This procedure resulted in the following four pre- and post-inoculation temperature combinations:

Temperature	
Pre-inoculation	Post-inoculation
18°C	18°C
18°C	30°C
30°C	18°C
30°C	30°C

The test plants were removed from the growth chambers on the fourteenth day after sowing at 5:00 p.m. Thus the duration of each of pre- and post-inoculation temperature treatments was 47 to 56 hours.

The results of the experiment and variance analysis are presented in Table 12. Pre-inoculation temperature had no effect on the susceptibility of plants.

Post-inoculation temperature significantly affected transmission. More plants were infected at 30°C than at 18°C. These results were obtained by both the methods of inoculation.

In the second experiment (Table 13) of this series, temperatures of 15 and 30°C were compared in a 2 x 2 factorial arrangment. The two factors were pre-inoculation temperatures (15 and 30°C) and post-inoculation temperatures (15 and 30°C). Only single-probe method of inoculation was used. The experiment was replicated 48 times. All other details of this experiment were like those of the first three-factorial experiment of this series. Again, pre-inoculation temperature had no influence on plant susceptibility. Post-inoculation temperature did alter the susceptibility of plants. More plants were infected at 30°C than at 15°C.

Welton (43, p. 121-122) pointed out that the pre-inoculation temperature effect in a pre-inoculation temperature X post-inoculation temperature factorial experiment might depend on a particular transition between the pre- and post-inoculation temperatures. Another experiment was, therefore, made to compare the pre- and post-inoculation temperatures of 15, 24 and 30°C in a 3 x 3 factorial experiment. In this experiment two growth

chambers were used for 15 and 24°C, and a greenhouse bench for 30°C. The bench was covered with black cotton-cloth from top and sides. Gro-Lux lamps were used on the bench to obtain light of the same intensity and quality as in growth chambers. In this experiment, procedures and details were the same as in the first experiment of this series except that (1) test plants were inoculated by the conventional method of inoculation only, and (2) there were three levels (15, 24, and 30°C) each of the pre-inoculation and post-inoculation temperatures instead of two (18 and 30°C) in the first experiment.

The results of this experiment and statistical analysis of data are shown in Table 14. Pre-inoculation temperature had no effect on the susceptibility of plants, whereas post-inoculation temperature did so. More plants were infected at 30°C than at 15°C or at 24°C.

A last experiment (Table 15) was made to investigate the effect of pre-inoculation temperature. The pre-inoculation temperature effect was superimposed on the fertilizer effect. Two levels of fertilizer (details not included in this thesis) were used with 15 and 30°C pre-inoculation temperatures, giving a total of four treatments. The fertilizer, at two different rates, was mixed

in soil. Lincoln peas were grown on a greenhouse bench for nine days. On the tenth day, the plants were moved into growth chambers at 15 and 30°C, respectively. The plants were inoculated on the twelfth day after planting. Only single-probe method of inoculation was used. Fertilizer did not alter plant susceptibility to virus infection, but pre-inoculation temperature had a considerable effect. More plants were infected at 15°C than at 30°C. This effect was obtained at both the fertilizer levels.

The results of these experiments followed the same pattern as obtained by Welton (43, p. 70-106). Pre-inoculation temperature for 47 to 56 hours considerably influenced plant susceptibility to BYMV infection when plants after inoculation were kept at a temperature of 22°C (night) and of about 27°C (day). Also, post-inoculation temperature for 48 to 56 hours had a definite effect on plant susceptibility. The pre- and post-inoculation temperature effects were, however, not additive when these were tried in a factorial design. Only post-inoculation temperature affected plant susceptibility. Pre-inoculation temperature effect was completely erased by post-inoculation temperature treatment. These tests also indicated that post-inoculation temperature treatments

for 24 hours and less had no effect on plant susceptibility.

Table 11. Effect of post-inoculation temperature on the susceptibility of Lincoln pea to BYMV isolate 1 infection over a 24-hour period.

A. Data

Replicates	Post-inoculation Temperature					
	18°C			30°C		
	1[a]	4	24	1	4	24
1	1[b]	0	1	2	1	1
2	1	0	2	1	2	2
3	1	1	0	1	2	1
4	0	1	2	0	1	2
4	1	2	1	2	2	2
6	2	2	1	1	1	1
7	1	1	0	1	2	1
8	2	1	1	2	1	2
9	2	1	2	1	0	2
10	0	1	1	2	2	0
11	2	1	1	1	2	1
12	0	1	1	1	1	2
13	1	2	0	2	2	0
14	2	1	2	0	0	1
15	2	1	2	2	0	0
16	0	1	1	2	1	2
17	1	1	0	0	1	1
18	2	1	1	1	1	2
19	1	1	1	1	1	0
20	1	0	1	0	2	1
21	1	2	1	0	0	2
22	0	1	0	1	1	1
23	1	2	1	0	1	1
24	1	0	0	1	1	1
25	1	0	0	0	0	2
26	2	0	1	2	0	1
27	1	1	1	1	1	2
28	1	2	1	1	1	0
29	0	1	0	1	0	0
30	2	1	2	2	1	1
31	0	2	1	1	1	2
32	1	2	1	0	0	2
Total	34	34	30	33	32	39

[a] 1, 4 and 24 = Time (hours) plants were exposed to different post-inoculation temperatures.

[b] Each number = plants infected out of two inoculated.

Table 11. Continued.

B. Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Total	191	95.48			
Replicates	31	15.81	0.51	1.02	NS
Treatments	5	1.42	0.28	0.06	NS
Post-inoculation temperature(Post)	1	0.19	0.19	0.04	NS
Duration	2	0.07	0.04	0.01	NS
Post X Duration	2	1.16	0.58	1.16	NS
Error	155	78.25	0.50		

Table 12. Effect of pre- and post-inoculation temperature on the susceptibility of Lincoln pea to BYMV isolate 1 infection by aphid inoculation.

A. Data

Replicates	Pre-inoculation Temperature 18°C				Pre-inoculation Temperature 30°C				Total
	Post[a] 18°C		Post 30°C		Post 18°C		Post 30°C		
	1st[b]	2nd	1st	2nd	1st	2nd	1st	2nd	
1	0[c]	0	0	1	0	0	0	0	1
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	0	0	1	1	0	0	1	0	3
5	0	0	0	0	0	0	0	0	0
6	0	1	0	1	1	0	0	0	3
7	0	0	0	1	0	0	0	1	2
8	0	1	0	0	0	0	0	0	1
9	0	0	0	1	0	0	1	1	3
10	0	0	0	0	0	1	0	0	1
11	0	0	0	0	0	0	0	0	0
12	0	1	0	0	0	0	1	0	2
13	0	0	0	0	0	0	0	1	1
14	1	0	0	1	0	0	1	0	3
15	0	0	0	0	1	0	0	0	1
16	0	0	0	0	0	0	0	1	1
17	0	0	0	0	0	0	0	1	1
18	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	1	1	2
20	0	1	1	0	1	0	0	0	3
21	1	1	1	1	0	0	0	1	5

Table 12. Continued.

A. Data - Continued

Replicates	Pre-inoculation Temperature 18°C				Pre-inoculation Temperature 30°C				Total
	Post[a] 18°C		Post 30°C		Post 18°C		Post 30°C		
	1st[b]	2nd	1st	2nd	1st	2nd	1st	2nd	
22	0	0	1	1	0	1	0	1	4
23	0	1	0	1	0	0	1	1	4
24	0	0	0	1	0	0	0	0	1
25	0	0	0	1	0	0	0	0	1
26	0	0	0	0	0	0	0	1	1
27	0	0	0	0	0	0	1	0	1
28	0	0	0	1	0	1	0	0	2
29	0	0	0	0	0	0	0	1	1
30	0	1	1	1	0	0	1	1	5
31	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	1	0	0	1
34	0	0	0	0	1	1	0	0	2
35	0	0	0	1	0	1	0	0	2
36	1	0	0	0	0	0	0	0	1
37	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	1	0	0	1
39	0	0	0	0	0	0	1	0	1
40	0	0	0	0	0	0	0	1	1
41	0	0	0	1	0	0	0	0	1
42	0	0	1	0	0	0	0	0	1
Total	3	7	6	15	4	7	9	13	64

[a]Post = Post-inoculation temperature
[b]1st and 2nd = Plants in a pair inoculated by an aphid.
[c]1 = plant infected, 0 = plant not infected.

Table 12. Continued.

B. Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Total	51.81	335			
Replicates	9.06	41	0.221	1.59	0.001
Treatments	2.91	7	0.416	2.99	0.01
Pre-inoculation temperature (Pre)	0.01	1	0.010	0.07	NS
Post-inoculation temperature (Post)	1.44	1	1.440	10.36	0.01
Inoculation Methods (M)	1.19	1	1.190	8.56	0.01
Pre X Post	0.00	1	0.000	0.00	NS
Pre X M	0.11	1	0.110	0.79	NS
Post X M	0.11	1	0.110	0.79	NS
Pre X Post X M	0.05	1	0.050	0.36	NS
Error	39.84	287	0.139		

Table 13. Effect of pre- and post-inoculation temperature on the susceptibility of Lincoln pea to BYMV isolate 1 infection by the single-probe method of inoculation.

A. Data

Replicates	Pre-inoculation Temperature 15°C Post[a] 15°C	15°C Post 30°C	30°C Post 15°C	30°C Post 30°C	Total
1	0[b]	0	0	1	1
2	1	1	1	1	4
3	1	1	0	0	2
4	1	1	0	0	2
5	0	1	0	0	1
6	0	0	0	2	2
7	0	0	1	0	1
8	1	0	0	1	2
9	0	1	2	2	5
10	1	1	1	0	3
11	1	1	0	1	3
12	0	0	0	1	1
13	0	2	0	0	2
14	0	1	1	1	3
15	0	1	1	0	2
16	0	1	0	1	2
17	0	1	1	0	2
18	0	0	0	1	1
19	0	0	0	1	1
20	1	1	1	0	3
21	1	1	0	0	2
22	0	0	1	0	1
23	0	1	0	0	1
24	1	1	0	1	3
25	1	0	0	1	2
26	1	0	0	1	2
27	0	1	1	0	2
28	0	1	0	2	3
29	0	0	0	1	1
30	1	0	1	1	3
31	0	1	0	1	2
32	0	0	0	1	1
33	1	1	1	0	3
34	0	1	1	1	3
35	1	0	0	1	2

Table 13. Continued.

A. Data - Continued

Replicates	Pre-inoculation Temperature 15°C Post[a] 15°C	15°C Post 30°C	30°C Post 15°C	30°C Post 30°C	Total
36	1	2	0	1	4
37	0	0	1	1	2
38	1	0	0	0	1
39	1	1	0	1	3
40	0	1	0	0	1
41	1	1	0	1	3
42	0	0	0	0	0
43	0	2	0	1	3
44	0	1	0	0	1
45	0	0	0	1	1
46	0	0	0	0	0
47	0	2	0	1	3
48	0	0	1	0	1
Total	18	32	16	31	97

B. Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Total	72.00	191			
Replicates	13.25	47	0.28	0.74	NS
Treatments	4.44	3	1.48	3.89	0.05
Pre-inoculation temperature (Pre)	0.05	1	0.05	0.01	NS
Post-inoculation temperature (Post)	4.38	1	4.38	11.53	0.001
Pre X Post	0.01	1	0.01	0.00	NS
Error	54.31	141	0.38		

[a]Post = Post-inoculation temperature.

[b]Each number = plants infected out of two inoculated.

Table 14. Effect of pre- and post-inoculation temperature on the susceptibility of Lincoln pea to BYMV isolate 1 by the conventional method of inoculation.

A. Data

Replicates	Pre[a] 15°C			Pre 24°C			Pre 30°C			Total
	Post[b]			Post			Post			
	15°C	24°C	30°C	15°C	24°C	30°C	15°C	24°C	30°C	
1	0[c]	2	1	1	1	2	0	1	2	10
2	1	0	1	2	1	2	1	1	0	9
3	1	0	1	2	0	0	1	1	1	7
4	0	0	0	0	1	1	2	1	2	7
5	0	0	0	0	0	1	2	0	1	4
6	1	1	1	0	1	2	2	1	1	10
7	0	1	2	1	0	0	0	0	0	4
8	0	0	1	0	1	1	0	1	1	5
9	1	1	1	1	2	2	1	1	1	11
10	0	1	1	0	1	1	0	0	1	5
11	1	0	1	0	2	2	1	2	1	10
12	2	1	2	1	1	1	0	0	1	9
13	1	0	0	0	1	0	1	2	1	6
14	1	0	2	1	0	1	0	1	1	7
15	1	0	0	1	1	0	1	0	0	4
16	0	1	1	1	0	1	0	1	1	6
17	0	1	1	0	1	1	0	0	2	6
18	1	1	1	0	1	2	1	0	1	8
19	0	0	1	0	0	1	0	0	1	3
20	1	1	1	0	1	0	1	0	0	5
21	1	0	1	1	0	0	0	0	0	3
22	0	0	1	1	2	2	0	1	0	7

Table 14. Continued.

A. Data - Continued

Replicates	Pre[a] 15°C Post[b] 15°C	Pre 15°C Post 24°C	Pre 15°C Post 30°C	Pre 24°C Post 15°C	Pre 24°C Post 24°C	Pre 24°C Post 30°C	Pre 30°C Post 15°C	Pre 30°C Post 24°C	Pre 30°C Post 30°C	Total
23	0	0	1	0	1	0	0	1	1	4
24	1	1	2	0	1	1	0	0	1	7
25	0	0	1	0	0	0	0	0	0	1
26	2	0	1	0	2	1	0	0	2	8
27	0	0	0	0	0	0	0	0	0	0
28	2	0	1	1	2	0	1	1	1	9
29	0	0	0	1	0	0	1	1	0	3
30	1	1	1	1	1	1	2	0	0	8
31	0	1	1	0	0	1	1	0	0	4
32	0	0	0	1	2	0	2	1	1	7
Total	19	14	29	17	27	27	21	18	25	197

[a]Pre = Pre-inoculation temperature

[b]Post = Post-inoculation temperature

[c]Each number = Plants infected out of two inoculated

Table 14. Continued.

B. Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Total	130.25	287			
Replicates	25.36	31	0.82	2.10	0.01
Treatments	6.97	8	0.87	2.23	0.03
Pre-inoculation temp (Pre)	0.47	2	0.24	0.62	NS
Post-inoculation temp(Post)	3.70	2	1.85	4.74	0.01
Pre X Post	2.80	4	0.70	1.79	NS
Error	97.92	248	0.39		

Table 15. Effect of fertilizer and pre-inoculation temperature on the susceptibility of Lincoln pea to BYMV isolate 1 infection by the single-probe method of inoculation.

A. Data

Replicates	High Fertilizer		Low Fertilizer		Total
	15°C[a]	30°C	15°C	30°C	
1	0[b]	0	0	0	0
2	1	2	0	0	3
3	0	0	1	1	2
4	0	1	1	0	2
5	0	0	0	0	0
6	1	0	0	0	1
7	0	0	0	0	0
8	1	0	0	0	1
9	1	0	1	0	2
10	3	1	0	1	5
11	2	0	2	0	4
12	1	1	0	0	2
13	0	0	2	0	2
14	0	1	0	1	2
15	1	0	1	0	2
16	1	0	2	0	3
17	2	1	0	1	4
18	0	0	1	1	2
19	1	0	0	0	1
20	1	1	2	1	5
21	3	0	2	1	6
22	0	0	0	0	0
23	1	1	1	1	4
24	0	0	1	1	2
Total	20	9	17	9	55

[a]15 and 30°C = pre-inoculation temperatures.

[b]Each number = plants infected out of three inoculated.

Table 15. Continued.

B. Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Total	51.49	95			
Replicates	16.24	23	7.06	15.69	0.0000 001
Treatments	3.95	3	1.32	2.93	0.05
Fertilizers (F)	0.09	1	0.09	0.02	NS
Pre-inoculation temperature (Pre)	3.76	1	3.76	8.36	0.005
FX Pre	0.10	1	0.10	0.02	NS
Error	31.30	69	0.45		

E. Vector-Virus Relationships of *M. persicae* and BYMV

1. Effect of Artificial Termination of Acquisition Probes on Transmission Efficiency.

Bradley (8, p. 80-81), Bradley and Rideout (10, p. 337) and Sylvester (42, p. 54-55) reported that artificial interruption of acquisition probes reduced transmission of stylet-borne viruses to one-half of that of naturally terminated probes. McLean (24, p. 1061) found little effect of artificial termination of acquisition probes on the transmission of feathery mottle virus of sweet potato by *M. persicae*.

An experiment was made to compare artificially and naturally terminated acquisition probes by *M. persicae* transmission of BYMV isolate 1. Naturally terminated probes were of 11- to 45-second duration and were obtained as described under materials and methods. Artificially terminated probes were of 15 $\pm$ 2 second duration. The probing aphids were disturbed by touching their antennae with a camel's-hair brush. This gentle disturbance was started after the thirteenth second of acquisition probe. The distrubed aphids stopped feeding and withdrew their stylets in one to four seconds. The experiment was replicated 125 times using 500 aphids.

The results and variance analysis of the experiment are shown in Table 16. There was no appreciable difference between the transmission efficiency of the two groups of aphids. Forty-three percent of the aphids with naturally terminated probes transmitted BYMV. Transmission efficiency of the artificially interrupted probes was 37 percent. This difference was not significant.

2. Relation of Duration of Acquisition Probe to BYMV Transmission.

The relation of duration of acquisition probes to BYMV transmission was studied in three experiments involving 375 aphids. Probes of 11 to 45 second duration were grouped into seven classes of five-second intervals (Table 17). Eighty percent of the aphids probed for 10 to 25 seconds. The duration of probes over the 11- to 45-second range had no appreciable effect on the rate of BYMV transmission. Similar results were reported by Swenson (37, p. 522) and Welton (43, p. 107) working with the same vector-virus combination.

3. Relation of BYMV Transmission to Number of Test Probes.

A 2 x 3 factorial experiment was made to study the effect of number of test probes on BYMV transmission.

The two factors were: (1) bean cultivars, and (2) BYMV isolates. Bachicha and Seminole beans were used as test plants. Three BYMV isolates included were: 1, 6 and 6-A. Isolate 1 has been mentioned in materials and methods. Isolate 6 was a subculture of isolate 1, maintained by mechanical transfer. Isolate 6-A was initiated from isolate 6, and was maintained by M. persicae transfer. Test probers of 10 seconds and longer, during the first five minutes of test feeding, were recorded. A summary of the results (plants infected out of 30 inoculated for each BYMV isolate-bean cultivar combination) follows:

Bean Cultivars	BYMV Isolates		
	1	6	6-A
Bachicha	9	10	5
Seminole	7	9	9

There was no difference in the susceptibility of the bean cultivars. All the BYMV isolates were transmitted as frequently and there was no interaction between bean cultivars and BYMV isolates. The data could be used, therefore, for studying the relation of BYMV transmission to number of test probes. The data, grouped into classes by number of test probes, are given in Table 18 and Figure 1. BYMV transmission increased as the number of

test probes increased. The relation between test probes and number of infected plants was linear as revealed by regression analysis.

4. Retention of BYMV by Feeding Aphids.

Retention of BYMV isolate 1 by feeding M. persicae was studied in two experiments. In one experiment, an aphid, after acquisition probe, was fed on a series of ten test plants. Each test plant was probed only once for 10 to 30 seconds. Test probes shorter than 10 seconds were disregarded. If the aphid did not stop probing by the end of 30 seconds, it was removed with a camel's-hair brush and transferred to the next test plant in the series. Seventy-five aphids were fed in this manner on 750 plants. Test probe time and the time that elapsed after the acquisition probe was recorded for each aphid. The following were calculated for each of the infective aphids:

1. time required to transfer an aphid through a series of 10 plants.
2. retention time which was the time elapsed between termination of the acquisition probe and beginning of test probe on the last plant infected in a series, and
3. actual probing time during the first five minutes of

test feeding.

The infective aphids were grouped according to retention time into classes of one-minute intervals.

The results of the experiment are given in Table 19. Twenty-seven of 75 aphids (36 percent) transmitted BYMV to one or more plants, and these aphids infected 49 of 750 plants (6.5 percent). There were no significant differences in the number of plants infected by each infective aphid.

The probability of transmission after the first plant was greatly reduced. Of the 27 infective aphids, 21 infected the first plant, 7 infected the second and 3 infected the tenth plant. Loss of virus by feeding aphids could be expressed exponentially. A straight line was obtained when the logarithm of infective aphids was plotted against retention time (Figure 2). Half-life of the retention of BYMV isolate 1 by feeding aphids was 3 minutes. Frazier and Sylvester (17, p. 233) found that loss of strawberry mottle and strawberry vein-banding viruses by non-feeding aphids could be expressed exponentially.

An aphid took from 8.88 to 21.83 minutes to probe the 10 plants in a series. Actual probing time during the first 5 minutes of test feeding of an aphid varied

from 0.18 minutes to 2.16 minutes.

In the second experiment, 50 aphids, which had probed a diseased plant, were each fed on a series of six test plants. An aphid was allowed to stay on a test plant for five minutes. Number and duration of test probes on each of the six test plants were recorded for each aphid. Record was also kept of the time elapsed between termination of the acquisition probe and beginning of each of the test probes.

The results of the experiment are shown in Table 20. Eighteen of 50 aphids (36 percent) transmitted BYMV. Only one aphid infected two plants, all other infective aphids infected just one plant each. Probability of infecting a second plant in this case was very low. Of the 18 infective aphids, 16 infected the first plant, one infected the second plant and another infected the third and fourth plants. No aphid infected the fifth or sixth plant. Actual probing time during the first 5 minutes of test feeding of an aphid varied from 0.28 to 4.37 minutes.

The efficiency of transmission in the above two experiments (Tables 19 and 20) was 36 percent. Transmission of virus to the first plants was less by the single-probe method than by the five-minute test feeding. The reverse

was true, however, of the second plants. More of the second plants were infected by the single-probe method than by the five-minute test feeding. The actual probing time on the first plant during the 5-minute test feeding was 2.79 $\pm$ 1.1 minutes as compared to 10 to 30 seconds in the case of single-probe method. Short test probing time and low transmission on the first plants were thus correlated with higher transmission on the second plants. Similar results were reported by Swenson (38, p. 476) using the same virus-vector combination.

The loss of BYMV by feeding aphids was much faster when an aphid fed on each plant for five minutes as compared to when an aphid probed a plant only once for 10-30 seconds. In the former case, only 13 percent of the infective aphids retained the virus at the end of 5 minutes after acquisition probe, and 6 percent at the end of 10 minutes. In the case of the single probe per plant, 37 percent of the infective aphids retained the virus at the end of 5 minutes after acquisition probe and 15 percent at the end of 10 minutes.

Table 16. Effect of artificially terminated and naturally terminated acquisition probes on the transmission of BYMV isolate 1 by M. persicae.

A. Summary of Data

Naturally Terminated Probes		Artificially Terminated Probes	
Plants Inoculated	Plants Infected	Plants Inoculated	Plants Infected
250[a]	107	250	97

B. Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Total	134.6	249			
Replicates	73.1	124	0.59	1.20	NS
Probes	0.9	1	0.90	1.83	NS
Error	60.6	124	0.49		

[a]Each number = Total of 125 replicates.

Table 17. Relation of duration of acquisition probe to the rate of BYMV transmission by *M. persicae*.

	Acquisition Probes (in seconds)						
	11-15	16-20	21-25	26-30	31-35	36-40	41-45
Number of aphids fed	163	83	62	21	20	11	15
Percent aphids	43.5	22.1	16.5	5.6	5.3	2.9	4.0
Number transmitted	61	37	25	10	7	5	7
Percent transmission	37.4	44.6	40.3	47.6	35.0	45.4	41.2

Table 18. Relation of BYMV isolate 1 transmission by *M. persicae* to number of test probes.

A. Summary of Data

Number of Test Probes	Number of Aphids Fed	Number of Aphids Transmitted	Percent Transmission
1	40	9	22.50
2	44	8	18.18
3	61	18	29.51
4	28	11	39.29
5	7	3	42.86

B. Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Total	35.66	179			
Test Probes	1.06	4	0.265	1.34	NS
Linear Regression	0.80	1	0.800	4.06	0.05
Deviation from linearity	0.26	3	0.087	0.44	NS
Error	34.60	175	0.197		

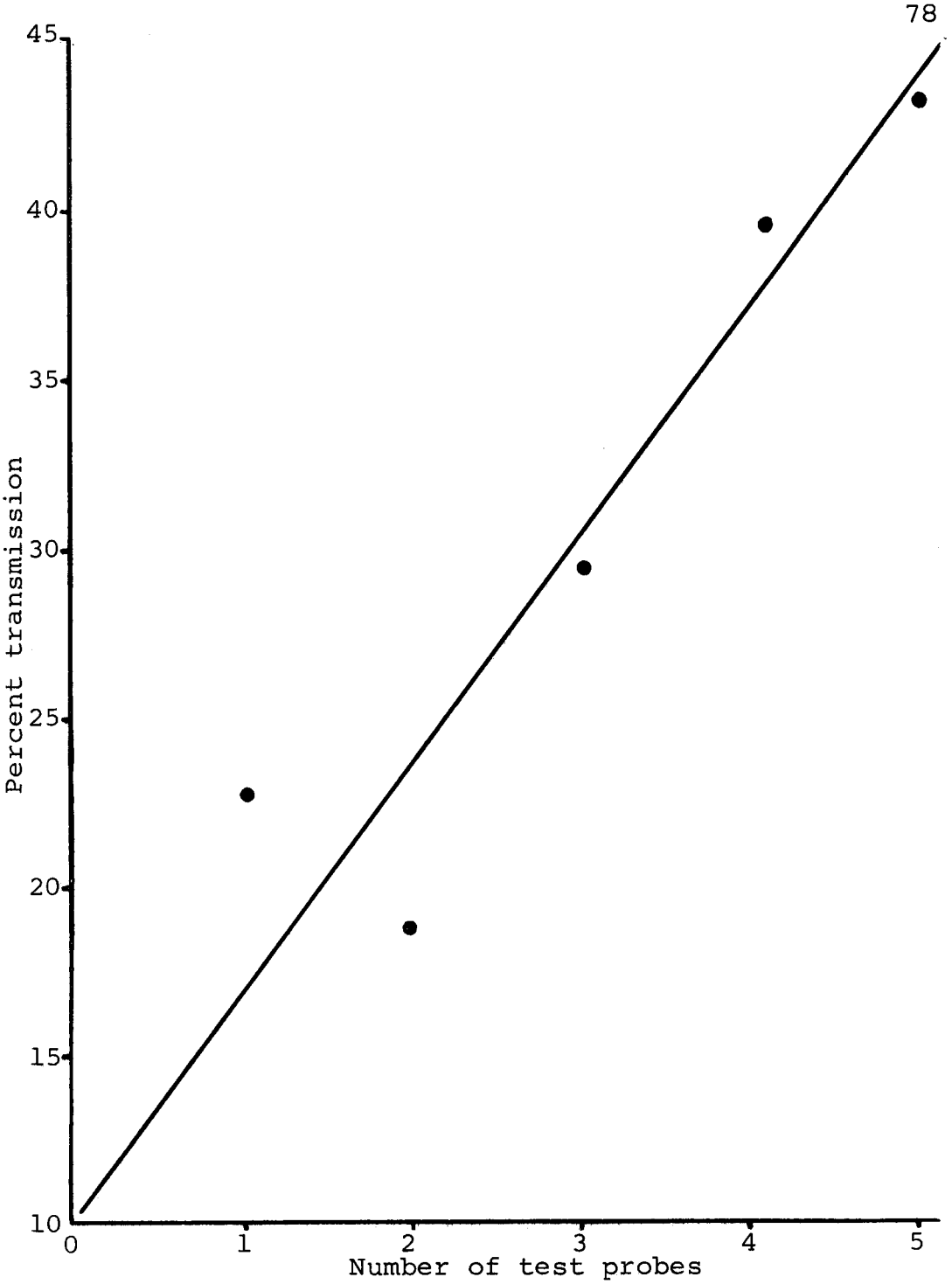

Figure 1. Relation of BYMV transmission by Myzus persicae to number of test probes.

Table 19. Retention of BYMV isolate 1 by feeding M. persicae when an aphid probed once on each of the ten plants in a series.

A. Serial Transmission Data

Aphid No.	Successive Plants in Test Feeding Series										Total Plants Infected	Completion Time[a]	Retention Time[b]	Probing Time[c]
	1	2	3	4	5	6	7	8	9	10				
1	0[d]	1	0	0	0	0	0	0	0	0	1	14.40	1.58	1.47
2	1	0	0	1	0	0	0	0	0	0	2	15.40	5.75	1.47
3	1	0	0	0	0	0	0	1	0	0	2	10.90	7.78	1.58
4	1	0	0	0	0	0	0	0	0	0	1	10.83	0.08	1.53
5	1	0	0	0	0	0	0	0	0	0	1	18.53	1.02	0.72
6	1	0	0	0	0	1	0	0	0	0	2	16.61	6.07	1.12
7	0	1	0	1	0	0	0	0	0	0	2	15.98	6.16	0.80
8	1	1	0	0	0	0	0	0	0	0	2	16.39	2.60	1.45
9	1	0	0	0	0	0	0	0	0	0	1	17.52	1.40	0.93
10	1	0	1	0	0	0	0	0	0	0	2	15.47	2.91	1.22
11	1	0	0	0	0	0	0	0	0	1	2	15.30	14.83	1.88
12	1	0	0	1	0	1	1	1	0	0	5	21.53	17.25	1.25
13	0	1	0	1	0	0	0	0	0	0	2	10.59	3.00	1.53
14	1	1	1	1	0	1	0	0	0	0	5	11.33	5.25	2.00
15	1	0	0	0	0	0	0	0	0	0	1	8.88	0.40	2.16
16	1	0	0	0	0	0	0	0	0	0	1	14.42	1.08	1.33
17	1	0	0	0	0	0	1	0	0	1	3	13.92	13.50	1.32
18	1	1	0	0	0	0	0	0	0	0	2	11.95	2.40	1.33
19	1	0	0	0	0	0	0	0	0	0	1	12.45	0.58	0.78
20	0	0	0	0	0	0	0	0	0	1	1	21.83	21.60	0.25

Table 19. Continued.

A. Serial Transmission Data - Continued

Aphid No.	Successive Plants in Test Feeding Series 1	2	3	4	5	6	7	8	9	10	Total Plants Infected	Completion Time[a]	Retention Time[b]	Probing Time[c]
21	1	0	0	0	0	0	0	0	0	0	1	12.58	0.45	1.18
22	0	0	1	0	1	0	0	0	0	0	2	14.54	5.08	1.47
23	0	1	0	1	0	0	0	0	0	0	2	18.30	4.33	1.12
24	1	0	0	0	0	0	0	0	0	0	1	17.73	1.39	0.73
25	1	0	0	1	0	0	0	0	0	0	2	15.92	4.40	1.42
26	1	0	0	0	0	0	0	0	0	0	1	17.80	0.39	1.25
27	1	0	0	0	0	0	0	0	0	0	1	20.23	3.78	0.18
Total	21	7	3	7	1	3	2	2	0	3	49	Mean		1.24
												Standard deviation ±		0.45

[a]Minutes required to probe ten plants in a series.

[b]Minutes elapsed between termination of acquisition probe and beginning of feeding on last plant-infected.

[c]Actual probing time during first five minutes of test feeding.

[d]1 = plant infected, 0 = plant not infected.

Table 19. Continued.

B. Serial transmission data arranged according to retention time

	Time (minutes) elapsed after acquisition probe												
	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	13-14	14-15	17-18	21-22	Total
Aphids retaining BYMV charge	27	22	17	13	12	10	7	5	4	3	2	1	
Last plant infected in a series	5	5	4	1	2	3	2	1	1	1	1	1	27

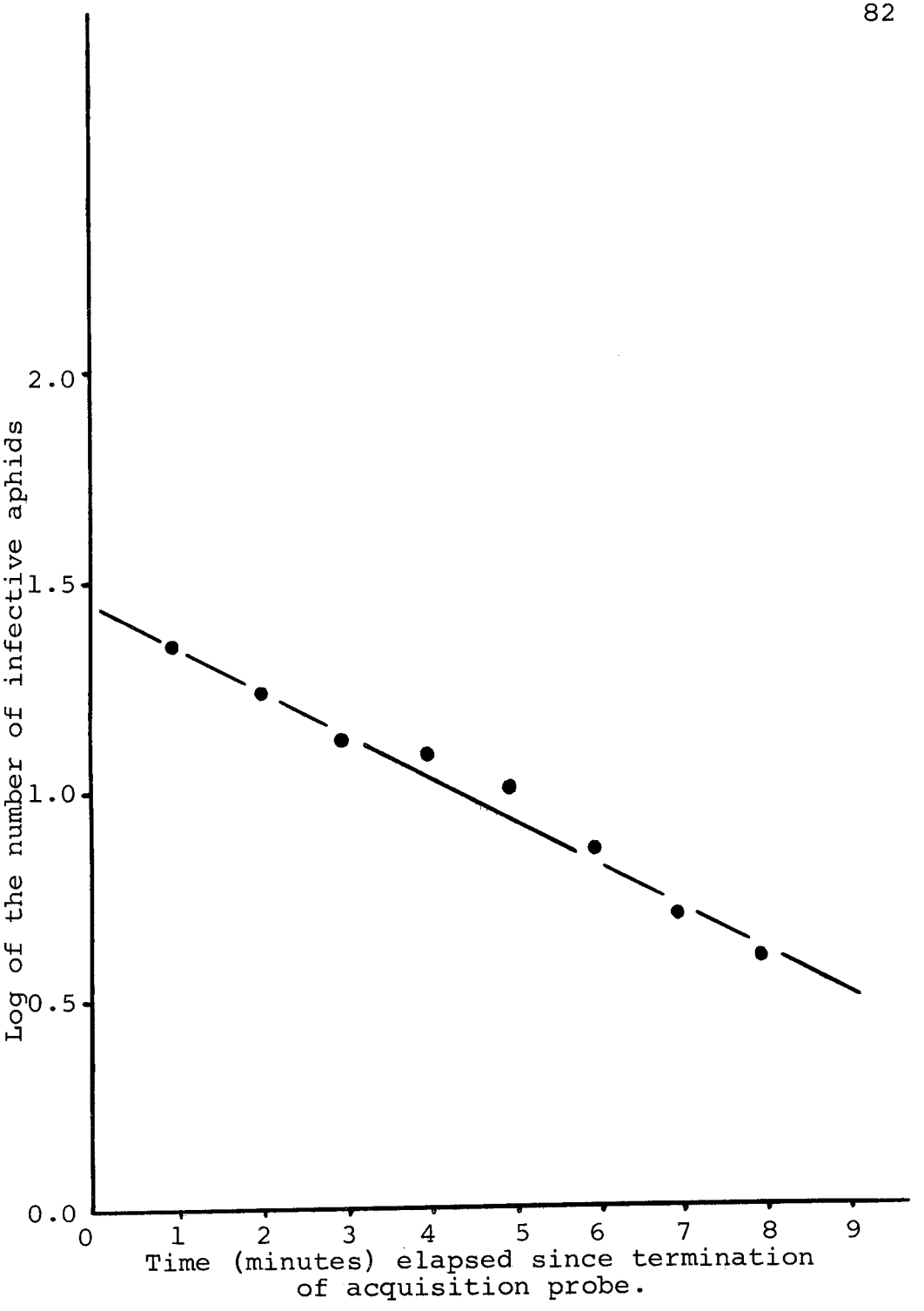

Figure 2. Retention of BYMV by feeding Myzus persicae.

Table 20. Retention of BYMV isolate 1 by feeding M. persicae when an aphid was allowed a test feeding of 5 minutes on each of the 6 plants in a series.

Aphid No.	Successive Plants in Test Feeding Series						Total Plants Infected	Minutes Probing on First Plant
	1	2	3	4	5	6		
1	0[a]	0	1	1	0	0	2	0.28
2	1	0	0	0	0	0	1	3.22
3	1	0	0	0	0	0	1	4.25
4	1	0	0	0	0	0	1	3.95
5	1	0	0	0	0	0	1	3.35
6	1	0	0	0	0	0	1	1.62
7	1	0	0	0	0	0	1	3.48
8	0	1	0	0	0	0	1	1.40
9	1	0	0	0	0	0	1	4.37
10	1	0	0	0	0	0	1	2.03
11	1	0	0	0	0	0	1	2.20
12	1	0	0	0	0	0	1	1.80
13	1	0	0	0	0	0	1	2.92
14	1	0	0	0	0	0	1	2.78
15	1	0	0	0	0	0	1	3.40
16	1	0	0	0	0	0	1	3.52
17	1	0	0	0	0	0	1	3.03
18	1	0	0	0	0	0	1	2.79
Total	16	1	1	1	0	0	19	Mean 2.79
								Standard deviation ±1.10

[a] 1 = plant infected, 0 = plant not infected.

F. Aphid Transmission Tests with Clover Yellow Mosaic Virus.

There have been no reports of vectors of clover yellow mosaic virus (CYMV) so far. Pratt (25, p. 660) found that the pea aphid, A. pisum, and the clover aphid, Anuraphis bakeri (Cowen) failed to transmit this virus. Three experiments were made to transmit CYMV with several aphid species. The materials and methods used in these tests were the same as mentioned for BYMV transmission tests unless otherwise specified.

A preliminary trial tested the transmission of CYMV by M. persicae. One of 100 aphids transmitted the virus. Six aphid species were included in the second experiment. Twenty-five aphids each of M. persicae, A. pisum, M. euphorbiae, M. rosae, A. fabae and C. aegopodii were used in five replicates. A different source plant was used for each replicate. None of 150 aphids of the six species transmitted the virus.

A final experiment was made to test the transmission of CYMV by M. persicae using 334 aphids. Dwarf Horticultural bean was the source plant for 100 aphids and broad bean for the other 234 aphids. Dwarf

Horticultural bean was included to find if the species of the source plant would affect aphid transmissibility. None of 334 aphids transmitted the virus.

The host reaction of CYMV in Vicia faba L., Phaseolus vulgaris L. cv. Bountiful, Gomphrena globosa L., Chenopodium amaranticolor Coste and Reyn., Cucumis sativus L., and Pisum sativum L. was like that described by Pratt (25, p. 658-659). It could be very easily confused with BYMV in some plants. Its symptom expression in Lincoln pea, Dwarf Horticultural bean, and Bountiful bean was similar to that of BYMV. Also, its primary symptoms in Chenopodium and secondary symptoms in broad bean, especially the symptoms on new sprouts, were like those of BYMV.

DISCUSSION

The pea aphid was usually an efficient vector of BYMV. Clone C was atypical in having a low transmission efficiency. The aphid species included in these tests could be arranged in the following order of descending efficiency of BYMV transmission: M. euphorbiae, A. pisum (clone B), M. persicae, A. fabae, A. pisum (clone C), M. rosae, T. riehmi, B. helichrysi, and C. aegopodii. The different positions of clones B and C of the pea aphid in such a ranking are quite important inasmuch as they indicate that differences in the inherent transmitting ability of clones of the same species were as large as some of the differences among different aphid species.

The differences in the BYMV transmitting ability of the pea aphid clones, along with the differences in their body size, fecundity and host preference, merit their recognition as biotypes. Inherent variation in the vector is likely to be as important as variation in the virus.

The low transmission efficiency of clone C might indicate that it would be less important in field spread

of BYMV. This may not necessarily be the case. Efficiency of virus transmission is not the only criterion in evaluating field spread of viruses by an aphid. Field spread of viruses by an aphid species or biotype is a function of transmission efficiency and numbers of aphids. The greater fecundity of clone C may more than compensate for its low transmission efficiency. Thus clone C may have greater potential as a vector of BYMV than clone B, in spite of less efficient transmission.

No evidence was found of differences in the inherent transmitting ability of the green peach aphid clones. There was a large variation among the clones in BYMV transmission. Variation among colonies of the same clone was, however, as much among different clones. Similar variation among identical clones of the green peach aphid was reported by Carpenter (11, p. 26). Such a variation among identical clones could not have been genetically based. It could have been due to colony plant effects, since these colonies were identical in all respects except the colony plants. It has been demonstrated that colony plants can considerably affect

BYMV transmission by aphids (41, p. 67)(38, p. 472-473). The variation among identical clones could have been caused by the same factors as in identical clones.

Aphid transmissibility of BYMV was lost on two occasions following a single mechanical transfer. The loss of transmissibility involved a change in the virus and not in the aphid, since the green peach aphid as well as the two pea aphid clones failed to transmit the virus. These aphids could transmit other isolates of BYMV. Also, the lack of aphid transmissibility could not have been due to an atypical source plant, as several plants were used as virus source.

Loss of insect transmissibility by plant viruses has usually followed maintenance of virus by mechanical transfer for several years (21, p. 67-70). This led to the impression that such a loss is gradual which does not appear to be the case with BYMV. Aphid transmissibility of BYMV was lost following a single mechanical transfer in 4 to 6 weeks in the present work and in 1 to 4 months in another case (36, p. 730). Swenson (39) found no reduction in aphid transmission when several BYMV isolates were maintained by mechanical

transfer for 3 to 4 years.

The loss of aphid transmissibility in such a short period could be explained only on the basis of mutation. Black (7, p. 466) and Swenson (36, p. 730) have discussed how a vectorless mutant may multiply to the exclusion of the insect transmissible virus. The symptoms of BYMV in broad bean plants, which were inoculated with both the aphid transmissible and the non-transmissible isolate, were like those of the latter. Also, aphid transmission of virus from this composite source was very low. This would indicate that the non-transmissible isolate had multiplied to the exclusion of the aphid transmissible isolate. Given more time or a few mechanical transfers, the vectorless isolate would have completely replaced the aphid transmissible isolate. A mutation resulting in a vectorless virus particle could lead to the complete loss of aphid transmissibility in this manner.

Mechanical transfer of a virus may not be a prerequisite for the loss of its transmissibility by insects. Vectorless isolates are not obtained under the laboratory conditions without resorting to mechanical

inoculation because the insect carries only the insect transmissible virus particles at each transfer. Particles of a vectorless mutant, if any, are left behind. Thus the concentration of the vectorless mutant does not build up. Reduction or loss of insect transmissibility might occur if a virus is kept in plants without any transfer for a sufficiently long time.

The plants to be used as virus source for insects should preferably be inoculated by insect transfer, since a single mechanical transfer may alter insect transmissibility of a virus. Further, a minimum number of source plants, preferably a single plant, should be used as virus source for insects in an experiment if the virus isolates and/or the source plants are not experimental variables.

Both pre- and post-inoculation temperatures considerably influenced susceptibility of Lincoln peas to BYMV infection by aphid inoculation. The effects of pre- and post-inoculation temperatures were not, however, additive. The number of plants infected depended entirely on post-inoculation temperature. The reason for this interaction between pre- and post-inoculation temperatures is not yet understood. At

this stage one can only say that post-inoculation temperature has a definite effect whereas pre-inoculation temperature effects can be detected only when post-inoculation temperatures are constant.

Artificial termination of acquisition probes did not have any appreciable effect on BYMV transmission by the green peach aphid. This was important. Only aphids terminating acquisition probes naturally within 11 to 45 seconds are used in BYMV transmission tests for reasons mentioned in aphid inoculation under methods. Other aphids are discarded. Most of the aphids usually terminate acquisition probes naturally within 11-45 seconds. Only a small number of aphids are discarded. Aphids do not behave, however, well on some days. One has to discard a large number of aphids because of too long probes. This renders inoculation of plants by aphids time consuming and tiresome.

One could interrupt the probing aphids artifically in the case of BYMV transmission by the green peach aphid on days when a large number of aphids probe longer than 45 seconds, since such interruption did not have any appreciable effect on BYMV transmission. If one has to interrupt acquisition probes artificially,

it should not be done at the end of 45 seconds. Aphids feeding that long would probably be feeding in deeper tissues, which results in a reduced transmission. The best time to disturb probing aphids would be between the fifteenth and thirtieth second of acquisition probe, which gives the highest transmission.

Transmission of BYMV by the green peach aphid increased with an increase in the number of test probes except in the case of two-test probe group. Swenson (38, p. 475), working with the same virus-vector combination found, however, that transmission was affected by the total probing time and not by the number of test probes. Duration of individual test probes was not recorded in my experiment. The total probing time could not, therefore, be calculated. It is possible, however, that larger number of test probes in my experiment resulted in a longer total probing time except in the case of two-probe group. The total probing time for the two-probe group may have been less than that for single probes. This may account for less transmission with two probes than with single probes.

SUMMARY

All eight aphid species included in these tests transmitted BYMV. B. helichrysi, T. riehmi and C. aegopodii of these species have not previously been reported as vectors of this virus. There were large differences among the species in BYMV transmitting ability.

Clones of the pea aphid included biotypes differing in BYMV transmission, fecundity, body size and host preference. No differences were found among the green peach aphid clones.

BYMV isolates differed in symptom expression and in the ease with which they were transmitted by aphids. Aphid transmissibility of BYMV was lost or greatly reduced following a single mechanical transfer. The vectorless isolate multiplied to the virtual exclusion of the aphid transmissible isolate when broad bean plants were inoculated simultaneously with both these isolates.

Different areas of broad bean leaves were not equal as sources of BYMV for aphids. More aphids transmitted the virus from the interveinal chlorotic areas than from the green areas along the veins.

Post-inoculation temperature for 48-56 hours had a considerable influence on Lincoln pea susceptibility to BYMV infection by aphid inoculation. More plants were infected at 27 and 30°C than at 15, 18, or 24°C. Post-inoculation temperature treatment for 24 hours or less did not have any appreciable effect. Pre-inoculation temperature for 47-56 hours also considerably influenced plant susceptibility to infection with BYMV by aphid inoculation. Twice as many plants were infected at 15°C as at 30°C. The effects of pre- and post-inoculation temperatures were not additive. The number of plants infected depended entirely on post-inoculation temperature.

Artificial termination of acquisition probes did not have any appreciable effect on BYMV transmission by the green peach aphid. No significant differences in virus transmission were found for aphids with acquisition probes in the 11- to 45-second range. Virus transmission increased with an increase in the number of test probes. Loss of BYMV by feeding green peach aphid could be expressed exponentially. Half-life of the retention of virus by feeding aphids was about three minutes.

Clover yellow mosaic virus could be easily confused with BYMV on the basis of symptom expression in Dwarf Horticultural bean, Bountiful bean, Lincoln pea, broad bean (secondary symptoms), and in Chenopodium (primary reaction). It was not transmitted by A. pisum, A. fabae, C. aegopodii, M. euphorbiae, M. rosae and M. persicae.

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