STUDIES ON THE MECHANISM OF APHID TRANSMISSION OF STYLET-BORNE VIRUSES (1)

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By

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Introduction

There are many kinds of the plant virus transmitted by the aphid, and the aphid has a very important role in the outbreak of virus diseases in the field. Many species of the insect vector have a suctorial mouth with the aphid being the most representative of them all. Among species of the aphid, *Myzus persicae* (Sulz.) can transmit many kinds of virus and has been reported to transmit over 50 (12). Plant viruses are divided into two groups from the standpoint of mechanism of insect transmission. That is aphid-borne and circulative virus (12). Most viruses belong to the former group with the greater part of these being injurious to crop production. Therefore the mechanism of aphid transmission of the stylet-borne virus has been studied by Bradley (1, 2, 6, 8), Day and Irzykieuicz (10), Kasai (11), Kennedy et al. (12), Nishi (14), Sylvester (18, 19), Swenson (17), van Hoof (20), van der Want (21), Watson (22), Yoshii (24) and other investigators, and various opinions have been presented. In 1952 Bradley (4) suggested that the aphid transmission of stylet-borne viruses was conducted by a mechanical process. Day and Irzykieuicz (1954) presented the "mechanical-inactivator behavior" hypothesis, that aphid transmission linked specificity with stylet contamination by virus particles, and aphid transmission was affected by the behavior and the saliva of vectors. In the same year, Sylvester proposed the "mechanical-inactivator-compatibility" hypothesis. He assumed that aphid transmission was due to a function of "compatibility" among virus, saliva and the host plant. Van der Want (1954) developed the "mechanical-surface adherence" hypothesis, that the stylet-borne virus attached on the outer surface of the stylet when a vector did not excrete a saliva. But there has been no clear evidence for this hypothesis until recently. Bradley (1, 6), Bradley and Ganong (5) showed that virus was absorbed on the 15μ length portion from the apex of the stylet, and if the portion was an inner part of the stylet, it would be the tip of the food duct or the salivary duct, and if the portion was of the outer parts of the stylet, it would be

on or within maxillae stylet and mandible stylet. These findings supported the hypothesis of van der Want.

Van Hoof (20) observed the path of the stylet in the epidermis of *Hyacinthus orientalis* L., and proved that aphids acquired the virus when they inserted their stylets between the transverse walls of epidermis. On the other hand, Swenson (17) and van der Want (21) reported that the aphid acquired the virus when the stylet was inserted into the epidermal cell. There are various opinions, as to what parts of the plant tissue stylet-borne virus are acquired by the aphid. Bradley (2, 7) suggested that aphids became mostly viruliferous when the stylet penetrated into outer layer of the tobacco leaf infected with potato virus Y, and aphids were less viruliferous when the stylet penetrated into their deep layer. On the other hand, Swenson (17) indicated that the acquisition rate of virus increased by the insert of the stylet during a 10 to 60 seconds interval. McLean and Kinsey (13) reported that it was questionable whether the saliva helped the insert of stylet or not, and also the process of salivaly secretion was not clear.

With the above background, our experiments have been carried out for elucidating the mechanism of aphid transmission of stylet-bone viruses. In this paper, we report on the relation between the insert behavior of the stylet of the aphid and transmission of virus.

Definitions

For these experiments, the following terms are used as defined below.

"Starvation period" is the period which aphids placed in shell vials before being placed on diseased plants.

"Probing" refers to the insert of the stylet into the host plant tissues several times for a biref time (the probing duration is within 30 seconds).

"Probing duration" refers to the time of the initial probing. The probing duration was measured from the time which the tip of the rostrum touched the leaf surface until it was removed.

"Feeding probe" refers to the behavior of the aphid inserting the stylet into the leaf and sucking the juice over 2 minutes.

Materials and Methods

Virus

An ordinary strain of bean yellow mosaic virus (BYMV-O) was used in these experiments.* This virus was isolated from gladiolus (*Gladiolus gandavensis* van Hoult) by Dr. Inoue. BYMV-O was maintained in broad bean (*Vicia faba* L.), and developed mosaic leaves were used as the virus source.

* BYMV-O was received from Dr. T. Inoue of Inst. Agr. Biol. Res. Okayama Univ.

Aphids

Green peach aphid (*Myzus persicae* (Sulz.)) was mainly used. *Aphis craccivora* Koch and *Acrythosiphon pisum* Harris were also used in probing behavior trials. Every aphid used was of the non-viruliferous apterous viviparae type which were reared on plants of *Raphanus sativus* L., *Vicia faba* L. and *Pisum sativum* L., respectively. Because in preliminary experiments no difference in transmission efficiency was found between winged and wingless aphids, we used apterous viviparae. Non-starved and starved aphids were used in the trials.

Plants

Broad bean (*Vicia faba* L. var. Wase Soramame) which BYMV-O was easily transmitted by *Myzus persicae* (Sulz.) was used as the host plant in many experiments. In the observation of probing behavior, *Phaseolus vulgaris* L. (var. Tsuru nashi Kintoki) and *Nicotiana tobacum* L. (var. Blight Yellow) were added. All plants were grown in the phytotrone at about 23°C or in the greenhouse at about 20°C, and were used at the stage at which four trifoliolate leaves developed.

Details of the methods will be given in each experiment.

Results

PROBING BEHAVIOR OF APHID

The behavior of probing of *Myzus persicae* (Sulz.) was observed as follows. When aphid is placed on the leaf surface, the rostrum is attached to the venter and then the aphid walks for several steps. The antennae are put forward and waves in walking. When the aphid stops, the rostrum vibrates towards the leaf surface and then its tip contacts the leaf surface. This contact is repeated 2 or 3 times. It is a tactile-like movement and has been described as tapping. The waving of the antennae then stops and the stylet is inserted into the leaf in touching the rostrum with the leaf surface. The antennae are once more moved forward and begin to wave. Simultaneously the rostrum is lifted up from the surface. A series of such behavior are repeated several times. This is called probing behavior. The duration for one probing is usually from 15 to 25 seconds. After the probing, the aphid touches the rostrum to the leaf surface again and sucks in a long period at a given site of the leaf. This action is the feeding probe.

TIME SPENT PRIOR TO INITIAL PROBING AETER PLACEMENT ON LEAF

After aphids were transfered on the leaf surface, the time which aphids spent prior to the initial probing was measured. Contact of the leaf surface and the tip of the rostrum was assumed as the beginning of the insert of stylet, because observation of stylet penetration into the plant tissue was not possible under our conditions.

In these experiments, the aphid-host plant combinations were as follows;

Table 1. The combination of host plant- aphid.

Group	Plants	Aphids
IA	*Vicia faba* L.	starved *Myzus persicae*(Sulz.)
IB	*Vicia fabu* L.	non-starved *Myzus persicae* (Sulz.)
IIA	*Vicia faba* L.	starved *Aphis craccivora* Koch.
IIB	*Vicia faba* L.	non-starved *Aphis craccivora* Koch.
III	*Nicotiana tobacum* L.	starved *Myzus persicae* (Sulz.)
IV	*Vicia faba* L.	starved *Acythosiphon pisum* Harris.
V	*Phaseolus vulgaris* L.	starved *Myzus persicae* (Sulz.)

The period of starvation was one hour. Above mentioned combinations are designated as IA, IB, IIA, IIB, III and IV group, respectively. 150, 150, 118, 61, 93 and 178 aphids were investigated for each group.

Results are presented in Fig 1. In IA group, 78.3 % of total aphids engaged in initial probing for less than 10 seconds. In groups of IB, IIA, IIB, III and IV, 3.5, 17.4, 2.1, 45.5 and 27.5% of the total of aphids engaged in initial probing for less than 10 seconds, respectively. Aphids which probed less than 20 seconds were 94.2, 10.5, 61.6, 10.4, 70.5 and 44.7 % for IA, IB, IIA, IIB, III and IV groups, respectively. Behavior of aphids of IIA, IIII and IV groups was similar to that of IA group. The tendency that many aphids begun to probe in a relatively short time after placement on the leaf seems to be a common habit of a starved aphid. Mean values of the time which was spent prior to the initial probing were 13.0, 52.6, 32.5, 71.9, 26.0 and 33.4 seconds for each group, respectively. From these mean values, it is clear that IA group began to do the initial probing in a very brief period. While, IB and IIB groups did not quickly make the initial probing, but probed after a long period of time. This tendency may be a common habit of the non-starved aphid. These results indicate that probing behaviors differ according to the kinds of aphid on the same host plant, and also that the same kind of aphid behaves differently due to kinds of host.

DURATION OF INITIAL PROBING

Duration of the initial probing was measured. Experiments were carried out in every combination of the above experiment and in addition in V group (*Phaseolus vulgaris* L. and starved *Myzus persicae* (Sulz.)).

Results are presented in Fig 2. The number of aphdis used were 150, 150, 118, 61, 193, 198 and 309 for IA, IB, IIA, IIB, III, IV and V group, respectively. Aphids which carried out the initial probing in 12 to 30 were 88.8, 74.7, 64.0, 65.6, 81.6, 78.4 and 71.6% of investigated aphids for IA, IB, IIA, IIB, III, IV and V groups, respectively.

Accordingly over 65% of the initial probing terminated within 12 to 30 seconds regardless of the combinations of the aphid and the host plant, and starvation did not influence the duration. Mean values of the duration of the initial probing for

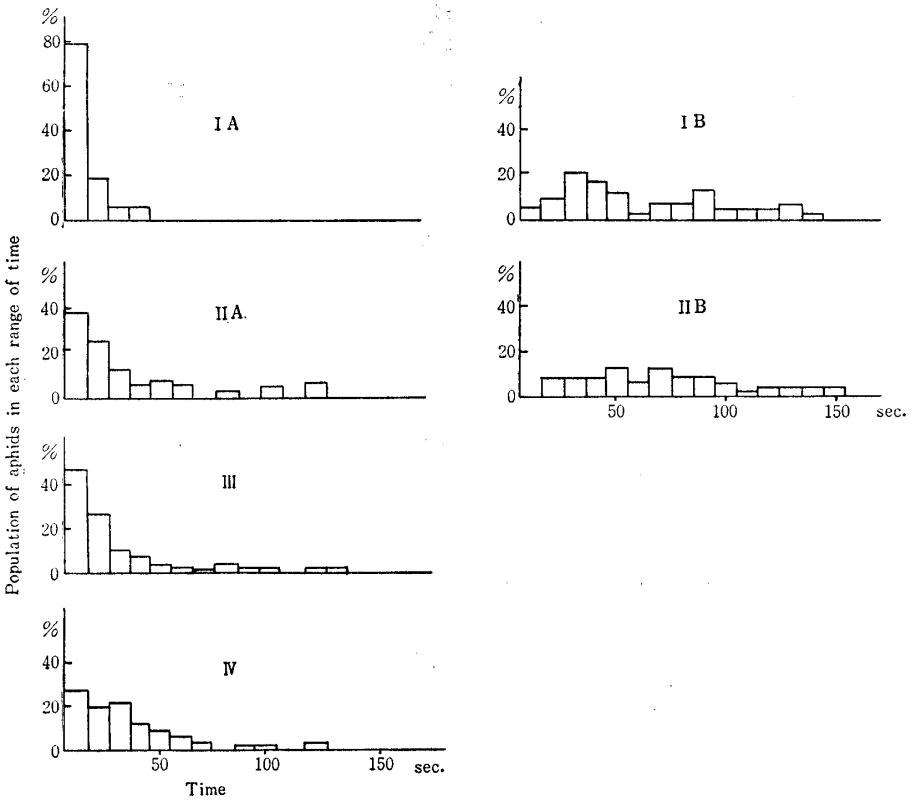

Fig. 1. Histograms of the time spent until the initial probe.
IA. *Vicia faba* L. - starved *M. persicae* (Sulz.)
IB. *Vicia faba* L. - non-starved *M. persicae* (Sulz.)
IIA. *Vicia faba* L. - starved *Aphis craccivora* Koch.
IIB. *Vicia faba* L. - non-starved *Aphis craccivora* Koch.
III. *Nicotiana tobacum* L. - starved *M. persicae* (Sulz.)
IV. *Vicia faba* L. - starved *Acythosiphon pisum* Harris.

each group were 27.3, 22.8, 25.8, 30.6, 19.6, 20.7 and 18.0 seconds, respectively. It was observed that the duration of the initial probing differed slightly with the kinds of the host plant or the aphid.

NUMBERS OF PROBING PERFORMED PRIOR TO THE FEEDING PROBE

Numbers of the probing done from placement of aphid on the leaf surface to beginning of the feeding probe were investigated.

Results are shown in Fig 3. The number of the aphids used were 70, 81, 92, 52, 100 and 110 for IA, IB, IIA, IIB, III and IV groups (above cited), respectively. In the case of IA group, 61.4% of aphdis probed only once before the feeding probe. But in groups of IB,III and IV, about 77% of aphids carried out the probing more than two times and these three groups indicated the same tendency on numbers of the probing. In every combination, aphids which performed the feeding probe immediately without the probing, were always observed, but a comparatively large number of aphids (about 30%) performed the feeding probe immediately in the

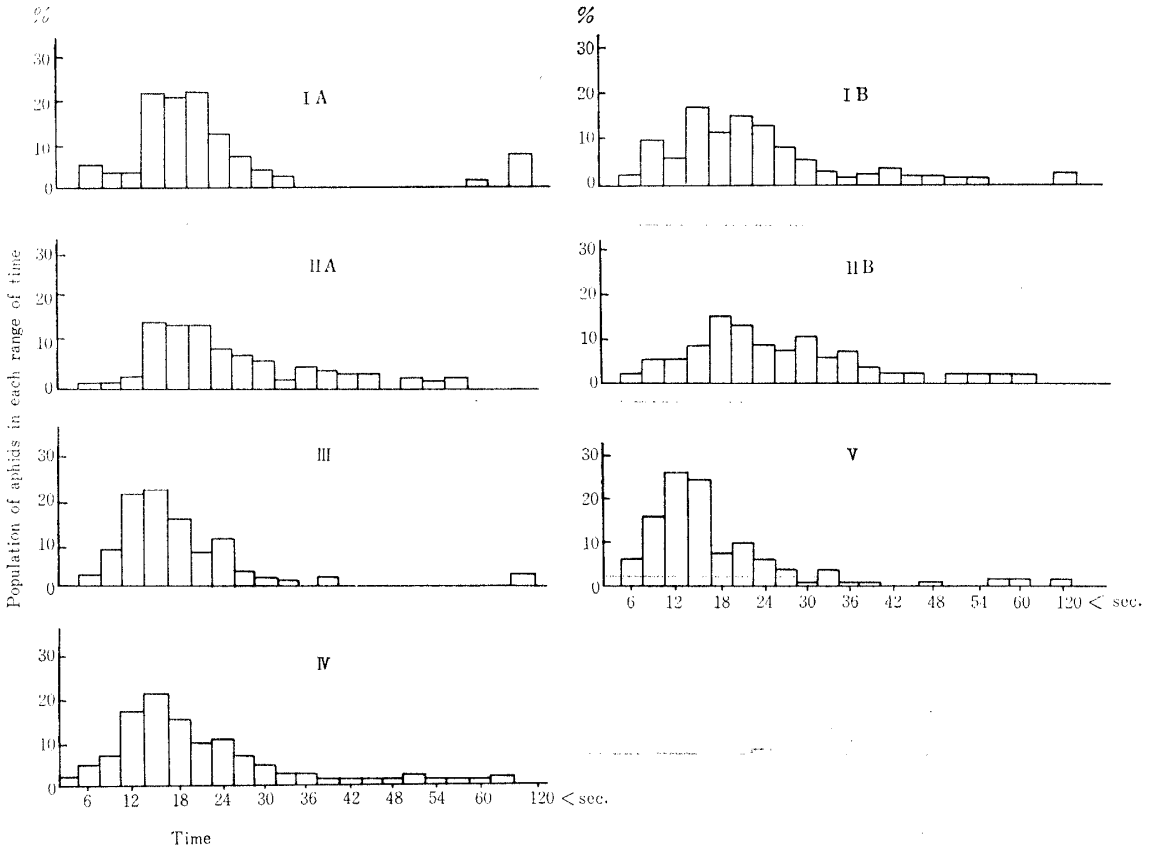

Fig. 2. Histograms of the duration of initial probing.

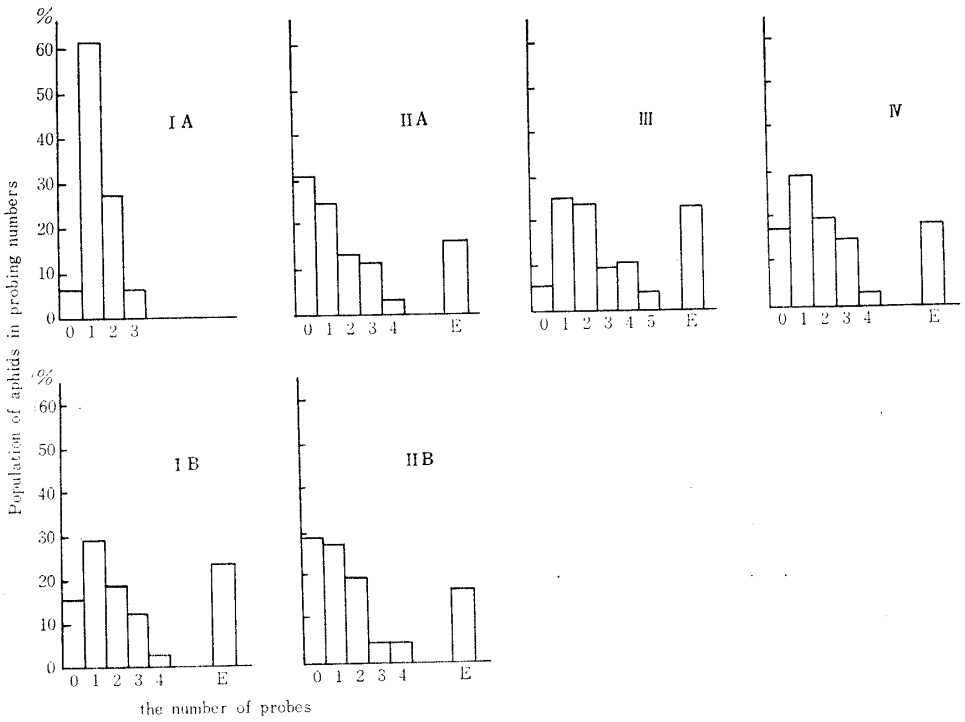

Fig. 3. Numbers of probing performed prior to feeding probe.
E : Aphids escaped from the leaf without the probing.

combination of broad bean and *Aphis craccivora* Koch. (namely IIA and IIB). This fact may be a special character of *Aphis craccivora* Koch. The largest number of probings done prior to feeding were 4 to 5 times in every case, whether or not the aphids were starved. Average numbers of the probing were 1.3, 1.5, 1.2, 1.0, 2.1 and 1.5 for each combination, respectively. These results show that a majority of the aphids probe once or twice on the average prior to the feeding probe, but some aphids perform the feeding probe immediately without probing. Further in this experiment about 20% of aphids escaped from the leaf without the probing in every group except IA group. This reason is not clear.

TIME SPENT PRIOR TO FEEDING PROBE

The time spent prior to the feeding probe from the placement of the aphids on the leaf surface was investigated. The number of the aphids used in IA, IB, IIA, IIB, and IV groups (as above cited) were 64, 60, 93, 50, 101 and 105, respectively.

Results are shown in Fig 4. In IA group, most of the aphids performed the feeding probe within 160 seconds. On the other hand, aphids in IB, IIB, III and IV groups spent variable times prior to the feeding probe, so the distribution pattern of the time was wider than IA group and there were unexpectedly many aphids which escaped without the feeding probe except in the case of IA. Distribution of the time for IIA group is the intermediate pattern between IA group and

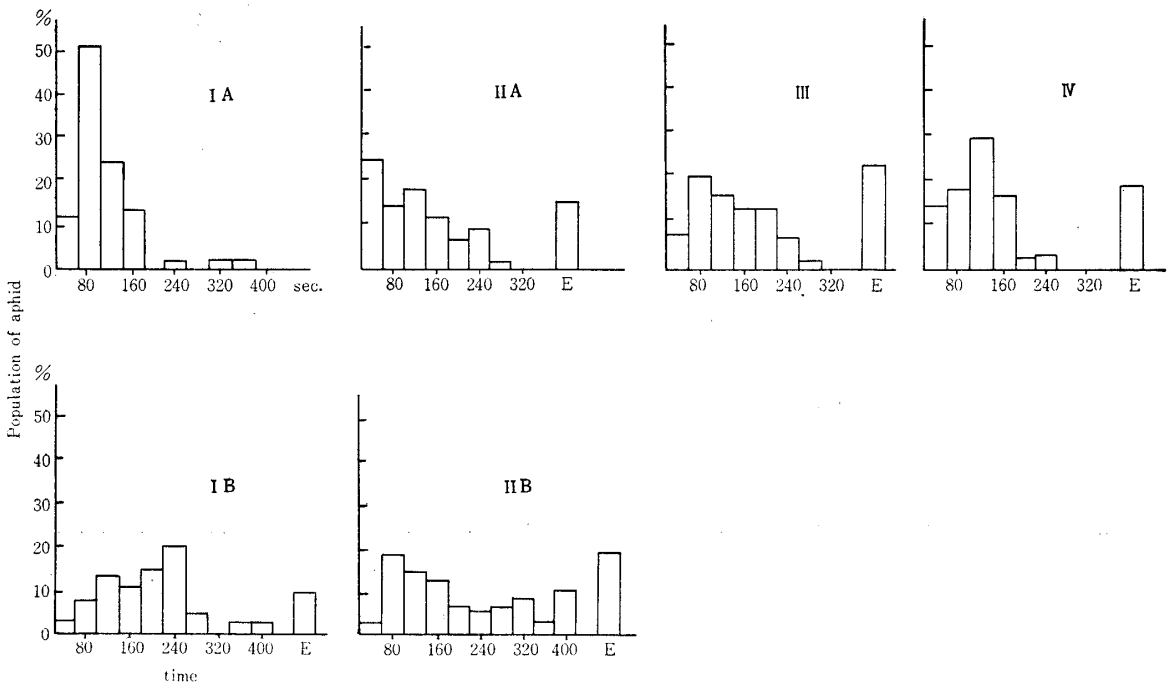

Fig. 4. Histogram of time spent prior to feeding probe
E : Aphids escaped from the leaf without the feeding probe.

Table 2. Relation between probing behavior of aphids and virus transmission.

Combination of host plant-aphid	Probing behavior and percentage of virus infected	Time spent prior to the initial probing		Duration of the probing			Transmission rate of the BYMV-0.
		10 sec.	20 sec.	15 sec.	15–30 sec.	30 sec.	
		%	%	%	%	%	%
IA	*Vicia faba* L. -starved *Myzus persicae* (Sulz.)	78.3	94.2	11.1	85.8	3.1	70.8
IB	*Vicia faba* L.-non-starved *M. persicae* (Sulz.)	3.5	10.5	17.5	69.1	13.4	11.1
IIA	*Vicia faba* L.-starved *Aphis craccivora* Koch.	17.4	61.6	8.2	59.0	32.8	22.1
IIB	*Vicia faba* L.-non-starved *Aphis craccivora* Koch.	2.1	10.4	14.4	47.7	37.9	0.0
III	*Nicotiana tobacum* L.-starved *M. persicae* (Sulz.)	45.5	70.5	33.0	58.2	8.8	non host
IV	*Vicia faba* L.-starved *Acythosiphon pisum* Harris.	25.7	44.7	26.4	62.3	11.3	25.0
V	*Phaseolus vulgaris* L.-starved *M. persicae* (Sulz.)	40.3	65.7	46.3	46.0	7.7	5.5

IB group. Mean values of the time were 101.5, 192.4, 117.0, 198.0, 133.4 and 112. 2 seconds, respectively. The time spent by IB and IIB groups was the longest of all. That is, non-starved aphids spent a long period prior to the feeding probe.

It is clear that the time spent prior to the feeding probe is affected by the kind of host plants or aphdis, and whether the aphids were starved or not. It appears that the time length is decided by numbers of the probing or the time lag between each probing.

RELATION BETWEEN PROBING BEHAVIOR OF APHIDS AND VIRUS TRANSMISSION

From the above experiments of the probing behavior, the relation among the time spent prior to the initial probing, the duration of the initial probing and the virus transmission can be arranged as shown in Table 2. Numbers of the aphid used for each group were 150, 150, 118, 61, 193 and 178 repsectively in the order as described in Table 1.

In the case of the combination of broad bean and *Myzus periscae* (Sulz.) or *Aphis craccivora* Koch., starved aphids always showed a high tranmission rate as compared with IA and IB groups, or IIA and IIB groups. And also starved aphids spent a relatively short time (less than 20 seconds) before the initial probing. That is, it is shown that many aphids probe immediately after placement on the leaf surface. And moreover, these were many of the aphids which had a probing duration of 15 to 30 seconds. There were few aphids which had a probing duration of less 15 seconds and over 30 seconds.

Considering the results, it is supposed that when the aphid probes immediately after the placement on the leaf surface and the probing duration is 15 to 30 seconds, the transmission rate is high. In both these factors, the probing duration or depth of insert of the stylet seems to be important to the infection. It is clear that when the insert of stylet is too shallow or too deep, the transmission rate decreases.

The V group in which only the host plant is different from IA and IB groups, showed the intermediate tendency between IA and IB groups, but the transmission rate was low. This seems to be caused by the fact that the probing duration tends to be short and the susceptibility of host plant low. No constant tendency was observed between kinds of host plants and aphids, because the kinds of plants used and aphids were very few. Thus it is not yet clear whether or not the above mentioned conclusions can be generalized.

RELATION BETWEEN TIME SPENT TO INSERT STYLET AND INSERTSPEED

This experiment was carried out to clarify the length of the inserted stylet for a given time.

After *Myzus persicae* (Sulz.) was starved for one hour, aphids were individually placed on the leaf of broad bean. The insert of stylet was terminated aritificially for a given period (15, 20, 30, 45, 60 and 90 seconds respectively). Timing was begun on contact of the leaf surface by the tip of the rostrum. Aphids were anaesthetized by a stream of carbon dioxide gas at a given period. Treatment of carbon dioxide was for 60 to 120 seconds. Or aphids were quickly killed by a dropping of Carnoy's solution of 50°C. The anaesthetized or killed aphids were carefully removed from the leaf by a soft hair brush, and then the length of stylet exposed from rostrum was measured under a microscope. We presumed that the stylet length measured showed the part of the stylet inserted in the leaf tissue. Bradley (6) also reported that stylet length measured according to this method would be the length of stylet inserted in practice. 261 aphids of *Myzus persicae* (Sulz.) were used.

Table 3 shows the relation among the number of aphid, the time of stylet insert and inserted stylet length. The stylet length, inserted within a given time, was variable according to the individual aphids. But the insert tended to deepen with the increase of time. The depth of insert is generally shallow up to 30 seconds. However, when the action is done for more than 45 seconds, the inserts are at considerable depth. But there were aphids which inserted slightly without relation to time.

Fig. 5 shows the relation between the mean length of inserted stylets and the insert times. The results of anaesthetized aphid coincide with the results of the killed aphid by hot Canroy's solution. This figure shows that insert of stylet is in the linear relationship of $Y = 0.526\ X - 5$ (X=time, Y=inserted stylet length).

Table 3. Relation among numbers of aphid, time of insert and length of inserted stylet.

Probing duration (sec.) / Method of anaesthesia / Length of stylets (μ)	15		20		30		45		60		90		120	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
< 5	20*	7	15	11	5	2	2							
5.1 — 10	6	4	10	5	11	3	1	4	1					
10.1 — 15	3	1	5	2	13	6	10	4	5					
15.1 — 20		1	1	3	6	2	6	1	7	1				
20.1 — 25					2	1	4	2	3	6	3		1	
25.1 — 30							0	2	6	2	0			
30.1 — 35							1	2	3	2	0	1		
35.1 — 40							0	1	0	0	2	1		
40.1 — 45							1		1	1	0	1		
45.1 — 50							2		0	1	2	0		
50.1 — 55									1	1	1	0	1	
55.1 — 60									0	0	2	0	2	
60.1 — 65									3	0	1	0	0	
65.1 <									1	1	1	1	2	
Mean length of the stylet	3.8	4.4	6.1	7.0	11.0	12.0	18.7	19.0	27.6	29.3	45.0	45.0	62.8	—

A : CO_2 anaesthetized. B : Insect killed by hot Carnoy's solution.
* : Numbers of aphid.

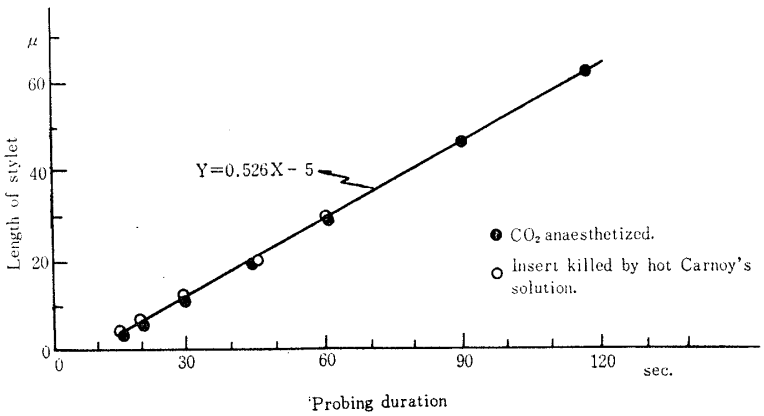

Fig. 5. Relation between time of stylet insert and length of stylet inserted into broad bean leaves. Aphid is *M. persicae* (Sulz.).

Namely this formula shows that aphids insert stylets into the leaf tissue at a constant speed. We could not really clarify, how many seconds the aphid spends from touching the rostrum on the leaf surface to stylet penetration into the plant epidermis. But if such time (preparation time) is always equalized, the speed that the stylet is actually inserted in the epidermis is calculated as 0.6 μ per second under our experimental conditions.

TIME TAKEN FOR THE STYLET TO PASS THROUGH THE EPIDERMAL TISSUE

The time for the stylet to pass through the epidermal cell was investigated.

At first for the object, the thickness of epidermal tissue of broad bean leaf (*Vicia faba* L.) was measured. These plants were cultivated in a glass house and their growth was seemingly normal. Sections of 5 mm square were cut off from the broad bean leaf completely expanded. The leaf sections were fixed in formalin-acetic-alchol (1:1:18) solution, and sectioned at 10 μ transversely by the usual method. The double stain with safranin and hematoxylin was used. Measurements were done on two sites of the outer membrane of epidermis (a) and the whole epidermis (b) as shown in Fig. 6. 300 cells were measured for (a) and 500 cells for (b).

Fig. 7 shows the distribution of values of each measurement. From the result of Fig. 7 (b), the thickness of the epidermis in about 70 % of the measured cells was from 10.4 to 18.6 μ. The minimum thickness was 5.3 μ. Therefore the time which is necessary to pass through the epidermis from touching the rostrum

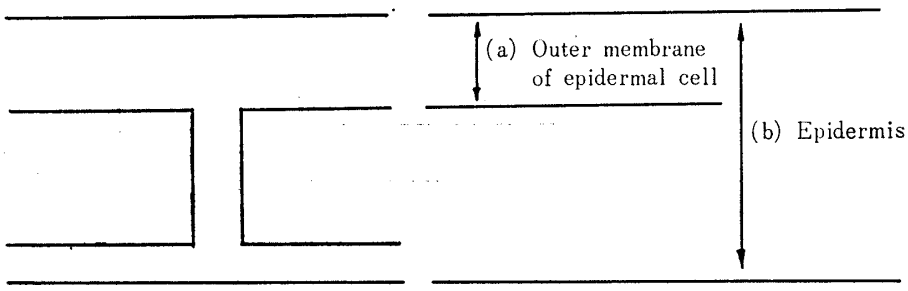

Fig. 6. Sites of measurement of the epidermal tissue.

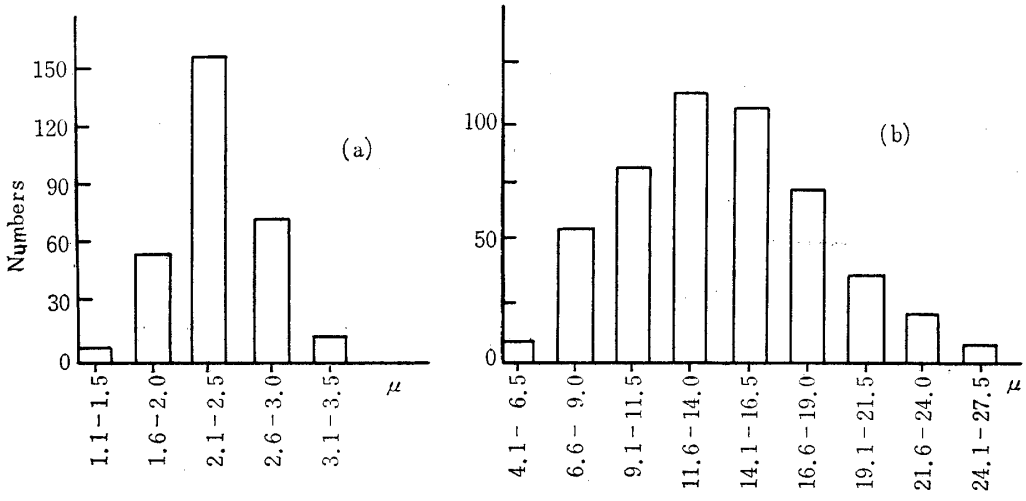

Fig. 7. Distribution of thickness of whole epidermis and outer membrane of epidermis of broad bean.

(a) : Outer membrane. (b) : Whole epidermis.

with the surface of leaf, appears to be from 24 to 41 seconds in many cases as calculated from the formula shown in Fig 5. And about 20 seconds is spent for the minimum thickness (5.3μ). About 37 seconds is spent in penetrating the mean thickness of the epidermis (namely 14.5μ). Further the mean thickness of the outer membrane of the epidermal cell was average 2.3 μ from Fig. 7 (a), so that *Myzus persicae* (Sulz.) might pass the mean thickness of the outer membrane of epidermis of the broad bean in about 14 seconds.

Further, direct observation was done to certify the above calculated values.

The epidermis of the broad bean (*Vicia faba* L.) was stripped and stuck on the cover glass. *Myzus persicae* (Sulz.) was put into a tube made of gum clay and the tube was set on the epidermis as shown in Fig 8 for the probing of aphid. The timing was begun on the contact of the epdiermis by the rostrum and terminated when the stylet just penetrated through the epidermis. Ten aphids were used for this experiment.

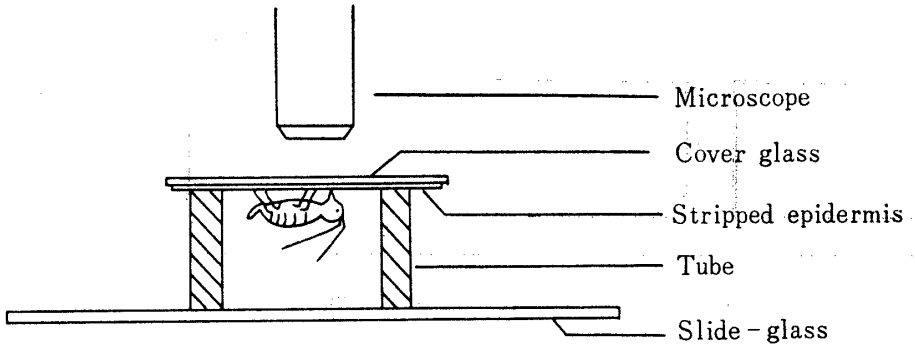

Fig. 8. Observation method of stylet insert through stripped epidermis.

Results are shown in Table 4. The times spent to penetrate through the stripped epidermis were divided into four groups 36–37, 38–39, 40–41 and 42–43 seconds, and numbers of aphid which belong to each group were indicated. The numbers of aphid used were small, but the result indicates that stylets passed through the epidermis from 36 to 43 seconds. This time coincided with the calculated value (namely 27–41 seconds).

Therefore it is concluded that *Myzus persicae* (Sulz.) can insert the stylet into the epidermis in a very short time.

Table 4. Time necessary to pass through stripped epidermis by stylet.

Groups of time (sec.)	36-37	38-39	40-41	42-43
Numbers of aphid	2	4	3	1

LENGTH OF STYLET INSERTED AND VIRUS TRANSMISSION

The relation between the length of stylet inserted and virus transmission was observed.

The aphid used was *Myzus persicae* (Sulz.). Aphids were starved for one hour before virus acquisition, and then transfered on broad bean plants infected with BYMV-O. Natural probing on infected plants were in 30 seconds. Afterwards this aphid was transfered from a diseased to a healthy broad bean leaf. The aphids were allowed to do initial probing of 20 to 30 seconds on the healthy plants and then they were anaesthetized immediately by the stream of carbon dioxide gas. Anaesthetized aphids were carefully removed from the leaves and the length of stylet exposed from the rostrum was measured under a microscope. Broad beans which aphids inserted stylets were investigated after 3 weeks for development of symptom. 52 aphids were used.

Results are shown in Table 5. It was possible that *Myzus persicae* (Sulz.) transmitted BYMV-O by stylet insert less than 5μ depth into the leaf. Inserted stylet length of these 5 aphids which transmitted the virus was really less than 3μ. The rate of transmission tended to reduce with the increased depth of stylet insert. The reason is unknown.

Table 5. Virus transmission and length of inserted stylet.

Range of length of inserted stylet (μ)	Number of aphids probed.	Number of aphids transmitted.	Rate of transmission (%)
< 5	25	5*	20.0
5—10	17	3**	17.0
10<	10	0	0.0

* 5 aphids inserted stylet less than 3μ depth. (2 aphids inserted stylet 2.5μ depth. 3 aphids inserted stylet 3μ depth.)
** 3 aphids inserted stylet 5 to 6μ depth.

Thus it is suggested that *Myzus persicae* (Sulz.) can transmit the virus in a very thin layer of the epidermis of a broad bean leaf.

VIRUS ACQUISITION-ACCESS PERIOD BY PROBING

Virus acquisition-access period by probing was investigated.

Myzus persicae (Sulz.) were starved for one hour. Then one aphid per plant was transfered on a broad bean leaf infected by BYMV-O. Acquisition-access periods were controlled artificially in 5, 15, 30, 45, 60, 75 and 90 seconds, respectively. Each period is the duration of the initial probing. Timing is the same as described in the probing duration. After the initial probing, each aphid was transfered from the infected leaf to a healthy broad bean. They were placed there for 24 hours for examination of acquisition and then were killed by spraying malation. The

experiment was replicated four times, and five plants were used for each treatment. Whether or not aphids transmitted the virus, was determined by the development of the symptom of the test plant after three weeks.

Results of the trials are shown in Table 6. It was indicated that *Myzus persicae* (Sulz.) could acquire BYMV-O by the probing of minimum 15 seconds. By an acquisition-access period of 5 seconds, the virus could not be acquired. It is presumed that the stylet was inserted to a depth of about 2.9μ in 15 seconds from Fig. 5. This depth indicates that stylets just penetrated through the outer

Table 6. Virus acquisition-access period.

Acquisition-access period. (sec.) / Replication.	5	15	30	45	60	75	90
I	0	1*	1	3	2	3	3
II	0	1	4	3	4	3	2
III	0	2	5	2	3	4	4
IV	0	3	3	4	3	4	4
An average transmission rate (%)	0.0	35.0	65.0	60.0	60.0	70.0	65.0

* : Number of infected plants in 5 inoculated plants.

membrane of epidermal cells of about 2.3μ thickness by the results of Fig. 7. Swenson (17) reported that virus was acquired by a probing of 10 seconds duration. So our result seems to coincide with his. The transmission rate of BYMV-O increased in the acquisition period of 30 seconds, but did not increase when the period became longer than 30 seconds.

INOCULATION-ACCESS PERIOD BY PROBING

In this experiment, the relation between the initial probing duration for inoculation and virus transmission was examined.

Myzus persicae (Sulz.) was starved for one hour, and then was allowed to do the initial probing of 30 seconds duration on broad bean leaf infected with BYMV-O to acquire the virus. Afterwards one aphid for a plant was transfered on the healthy plants to examine whether or not the aphids can inoculate the plant by one probing. Duration of the initial probing for the inoculation was natural. Experiments were replcated three times and 50 aphids were used.

Results are shown in Table 7. When viruliferous *Myzus persicae* (Sulz.) was placed on healthy broad bean leaf and probed immediately, aphids could transmit the virus with only one probing of a shrot duration of 10 to 15 seconds. About 40% of the test plants were infected with one probing of a duration of 16 to 20 seconds, and over 50% of test plants were infected with one probing of over 20 seconds dura-

Table 7. Inoculation-access periods by probing.

Time of naturally terminated probing (sec.) / Replication	10-15	16-20	21-30	31-35.....60
I	2/6	4/14	10/19	2/3.....6/8
II	2/8	7/15	8/15	2/4.....4/8
III	0/4	8/16	9/16	1/2.....5/10
An average transmission rate (%)	19.4	39.9	54 1	55.6.....57.7

Denominator=number of plants inoculated (=number of aphids measured)
Numerator=number of plants infected (=number of aphids which could infect)

tion. As aphids which probed in 35 to 60 seconds were not observed, results for this period are unknown. Also insects which spent over 60 seconds seemed to do immediately the feeding probe without an initial probing considering the results of fig. 3.

Discussion

When aphids are placed on leaf surfaces of host plants, aphids insert stylets into the tissue after tapping 2 or 3 times. Bradley (1) reported that the time spent prior to the initial probing was less than 30 seconds after *Myzus persicae* (Sulz.) was placed on the leaf surface and that 1% of the aphids used spent over 60 seconds. Furthermore, Bradley and Rideout (5) reported that this behavior was observed not only on *Myzus persicae* (Sulz.), but also on other species such as *Macrosipum euphorbiae* (Thos.) and *Aphis nasturtii* Kltib. But Chalfant (8) reported that *Brevicoryne brassicae* (L.) did not show such behavior. Whether the aphid was starved or not may effect markedly such behavior. Our experimental results indicate that the time spent prior to the initial probe was short for starved aphids, but that non-starved aphids spent a relatively longer period. That is, the presence or absence of starvation influences markedly the time spent prior to the initial probing. After the tapping, most of the initial probing was terminated in 30 seconds. There were similar tendencies in all examined combinations whether the aphids were starved or not. Generally, an aphid makes a feeding probe after probing, and most of the aphids always perform the probing once or twice before the feeding probe. But some aphids always performed the feeding probe immediately without the initial probing. It is unknown, by what object the aphids probe. It is assumed that they select sites for a feeding probe or a favorite host. The time spent, up to the feeding probe after being transfered on the leaf, varied according to the species of the host or the kind of aphid, and whether the aphid

was starved or not. For example, as shown in Fig. 1, 3 and 4, probing behaviors differed according to the kinds of aphid on the same host plants, and if the host plant is different, the same kind of aphids showed different probing behaviors. On the relation between the probing behavior of aphids and virus transmission, Sylvester (19) reported that only one probing was better for the virus transmission. Whereas, Zettler and Wilkinson (25) recently showed that the final probing having a duration of 11 to 20 seconds after several probings, was important for the virus transmission. The relations among the time prior to the initial probing, the duration of the initial probing and rate of the virus transmission were observed. From these results, it appears that cases of high virus transmission rate have a tendency that finds the time spent prior to the initial probing quite short and that further most of aphids have an initial probing of 15 to 30 seconds. On the contrary, in cases of low virus transmission rate such a tendency was in reverse. The number of starved aphids which performed the probing in 15 to 30 seconds showed higher rate of transmission and were many more than that of non-starved aphids. In cases of a low transmission rate, there were also many aphid with a brief probing duration of less than 15 seconds. Therefore an initial probing having a very brief duration does not seem to be suitable for the transmission.

When an aphid inserts the stylet as in the probing behavior described above, at what site of the leaf surface does the aphid insert the stylet ? For this question, van Hoof (20) suggested that *Myzus persicae* (Sulz.) and *Aphis fabae* Scop. usually inserted the stylet between the transverse walls of the epidermal cell. Bradley (6) also obtained the same result on tobacco. These results seems to show the insert site for the feeding probe, because they did not show the site of probing. We observed the similar result for the feeding probe of *Myzus persicae* (Sulz.) (unpublished). Swenson (17) and van der Want (21) assumed that on the probing the stylet was likely inserted into the cell. While van Hoof (20) and Bradley (6) assumed that aphids inserted stylets at the middle lamella of the epidermal cells on the probing the same as in the feeding probe. But they have not shown the evidence for their presumption. The probing site seems to be important to virus transmission, but clarification of the probing site was impossible under microscopic observation. Afterwards by using an electron microscope, we discovered that the initial probing was usually carried out on the surface of epidermal cell (unpublished).

Concerning the infection, an important problem is, to what depth does the aphid insert the stylet on an initial probing ? In the combination of *Myzus persicae* (Sulz.) and broad bean (*Vicia faba* L.), as indicated in Fig. 5 the speed of stylet insert was 0.6μ per second. As the thickness of broad bean leaf epidermis was mostly from 10.4 to 18.6 μ, the calculated time of 27 to 41 seconds is necessary from the touching of the rostrum on the leaf surface to the penetration through this thickness. This time coincides with the measured value of 36 to 43 seconds that *Myzus persicae* (Sulz.) required for penetration of stylet through stripped

epidermal cells. Moreover it is calculated that aphids spend about 14 seconds from the touching of the rostrum on the plant surface to the penetration through the outer membrane of the epidermal cell (its thickness is average 2.3 μ). This calculated value (about 14 seconds) coincides with the shortest time (15 seconds) that *Myzus persicae* (Sulz.) acquires the virus from broad bean in the experiment of virus acquisition-access period from the infected tissue. Furthermore as it is assumed that the stylet is inserted about 2.9μ depth in 15 seconds, we thus presume that stylets are able to penetrate through the outer membrane (average 2.3 μ) of epidermal cells during this time.

Simons (15) reported that pepper veinbanding mosaic virus was acquired in minimum of 5 seconds by one initial probing. But Simons (15) and Swenson (16) indicated that the insert during 10 to 60 seconds was most optimum for a stylet-borne virus acquisition. Our data also indicated that an insert of 15 to 30 seconds duration showed the highest level of the virus acquisition, and that on the contrary, an insert of a longer duration did not show an increase of the virus acquisition. Watson (23) and Bradley (4) reported that if the stylet was inserted continuously over 5 minutes, the acquisition rate of beet mosic virus by *Myzus persicae* (Sulz.) decreased rather than the short duration probing. Our results on the acquisition rate also showed a tendency similar to theirs (unpublished).

The relation between the virus transmission and the length of stylet inserted showed that plants were really infected by stylet insert less than 3 μ depth, as the example of the shortest depth. This depth corresponds to stylet insert in about 15 seconds considering from the time. That is, this time is the time that the stylet just penetrated through the outer membrane of the epidermal cell or in other words barely protruded the tip of stylet into the epidermal cells. Therefore, it is concluded that stylet-borne virus could be acquired by the stylet penetrating through the outer membrane of epidermal cells into the epidermal cell. The same conclusion is obtained on the inoculation-access.

In conclusion, it was elucidated that in transmission of BYMV-O by *Myzus persicae* (Sulz.), the feeding probe was not only important, but even more important was the probing through the outer membrane of epidermis into the cell.

Summary

This report presents recent experimental results that were carried out on the relations between the probing behavior of the aphid and the transmission of plant virus. In this experiment, *Myzus persicae* (Sulz.), bean yellow mosaic virus and braod bean were mainly used as the materials.

1) Many aphids spent a relatively short time (less than 20 seconds) prior to the initial probing after placement on the leaf surface, while non-starved aphids spent a relatively longer period.

2) There were many aphids which had a probing duration from 15 to 30

seconds and there were few aphids which had a probing duration of less than 15 seconds or over 30 seconds.

3) The majority of aphids probed once or twice on the average prior to the feeding probe, but some aphids performed the feeding probe immediately without the initial probing.

4) Most of the starved aphids performed the feeding probe within 160 seconds after placement. On the other hand, non-starved aphids spent a longer period prior to the feeding probe.

5) In the case of the combination of borad bean and *Myzus persicae* (Sulz.), starved aphids always showed a high transmission rate.

6) When the aphid probes immediately after placement on the leaf surface and the probing duration is 15 to 30 seconds, the transmission rate is high. The probing duration or depth of insert of the stylet is important to the infection.

7) The relation between depth of insert of the stylet and probing duration is indicated by the formula of $Y=0.526\ X-5$ (X=time, Y=inserted stylet length). Namely this formula shows that *Myzus persicae* (Sulz.) insert the stylet into the leaf tissue at a constant speed. Therefore the speed that the stylet is inserted in the epidermis is claculated as 0.6μ per second under our experimental conditions.

8) As the thickness of broad bean leaf epidermis was mostly from 10.4 to 18.6 μ, the time which is necessary to pass through the epidermis from the touching the rostrum to the surface of leaf, appears to be from 24 to 41 seconds as calculated from the formula. This time nearly coincides with the measured value of 36 to 43 seconds actually needed for the penetration of the stylet of *Myzus persicae* (Sulz.) through stripped epidermal cells.

9) Aphids spend about 14 seconds from touching the rostrum on the plant surface to penetration through the outer membrane of the epidermal cell (its thickness is average 2.3 μ). This calculated value (about 14 seconds) coincided with the shortest time (15 seconds) that *Myzus persicae* (Sulz.) acquired the virus from broad bean in the experiment of virus acquisition-access period from the infected tissue.

10) Plants were really infected by stylet insert less 3μ length into the epidermal cell. This depth corresponds to stylet insert in about 15 seconds.

11) *Myzus persicae* (Sulz.) can acquire and inoculate BYMV-O by a probing of a minimum of 15 seconds. An insert of 15 to 30 seconds duration showed the highest level of acquisition.

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References

1) Bradley, R.H.E. (1952). Ann. Appl. Biol., **39**, 78.
2) Bradley, R.H.E. (1953). Nature, **171**, 755.
3) Bradley, R.H.E. and D.W. Rideout (1953). Can. J. Zool., **31**, 333.
4) Bradley, R.H.E. (1954). Can. J. Zool., **32**, 64.
5) Bradley, R.H.E. and R.Y. Ganong (1954). Can. J. Microbiol., **1**, 784.
6) Bradley, R.H.E. (1964). Plant virology., Gainesville, p. 148–174.
7) Bradley, R.H.E., E.S. Sylvester and C.V. Wade (1964). Can. J. Entomol., **94**, 635.
8) Bradley, R.H.E. (1966). Virology, **29**, 396.
9) Chalfant, R.B. (1959). Dissertation abstr., **20**, 432.
10) Day, M.F. and H. Irzykiewicz (1954). Aust. J. Biol. Sci., **7**, 251.
11) Kasai, Z. (1954). Virus., **4**, 337.
12) Kennedy, J.S., M.F. Day and V.F. Eastop (1963). Commonwealth Inst. Entomol., p. 114, London.
13) McLean, D.L. and M.G. Kinsey (1964). Nature, **202**, 1358.
14) Nishi, Y. (1963). Bull. Kyushu Agri. Exp. Stat., **8**, 351.
15) Simons, J.N. (1956). Phytopath., **46**, 53.
16) Swenson, K.G. (1960). Ann. Entomol. Soc. Amer., **53**, 521.
17) Swenson, K.G. (1962). Aust. J. Biol. Sci., **15**, 468.
18) Sylvester, E.S. (1949). Phytopath., **39**, 417.
19) Sylvester, E.S. (1950). Phytopath., **40**, 737.
20) van Hoof, H.A. (1958). van Putten and Oortmeijer, Alkmaar, Netherland., 108 p.
21) van der Want, J.P.H. (1954). H. veenman and Zonen, Wageningen, Netherland., 84 p.
22) Watson, M.A. (1938). Proc. Roy. Soc., **125**, 144.
23) Watson, M.A. (1946). Proc. Roy. Soc., **133**, 200.
24) Yoshii, H. (1966). Ann. Phytopath. Soc. Japan, **32**, 46.
25) Zettler, F.W. and R.E. Wilkinson (1966). Phytopath., **56**, 1079.