BEAN YELLOW MOSAIC VIRUS TRANSMISSION BY MYZUS PERSICAE

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Summary

Efficiency of bean yellow mosaic virus (BYMV) transmission from broad bean source plants varied with age of leaf on which aphids, *Myzus persicae* (Sulz.), fed. Duration of infection in the source plants did not affect transmission. Observations of acquisition feeding behaviour yielded results which are interpreted to indicate that most transmission occurs with virus acquired intracellularly.

Aphids from thriving *M. persicae* colonies on young leaves of *Brassica pekinensis* Rupr. transmitted BYMV to more plants than did a comparable group of aphids from mature leaves. Likewise, aphids from thriving colonies on young leaves transmitted the virus to more plants than did aphids from older colonies on heavily infested, deteriorating plants. These differences in transmission were not explained by comparable transmission tests with adult apterae and fourth instar alatiform nymphs nor by the feeding behaviour of aphids on healthy test plants. No differences in transmission were found between apterous adults and fourth instar alatiform nymphs. The number of plants infected in successive replicates by aphids from uniform colonies was more consistent than that by aphids from variable colonies.

Transmission increased with increasing duration of probes on healthy test plants. The number of probes was not as important as total probing time. Non-feeding *M. persicae* gradually lost the capacity to transmit BYMV following the acquisition feeding. Virus was retained for at least 4 hr.

I. INTRODUCTION

Aphid transmission of bean yellow mosaic virus is characterized by a high degree of variation within and among experiments (Swenson 1960). The magnitude and frequency of the variation indicated the existence of important unknown factors affecting the transmission of this virus. Effects of changing temperature and light intensity on susceptibility of plants to mechanical inoculation with viruses suggested that variations in the greenhouse environment might cause similar effects on BYMV transmission by aphids. Numerous experiments showed, however, that large changes in environment or nutrition were necessary to bring about relatively small changes in the susceptibility of test plants to inoculation with BYMV by aphids. The condition of the colony from which aphids are obtained for transmission tests, on the other hand, is constantly changing, even in a uniform environment, due to the feeding of the aphid population which increases until the point of plant deterioration is reached. Preliminary experiments indicated that variation in the condition of the colonies was a major source of transmission vari-

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ation (Swenson and Sohi 1961). This idea is not new, although full appreciation of the idea and experimental data have been lacking.

Watson (1936) used aphids to inoculate tobacco plants with henbane mosaic virus over a 2-year period and noted a seasonal trend in number of infected plants. More plants were infected in winter than in summer. This was attributed to increased plant susceptibility at lower light intensities and shorter days. Results of transmission experiments were combined in weekly totals which were highly variable. Lack of correlation between the weekly totals and meteorological conditions was attributed to "biological errors due to variation in aphid cultures and technique", among other things.

Myzus persicae (Sulz.) reared on peach transmitted lettuce mosaic virus to fewer plants than did *M. persicae* reared on mustard, sugar beet, or radish (Sylvester 1955). The aphids reared on peach were from different stock than those reared on the other plants and, therefore, may have had an inherently different ability to transmit the virus. *M. persicae* reared on chard transmitted southern cucumber mosaic virus to more plants than did *M. persicae* reared on pepper (Simons 1955). The difference was small, however, and well within the scope of variation possible among samples from aphids with equal transmission efficiency. Simons considered transmission differences due to aphid host plants to be relatively unimportant in his experiments. MacKinnon (1961) showed that more plants were inoculated with turnip latent mosaic virus by *M. persicae* reared on detached leaves of *Physalis floridana* Rydb. infected with potato leaf roll virus than by aphids reared on either detached leaves of *Datura stramonium* L. infected with potato leaf roll virus or on detached leaves of healthy rape plants. Also, more plants were inoculated with turnip latent mosaic virus when aphids were reared on detached leaves of healthy rape plants than when reared on detached leaves of healthy *D. stramonium*.

The purpose of the work reported herein is to substantiate the idea that aphid colony condition can greatly influence BYMV transmission and to obtain information on the nature of the effect on transmission.

II. Methods and Materials

(*a*) *Definitions*

A "source plant" was a diseased plant on which aphids were placed to acquire virus. Aphids were removed from the colony plants and placed in shell vials for a period of "preliminary starvation" before being placed on the source plant. "Acquisition probes" refers to probes on the source plant. It is used in preference to the usual term, acquisition feeding, because all these probes were of 11–45 sec duration and terminated naturally. Acquisition probes were measured from the time the tip of the rostrum touched the leaf surface until it was removed. Healthy plants on which aphids were placed following the acquisition probe are designated "test plants". "Prepenetration time" was the period which elapsed after the aphid was placed on the test plant until it began to probe. Probes on the test plant are designated "test probes" and were measured in the same way as were the acquisition probes. The total time on the test plants is referred to as the "test feeding".

(b) *Materials*

Some of the experiments were made at Canberra and others at Corvallis, Oregon, U.S.A. The bean yellow mosaic virus (BYMV) used in the Corvallis experiments has been described before (Swenson 1960). The virus used in the Canberra experiments was obtained from naturally infected *Trifolium subterraneum* L. in Canberra. Symptoms produced on several species of plants indicated that it was BYMV. Some properties of this virus are described in Section III.

Myzus persicae, reared on *Brassica pekinensis* Rupr. unless otherwise indicated, was the only aphid species used in transmission tests. To minimize genetic variation new colonies were usually initiated by transfer of a single aphid from an old colony.

Vicia faba minor Beck. was used for source plants in the Canberra experiments and *V. faba major* L. was used in the experiments at Corvallis. Test plants were usually either pea, *Pisum sativum* L. cv. Greenfeast, or bean, *Phaseolus vulgaris* L. cv. Dwarf Horticultural.

(c) *Methods*

Aphids were removed from colony plants and placed in vials for starvation periods of 15 min or more. Maximum starvation effect on BYMV transmission was obtained in 15 min (Swenson 1960). Acquisition probes were of 11–45 sec duration, a range within which variation in transmission due to acquisition feeding should have been negligible (Swenson 1960). Only one aphid was placed on each test plant in all experiments except those on host range of the virus. Aphids were left on the test plant for at least 2 hr when the test feeding was not an experimental variable.

All experiments were replicated and, when several treatments were included in an experiment, a randomized block design was used with order of inoculation of plants representing the different treatments randomized within each replicate. The χ^2 test is theoretically more appropriate to virus transmission data than is the F test, but in practice little difference results from the test selected (Li 1957, p. 419). Analysis of variance and the F test were used in all experiments in which several treatments were included because of greater simplicity than the χ^2 test, especially for the analysis of factorial experiments. The probability values, P, represent the probability of obtaining a larger χ^2 or variance ratio if the null hypothesis were true.

III. Results

(a) *Properties of the Canberra Bean Yellow Mosaic Virus*

Preliminary host range trials indicated that the virus was a BYMV isolate. A positive reaction was obtained in a ring precipitation test with BYMV antiserum obtained from Dr. A. H. Gold, University of California, Berkeley.

Systemic symptoms followed mechanical inoculation of *Phaseolus vulgaris* cv. Pinto. Only chlorotic local lesions, 2–3 mm in diameter, occurred on the bean varieties Canadian Wonder, Gower's Special, and Hawkesbury Wonder. No local lesions occurred on Pinto. It was transmitted by *Myzus persicae* or mechanical inoculation to *Trifolium pratense* L., *Medicago denticulata* Willd., *Crotalaria spectabilis*

Roth., and Greenfeast pea, and returned to *V. faba minor* and Canadian Wonder bean where the original symptom type occurred. Necrotic local lesions resulted on *Ipomoea purpurea* (L.) Roth., a plant not previously reported susceptible to BYMV. The virus was recovered to *V. faba minor* by inoculation from single lesions. *Chenopodium amaranticolor* Coste & Reyn was also susceptible to the virus. No infection was obtained in *Datura stramonium* L. or *Cucumis sativus* L., each of which is susceptible to at least one of the other known sap-transmissible viruses of legumes in Australia.

(*b*) *Source Plant Effects*

(i) *Leaf Age.*—A factorial experiment on BYMV transmission at Corvallis included a comparison of broad bean leaves of different ages as sources of virus. Transmission from the youngest leaf (first leaf), which had unfolded sufficiently so that aphids could be placed on the upper surface, was compared with transmission from the leaf preceding it (second leaf). The experiment consisted of six replicates with 15 plants for each treatment in each replicate. The mean acquisition feeding for the 90 aphids on the first leaf was 25·4 sec and for the aphids on the second leaf, 26·8 sec. Twenty-four of 90 bean plants were infected by aphids that fed on the first leaf compared to 38 of 90 by aphids that fed on the second leaf ($P<0·05$). Consequently, aphids were placed on the leaf corresponding to the second leaf in all experiments.

(ii) *Duration of Infection.*—Broad bean plants infected with BYMV used for transmission tests developed a yellow mosaic symptom. Infected plants bloomed and produced pods without change in symptoms. A test was made at Corvallis to learn if availability of virus to aphids was as constant as the symptoms. The first of three groups of broad bean plants was inoculated on June 21, the second on July 8, and the third on August 5. In replicated transmission tests made from August 26 to September 1, 31 of 60 bean plants were inoculated by aphids that fed on the first group of source plants, 26 of 60 by aphids from the second group, and 29 of 60 from the third group. Differences in duration of infection up to 6 weeks obviously had no appreciable effect on the rate of aphid acquisition of BYMV from broad bean.

(*c*) *Acquisition Feeding*

Some aphids moved the tip of the rostrum about the leaf surface before probing, in a manner which can be described as tapping. Comparable transmission tests at Canberra, using *V. faba minor* test plants and aphids having naturally terminated acquisition probes of 11–45 sec duration, resulted in 36 of 45 plants infected by aphids which probed directly on the source plant and 30 of 45 plants infected by aphids which first tapped the leaf surface in several places. The difference in transmission was not significant, but a large number of tapping aphids were discarded in the process of obtaining 45 aphids with acquisition feedings of 45 sec or shorter duration.

Non-tapping aphids were much more likely to terminate acquisition probes within 45 sec than were aphids which tapped the leaf surface before probing. Aphids

apparently discerned differences in the leaf by tapping and this may be the means of locating transverse cell walls. In one experiment, 11 of 13 aphids which probed directly terminated the probes within 45 sec compared to termination within this time by 2 of 14 tapping aphids. Non-tapping aphids probably make most penetrations intracellularly. In *Datura stramonium*, for example, the leaf area occupied by transverse cell walls was 7·0% in leaves 4 in. long and 7·4% in leaves 8 in. long (M. F. Day, personal communication). Aphids which did not tap the leaf surface before probing would have been much more likely to insert the stylets into a cell than into a wall between cells. Work on the path of aphid stylets in plant tissue has been based on feeding periods of several minutes or longer and has indicated that stylet penetration is predominantly intercellular. No stylet tracks were left, however, by *M. persicae* which probed less than 45 sec in sugar-beet leaves (Esau, Namba, and Rasa 1961).

BYMV transmission was not affected by variation in duration of acquisition probes within the 11–45-sec range but decreased when acquisition probes were longer than 45 sec (Swenson 1960). Comparable transmission tests must be based on comparable acquisition probes and these cannot be obtained by forcible termination (Sylvester 1954). Therefore, the comparison of transmission by tapping and non-tapping aphids was not necessarily a comparison between intercellular and intracellular acquisition of virus because all acquisition probes were terminated naturally within 45 sec. The tapping aphids may have ceased probing for the same reason as non-tapping aphids—they had not found a transverse cell wall.

Van Hoof's (1958) conclusion that aphids acquired BYMV from the middle lamella was based on limited observations of aphid probing in detached epidermal strips and on the intercellular path of stylet tracks. My experiments indicate that, if tapping is the mechanism aphids use to find transverse cell walls, most transmission is of virus acquired intracellularly. This conclusion, as qualified, is based on the small portion of leaf area occupied by transverse cell walls, the much greater tendency of tapping aphids to probe longer than 45 sec on source plants, and less transmission when acquisition probes are longer than 45 sec.

(*d*) *Effect of Aphid Colony Plant*

Three experiments were made at Corvallis to determine the effect of condition of aphid colony plants on BYMV transmission. In the first experiment, transmission by *M. persicae* from recently established uncrowded colonies on young leaves of *B. pekinensis* was compared with that by aphids from heavily infested deteriorating plants. In the second experiment, transmission by aphids from young leaves of *B. pekinensis* plants, on which a high rate of reproduction was still occurring, was compared with that by aphids from mature leaves of the same plants. In the third experiment, transmission by aphids from young leaves of new uncrowded colonies on *B. pekinensis* was compared with that by aphids from mature leaves of the same plants and with transmission by aphids from old deteriorating colonies on *B. nigra* Koch.

Each experiment consisted of five replicates of 12 plants per treatment. A different colony was used in each replicate for each treatment. Aphids would,

therefore, represent the experimental variables, i.e. colony condition, and not merely reflect the peculiarities of a single colony, as might have happened if all aphids for each colony condition in an experiment were taken from one colony. An attempt was made to use only apterous adults or fourth instar alatiform nymphs. The following results were obtained:

Experiment	Colony	Transmission
1	New	To 37 of 60 plants
	Old	To 23 of 60 plants
2	Young leaves	To 37 of 60 plants
	Mature leaves	To 23 of 60 plants
3	New; young leaves	To 19 of 60 plants
	Old; mature leaves	To 11 of 60 plants
	Old	To 9 of 60 plants

The probability of obtaining such differences between aphid colonies without a real difference in virus transmission was less than 5% in experiments 1 and 2, and less than 1% in experiment 3.

(e) *Effect of Aphid Stage*

Attempts were made to use only apterous adults or fourth instar alatiform nymphs in the experiments on the effects of colony condition. Adult apterae could readily be selected from uncrowded colonies. The proportion of adult apterae to alatiform nymphs was low in crowded colonies, however, and it was difficult to be certain if apterous forms were adults or nymphs since little reproduction occurred. Consequently, crowded colonies were represented in the experiments by a high proportion of fourth instar alatiform nymphs. The differences between new and old colonies might, therefore, be explained if the different aphid forms did not transmit equally. Conflicting results have been obtained by others in experiments comparing virus transmission by different aphid forms or stages (Broadbent 1960).

(i) *Experiment* 1.—*M. persicae* was reared at Canberra on *Brassica rapa* L. (turnip), *Datura stramonium*, and *Malva parviflora* L. Each species was represented by five aphids per replicate in an experiment of 30 replicates. The aphids had one test probe of 11–120 sec duration in the first five replicates and were left on the test plant for 2 hr or longer in the last 25 replicates. The stage of each aphid was noted. Alates, apterous adults, or fourth instar alatiform nymphs were used in most cases, but occasionally younger aphids had to be included. Aphids from *Malva parviflora* transmitted virus to 91 of 150 plants (61%). Aphids from *D. stramonium* transmitted to 103 of 150 plants (69%), as did aphids from *B. rapa*. These differences are not significant. Two conclusions are possible concerning colony–plant effect: (1) No differences in BYMV transmission result when *M. persicae* is reared on these three species. (2) Aphids were taken from plants which frequently were not infested to an equal degree and, therefore, the different plant species were not appropriately compared. Fourth instar alatiform nymphs transmitted virus to 132 of 194 plants (68%). Apterous adults infected 121 of 167 plants (72%) and alates infected 21 of

29 plants (72%). Twenty-five of 75 plants were infected in replicates 1–5 compared to 270 of 375 plants (72%) in replicates 6–30.

(ii) *Experiment* 2.—*M. persicae* were reared on *Malva parviflora* at Canberra. The colonies were allowed to become crowded to the extent that apterous adults and fourth instar alatiform nymphs were produced on the same leaves. The same number of aphids of each stage was taken from each leaf used to supply aphids for the experiment. Differences in transmission should thus have reflected true differences in transmitting ability between stages, and not the condition of the leaves from

TABLE 1

RELATION OF APHID STAGE AND NUMBER OF TEST PROBES TO TRANSMISSION OF BYMV BY M. PERSICAE

Treatment	Plants Infected	Treatment	Plants Infected
Apterous adults		Alatiform nymphs	
One probe	10 of 40	One probe	15 of 40
5-min test period	25 of 40	5-min test period	29 of 40

Analysis of Variance

Source of Variation	Sum of Squares	Degrees of Freedom	*F*	*P*
Total	88	31		
Replicates	28	7	2·70	0·05
Treatments	29	3	6·53	0·005
Test feeding (TF)	26	1	17·57	0·001
Aphid stage (AS)	3	1	2·03	n.s.
TF × AS	0	1	—	—
Error	31	21		

which the aphids were obtained. Half the aphids of each stage were allowed one test probe of 10–60 sec duration. This single probe was interrupted at the end of 60 sec if not already terminated naturally. The remaining aphids were watched for 5 min on the test plant and the number of probes recorded. The aphids were removed at the end of 5 min even if they were then in the act of probing and such interrupted probes were included in the count. There were thus four treatments and the experiments consisted of eight replicates of 20 aphids each (five aphids per treatment).

The difference in BYMV transmission between the two aphid stages was not significant (Table 1). Fewer plants were infected by aphids with one test probe of 10–60 sec duration (34%) than by aphids with a 5-min test feeding (74%), indicating that transmission increased with increasing duration of the test feeding

period. The mean number of probes per aphid during 5 min for apterous adults was 2·17 and it was 2·23 for fourth instar alatiform nymphs. The non-significant differences in transmission and in numbers of probes between aphid stages, as well as the complete lack of interaction between aphid stage and probe number, permitted the combination of all data from 5-min test feedings, with the following results:

Number of Probes	Plants Infected
1	17 of 23 (74%)
2	31 of 44 (70%)
3	4 of 10
4	1 of 2
5	1 of 1

Too few aphids probed more than twice during 5 min on the test plants for adequate comparisons. Aphids that probed once during 5 min spend about as much time probing as those that probed twice, indicating that the factor affecting transmission was total probing time and not the number of probes.

(iii) *Experiment* 3.—Aphids were reared on *B. pekinensis* at Canberra. The colonies were allowed to become crowded to the extent that apterous adults and fourth instar alatiform nymphs were produced on the same leaves. Equal numbers of aphids of each stage was taken from each leaf used to provide aphids for the experiment. Each aphid was allowed one test probe of 10–60 sec duration and then transferred to a second plant where it was left for 2 hr or longer. There were 12 replicates, each including five aphids of each stage. Alatiform nymphs transmitted BYMV to 19 of the 60 plants on which the first test probe was made compared to 22 of 60 by apterous adults. Forty-five of 60 of the second test plants were infected in both groups.

(*f*) *Effect of Aphid Behaviour during Test Feeding*

Effects of colony plant conditions were not explained by differences in virus transmission between aphid stages. Condition of the colony plant, however, might have influenced aphid behaviour on the test plant and have caused transmission differences in this way. Further studies on aphid feeding behaviour on test plants were made at Canberra.

Greenfeast peas were used as test plants at the stage when the second leaf had expanded. Aphids were always placed on the upper surface of a leaflet of the second leaf. Prepenetration time, site, and duration of the first probe were recorded. Maximum duration of the first probe was 15 min after which the aphid was removed if probing had not already terminated naturally. Each aphid was then transferred to a second plant where it remained for 2 hr or longer. The experiment took place during a 21-day period and consisted of 23 replicates of 10 aphids each. All aphids used in each replicate were removed from the colony plant at the same time and placed in shell vials. The first aphid was used after a starvation period of 15 min. Transfer of the 10 aphids per replicate from the scource plant to the first and second test plants required 1–4 hr, depending primarily on how many aphids had to be watched for 15 min on the first plant.

Factors of particular interest (site and duration of probes) were not represented by samples of equal size and were not equally distributed among the replicates. These reflected differences in aphid behaviour and could not have been standardized because the effect of variation in behaviour was the primary object of investigation. Such results are not, however, amenable to satisfactory statistical analysis.

(i) *Duration of Test Probes.*—The virus was transmitted with probes of 9 sec duration and only one aphid probed for a shorter time (8 sec.) Aphids that probed 8–60 sec on the first plant infected a considerably lower proportion of the first test plants than did aphids that probed for 15 min (Table 2), showing once more that transmission increased with increasing duration of test probes. Intermediate probing times gave intermediate transmission. These results do not, however, account for transmission variation. Short probes and low transmission on the first plants

TABLE 2

RELATION OF DURATION OF PROBES ON TEST PLANTS TO TRANSMISSION OF BYMV BY M. PERSICAE

Duration of Probe (min)	No. of Aphids	First Plant Infected		Second Plant Infected		Both Plants Infected	
		No.	%	No.	%	No.	%
<1	144	49	34	83	58	96	67
15	54	36	67	12	23	40	74
Intermediate	32	12	38	15	47	32	69

were correlated with higher transmission on the second plants, indicating that total probing time is the determining factor in transmission and was probably the same for all three groups of aphids (Table 2).

(ii) *Site of Test Probes.*—In all, 75% of the aphids made the first probe on either the upper or the lower surface of the second leaf. Probes by the remaining aphids were distributed among other parts of the plants, but with too few in each area for useful comparisons. Aphids which probed the upper surface of the second leaf transmitted BYMV to fewer plants than did aphids which probed the lower surface (Table 3). The significance of the difference is difficult to evaluate but it cannot be explained by differences in duration of test probes at different sites. The number of aphids probing on the lower second leaf varied from replicate to replicate but there was no correlation with transmission within replicates (correlation coefficient = $-0{\cdot}376$, not significant).

(iii) *Prepenetration Time.*—Further information concerning the relation of transmission to aphid behaviour was obtained from prepenetration times on the first plants. Transmission increased with increasing prepenetration time (Table 4). This was unexpected because infective non-feeding aphids gradually lose ability to transmit BYMV following the acquisition feeding. Therefore, transmission was

expected to decrease somewhat with increasing prepenetration time. A possible explanation for these results is that the data constitute an aberrant sample. This is unlikely because of the large number of aphids involved. Another explanation is that some aphids which had the tip of the rostrum in contact with leaf surface did not make an acquisition probe. These aphids might have probed more readily

TABLE 3

RELATION OF SITE OF PROBES ON FIRST TEST PLANT TO TRANSMISSION OF BYMV BY M. PERSICAE

Site	No. of Aphids	Mean Duration of Probes (sec)	No. of Plants Infected
Upper second leaf	108	304	42 (39%)
Lower second leaf	65	244	32 (49%)
All other areas	57	215	23 (40%)

on the test plant and, consequently, the group of aphids with longer prepenetration times would have included a lower proportion of aphids without acquisition probes. Duration of prepenetration times varied among the replicates but no correlation was found between prepenetration time and transmission within replicates (correlation coefficient = 0·0639, not significant).

TABLE 4

RELATION OF PREPENETRATION TIME TO TRANSMISSION

Time (sec)	Total No. of Aphids	No. of Transmitting Aphids*
0–60	138	89 (64%)
61–120	47	33 (70%)
121–300	37	29 (78%)
300+	8	6 (75%)

* One or both plants infected.

(iv) *Inoculation Sequence.*—The relation of sequence of inoculation in each replicate to transmission in the 23 replicates is given in Table 5. There was obviously no relationship between inoculation sequence and transmission. This provides information on three points: (1) The previous finding that extension of the period of starvation beyond 15 min did not affect BYMV transmission (Swenson 1960) was confirmed. These data also indicate (2) that the probability of acquiring virus

from the source plant and (3) the susceptibility of the test plants did not change during the period required for inoculation of the plants in each replicate. One could assume that two, or all three, of these changes did occur with counteracting effects but additional evidence would be required before giving much credence to such an assumption.

TABLE 5

RELATION OF SEQUENCE OF INOCULATION WITHIN REPLICATES TO TRANSMISSION

Aphid No.	1	2	3	4	5	6	7	8	9	10
No. of transmitting aphids*	14	15	19	18	17	13	17	17	13	14

* One or both plants infected.

(*g*) *Virus Retention by Non-feeding Aphids*

Aphids were given the usual starvation and virus acquisition periods. They were then held in shell vials for various periods before transfer to healthy bean plants for comparison of transmission with that by aphids transferred directly to the test plants (0 retention). The experiment (at Corvallis) consisted of 10 replicates with six plants per retention period in each replicate. Groups of aphids representing the different periods, including the aphids transferred directly to test plants, were given acquisition feedings in a randomized order within replicates. The following results were obtained:

Retention time (min)	0	5	15	30	60	120	240
Transmission (%)	50	35	32	25	19	25	13

(*h*) *Interruption of Test Feeding*

Bradley (1952) and Sylvester (1954) found that forcible termination of the test feeding did not reduce the transmission of henbane mosaic virus and *B. nigra* virus by *M. persicae* below that by aphids having naturally terminated test feedings of approximately the same duration. Further information was obtained on this point because the interpretation of much of the data reported in this paper depended on the assumption that this would also be true of BYMV transmission.

The transmission by 50 aphids with interrupted test probes was compared with that by 50 aphids with naturally terminated test probes (at Canberra). Probes were of 10–35 sec duration. Eighteen plants were infected by aphids with interrupted test probes; 21 by aphids with naturally terminated test probes. The assumption of no difference in transmission resulting from interruption of test probes was further substantiated by these results.

(*i*) *Transmission Variation*

A final experiment at Corvallis compared transmission by aphids from uniform colonies with transmission by aphids from variable colonies. Adult *M. persicae*

were placed on *B. pekinensis* plants in the three-leaf stage and removed 4 hr later. One to five nymphs were deposited on each plant. A week later the aphids were redistributed so that each plant had the same number of aphids before reproduction began. Plants were kept in growth chambers before and after infestation with aphids. The growth chambers were operated at 16°C during the night (12 hr) and 26°C during the day (12 hr). Relative humidity was 81–84% during the night and 62–74% during the day. The photoperiod was 12 hr and light intensity was

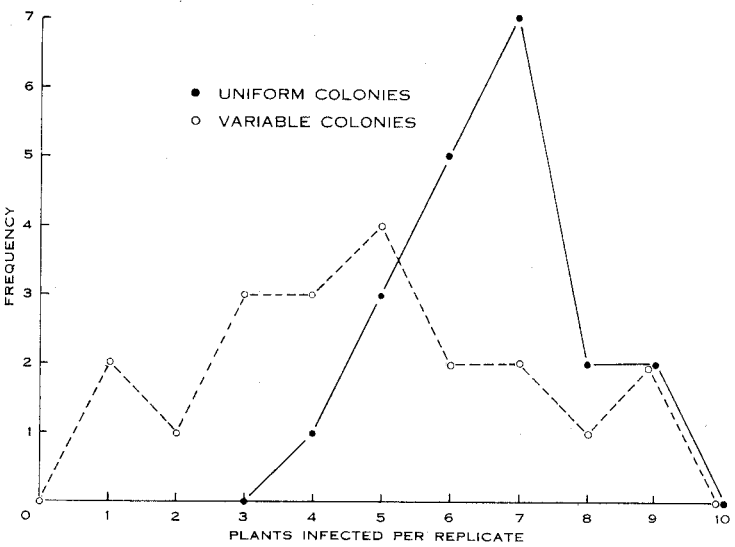

Fig. 1.—Frequency distribution of plants infected per replicate by aphids from uniform and variable colonies.

2700 f.c. (measured by photometer). Source of light was Sylvania VHO fluorescent lamps and incandescent lamps. Aphids from these plants were used in transmission experiments before crowding occurred and while the plants were still growing vigorously.

Aphids from variable colonies on *B. pekinensis* were obtained by keeping colonies in greenhouse rooms with different environments and in different types of cages. The colonies also varied in the degree of infestation and in plant age. Each replicate was represented by aphids from a different colony.

The starvation period was at least 15 min, the acquisition feeding was 11–45 sec, and the test feeding on pea plants, cv. American Wonder, was at least 2 hr. Four different *V. faba major* source plants were used and 1½ days were required to complete the aphid transfers. The experiment consisted of 20 replicates with each type of colony represented by 10 aphids in each replicate. Aphids from variable colonies were fourth instar alatiform nymphs or apterous adults. Second and third instar nymphs were used from uniform colonies because of better feeding behaviour on source plants.

Results are presented as a frequency distribution (Fig. 1). For example, 7 out of 10 plants were infected in each of seven replicates by aphids from uniform colonies. The mean number of infected plants per replicate was 6·6 for uniform aphids and 4·85 for variable aphids, a significant difference. Use of the χ^2 test as an index of dispersion, modified for samples of limited size (Goulden 1952, p. 371), indicated that the distribution of transmissions within replicates by uniform aphids was well within the range probable from samples with a common mean. This amount of variability could easily result from variation around a common mean probability of acquisition of virus. The same test applied to the transmission data for variable aphids indicated that such a distribution would be obtained less than once in 1000 times from samples with a common mean. These results show that the condition of the aphid colonies was an important factor in transmission, affecting both the amount and uniformity of transmission.

IV. Discussion

Results of several experiments showed that probability of transmission increased with increasing probing time on test plants. They also indicated that total probing time was as important as number of probes. Wildman (1959) suggested the idea of susceptible sites to explain the variation in susceptibility to tobacco mosaic virus of areas of a single tobacco leaf. According to this concept, the more leaf tissue an aphid contacts in probing, the more likely it is to place virus in a susceptible site. An aphid can contact more leaf tissue by probing at several different places or by probing longer in one place.

In the the experiment on aphid behaviour on the test plant, duration and site of first probe and prepenetration time were all highly significant inter-replicate variables. No relationship was observed, however, between behaviour and light intensity, temperature, relative humidity, or barometric pressure. Probably the condition of the aphid colony determines test feeding behaviour to some extent. Aphids from thriving colonies usually probed less readily on the source plant than did aphids from crowded deteriorating colonies.

When transmission of a stylet-borne virus, such as BYMV, is less than complete, the problem is to decide if some aphids did not transmit because they failed to acquire virus or because they did not succeed in inoculating test plants with virus they were carrying. Work presented herein on prepenetration time indicated that some aphids did not acquire virus because they failed to probe on the source plant, although their activities could not be distinguished from those that did probe.

Good evidence also exists that many aphids acquire virus but do not transmit it. The constancy of infected broad bean as source plants over a period of weeks and the frequent inconstancy of transmission during even much shorter periods is evidence of this. Comparison of aphids from uniform colonies with aphids from variable colonies resulted in more transmission by the uniform aphids than by the variable aphids even though they fed on the same source plants.

Consideration of colony–plant effects on transmission complicates the situation. The first three experiments on colony condition showed that the type of leaf or plant

affected virus transmission. Comparison of virus transmission by aphids from uniform colonies and variable colonies indicated that aphid colonies are the source of the high degree of variation in BYMV transmission described by Swenson (1960). Whether colony–plant effect is due to some unknown behavioural factor or some change in the ability of the aphids to transmit virus, variation in transmission may be greatly reduced if aphids for transmission tests are from colonies uniform with respect to plant age, degree of infestation, and environment. In my experiments and those of MacKinnon (1961), more transmission was obtained by aphids from plants that would be regarded as better host material for the aphids (Kennedy 1951; Baker 1960). Differences in transmission between different aphid stages or forms (Broadbent 1960) may reflect the condition of the plants from which aphids were obtained rather than intrinsic differences between stages or forms. Variability in transmission described for other aphid-borne viruses (Watson 1936; Hamlyn 1955; Sylvester 1956; Frazier and Sylvester 1960) may be also due to similar colony–plant effects.

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