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Tohoku Journal of Agricultural Research
Vol. 21 No. 1, March 1970
Printed in Japan

Studies on the Mechanism of Aphid Transmission of Stylet-Borne Virus. (V) The Relation between the Stylet Insertion Site on the Diseased Leaf and Virus Acquisition

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(Received, March 31, 1970)

Summary

These experiments were carried out for elucidating the relation between the stylet insertion site on the infected leaf and virus acquisition.

1. There is no difference in virus acquisition between probing in the upper epidermis and in the lower epidermis of a leaf infected with bean yellow mosaic virus (ordinary strain).

2. In an infected leaf, the virus content in the epidermal cell is lower than in the mesophyll cell. Virus acquisition by aphids from stripped epidermal cells was very low. This transmission rate was about 6.7% and about one third of the acquisition rate from mesophyll cells where the epidermis had been stripped.

3. Many particles of this vrius were observed in the mesophyll cells of the yellow parts of mosaic leaves. The virus content in the green parts was not only lower than in the yellow parts but in green parts there are cells in which no virus particles were found.

4. Many virus particles were recognized in the epidermal cells of the yellow parts, but they were fewer than in the mesophyll cells. Few virus particles were found in the epidermal cells of the green parts.

5. The acquisition rate of virus by probing, was 66.7% from the yellow part and 16.7% from the green part, also the virus acquisition rate from the epidermal cells of the green part was very low.

6. The total area of yellow parts in the mosaic leaves was from 59 to 87% (average 72.1%) of the leaf surface and 60 to 87% of the examined aphids inserted their stylets in the yellow parts.

7. A parallel relation was observed between the acquisition rate of the virus and the virus concentrations.

From the results above mentioned, it is concluded that the acquisition rate by probing is influenced remarkably by the insertion site in the epidermis of the mosaic leaf.

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Misawa and I (4,8) reported that in the case of *Myzus persicae* (Sulz.) on broad bean leaf, the duration of one probing was usually 15 to 30 seconds and that such probings were usually carried out two or three times on the surface of the epidermal cells. Moreover virus was transmitted most effectively by probing.

A broad bean leaf systemially infected with BYMV-O shows a mosaic pattern consisting of green and yellow parts. Any difference in virus acquisition rate of the two parts when the aphid inserts the stylet is of interest regarding virus transmission.

Bradley (3) suggested that when the stylet penetrated into the tobacco leaf infected with potato virus Y, the acquisition of virus is better from the tip area of the leaf than from the middle or the base areas. Also he indicated that the interveinal area is better than the vein. In comparing the mesophyll and epidermis areas, Bawden et al. (2) reproted that the virus concentration in the epidermis area is higher than in the mesophyll area, and that when the aphids were allowed to feed for long periods, they inserted their stylets into the mesophyll cells containing low virus concentrations, thus the transmission rate decreased. But, Hitchborn (6) van Hoof (10) and Namba (9) reproted that the virus concentration in the epidermal tissue is not higher than in the mesophyll tissue.

The experiments presented here were carried out to elucidate the relation between the yellow and the green parts of the infected leaf in virus acquisition by the aphid.

Materials and Methods

The ordinary strain of bean yellow mosaic virus (BYMV-O) used was the same as described in the first reprot (8)

The aphids used were non-viruliferous green peach aphids (*Myzus persicae* (Sulz.)) of apterous viviparae which had been reared on plants of *Raphanus sativus* L.

Broad bean (*Vicia faba L. var. Wase Soramame*) was used as the experimental plant and was used at the growth stage of four trifoliolate leaf development.

Details of the methods will be given in each experiment.

Results

1. Virus acquisition from the upper and the lower surface of a leaf.

Acquisition rate of the virus by probing was compared between the upper surface and the lower surface of the mosaic leaf.

Aphids were allowed to acquire the virus by initial probings of 15 to 30 seconds duration from one of the two surfaces of the mosaic leaf. After the insertion, the aphids were transfered separately to a healthy broad bean. The rate of virus acquisition was exmained by the development of symptoms in infected plant. The

experiment was replicated three times, and ten plants were used for each treatment.

Results of the trials are shown in Table 1. There was no difference in the virus acquisition by probing between the upper epidermis and lower epidermis of the infected leaf. Namely an aphid's virus acquisition is equal whether probing on the top or bottom epidermis of a leaf.

TABLE 1. *Virus acquisition from upper and lower surfaces on the mosaic leaf.*

Acquisition site \ Replication	I	II	III	An average transmission rate
Upper epidermis	5/10	5/10	6/10	53.3%
Lower epidermis	5/10	6/10	5/10	53,3

2. Virus concentrations in the mesophyll tissue and the epidermal cells.

The virus concentrations in the mesophyll tissue and the epidermal cells were compared.

Mesophyll tissue where the epidermis had been stripped from the upper and lower surfaces of the mosaic leaves (which were grown for two months in phytotron at 24°C after inoculation), and the epidermis stripped from the lower surface were used for the measurements. As the epidermis stripped from the upper surface of the leaf was alway accompanied with mesophyll cells, only the epidermis of the lower surface was used. The epidermis strips were ground with a fifth of their weight of 0.1 M phosphate buffer at pH 7.0 and the squeezed juice was used for the assay of the concentration of the virus. Leaves of *Chenopodium amaranticolor* L. as the local lesion host were infected for a measurement of virus concentration, and the number of developed local lesion was determined by the half leaf method. Ten leaves were used for one experiment.

Results are shown in Table 2. If the number of developed local lesion indicates the concentration of the virus in the tissue, the mesophyll tissue appears to contain larger quantity of the virus than that of the epidermis. Let 100 denotes the virus concentration of the mesophyll cell in the mosaic leaves, then that of the epidermal cell shows values of 32 to 37.

3. Acquisition of the virus from the epidermal cell and the mesophyll cell.

The lower epidermis of the diseased leaf was stripped and was attached on the slide glass. One group of aphids were allowed to probe on the stripped epidermal cell by the same method as shown in the first report (8). And another group of aphids were allowed to probe on the mesophyll cell from where the epidermis was

TABLE 2. *The comparison of virus concentrations in both tissues in the mosaic leaf.*

Estimated site \ Replication	I	II	III
Yellow part of the mesophyll tissue	534[a] (100)	842 (100)	673 (100)
Yellow part of the epidermal tissue	171 (32)	312 (37)	276 (35)

a) Number of local lesions formed in 10 leaves of *Chenopodium amaranticolor* L.. A parenthesized number is index.

stripped. After 20 to 30 seconds after the tip of the rostrum touched the epidermal cell or the mesophyll cell, aphids were transferred separately one by one on the healthy plant. They were placed there for 24 hours and then killed by spraying malathion. The infection rate of infected plants by both groups of the aphid was determined by the development of the symptom after three weeks. For a comparison, aphids were allowed to acquire the virus by the probing from the diseased leaf which was not treated. The experiment was replicated three times, and ten plants were used for each treatment.

As shown in Table 3, the virus acquisition by aphids from the stripped epidermal cells were not good, and the transmission rate was about 6.7%. On the other hand, the virus acquisition by the aphid from the naked mesophyll cell was better than from the epidermal cells, about 16.7%.

TABLE 3. *Virus acquisition by aphids from stripped epidermal and mesophyll cells.*

Probed tissue \ Replication	I	II	III	An average transmission rate
Stripped epidermal cells	1/10	0/10	1/10	6.7%
Stripped mesophyll cells	2/10	1/10	2/10	16.7
Untreated leaf (control)	6/10	5/10	5/10	53.3

Also, the virus acquisition rate by the aphid from non-treated diseased leaf indicated a value of 53.3%. Lower rate of virus acquisition from the stripped epidermal cell or the naked mesophyll cell seems to be caused partly by any physiological damage by the operation of stripping. But, if the physiological damage is the same at the epidermal cell and the mesophyll cell, it appears that the virus acquisition rate from the mesophyll cell is approximately two and a half times more to that of the epidermal cell. Results of previous section indicates that the concentration of the virus in the mesophyll cell is 2.5 to 3.0 times higher than

that of the epidermal cell. Namely there is a parallel relationship between the rate of virus acquisition and the virus content of the infected cell. Although the facility of virus acquisition may be based partly on the different characters of the cells, it is considered that different acquisition rates are caused mainly by different contents of the virus. Namely, the aphid seems to acquire the virus easily from the cells containing high concentration of the virus. Moreover above results shows that the aphid can acquire the virus directly from the naked mesophyll cell by the probing within 30 seconds.

4. Distribution of the virus in the mosaic leaf.

It has been known that systemically infected leaves of broad bean shows a mosaic symptom consisting with the yellow part and the green part. So if these parts contain different quantity of the virus, the acquisition rate of the virus which the aphid is able to obtain from each part respectively, is presumed to be different. In order to clear this presumption, the distribution of the virus was observed with an electron microscope on the epidermal cell and the mesophyll cell of the yellow and green parts of the mosaic leaves.

Each tissue was sampled from the yellow part and the green part of the mosaic leaf, respectively and fixed. Fixation, embedding and staining were made by the method reported previously (5). Cross sections were made and observed with an electron microscope.

The fine structures of the epidermal cell of the yellow part of the mosaic leaf are shown in Figures 1 and 2. Many virus particles were recognzied in the epidermal cell of the yellow part. But there is a tendency that the content of virus particles was few in the epidermal cell of the yellow part than in the mesophyll cell. The structure of the epidermal cell was not changed remarkably by the infection of the virus. On the other hand, virus particles were not found in the epidermal cell of the green part of the mosaic leaf (Figure 3).

The fine structures of the mesophyll cell of the yellow part of mosaic leaf are shown in Figures 4 and 5. Many virus particles were found in every mesophyll cells. These virus particles are scattered in the cytoplasm, and the virus forms bundles of the flexuous particle or aggregates of the particle (Figure 5). Many osmiophillic globules were found always in the site where the virus particles was found in the cytoplasm. These globules are surrounded by the virus particle (Figures 4 and 5). Virus particles could not be found within chloroplast and nuclei.

The sectioned mesophyll cells of green part of the mosaic leaf are shown in Figures 6 and 7. Virus particles were observed very few in the green part than in the yellow part, and besides there were cells which the virus was not observed.

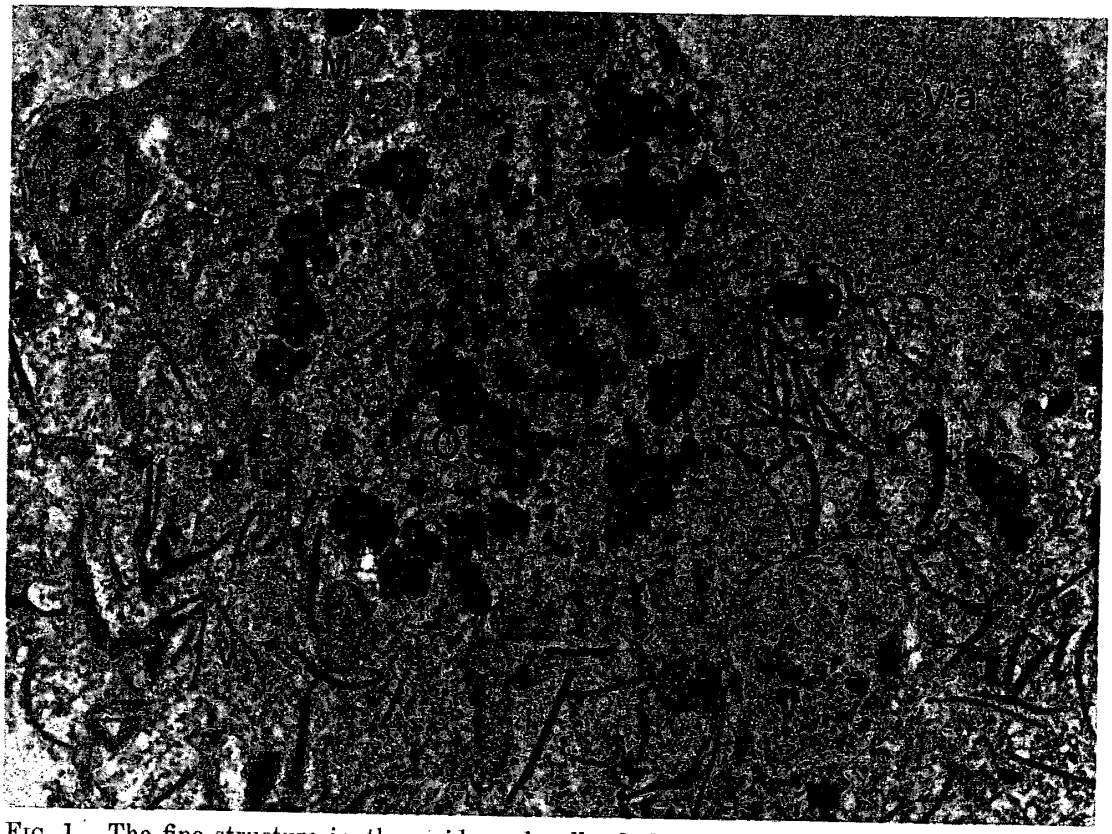

FIG. 1. The fine structure in the epidermal cell of the yellow part of the mosaic leaf. ×10,500

FIG. 2. The fine structure in the epidermal cell of the yellow part of the mosaic leaf. ×10,500

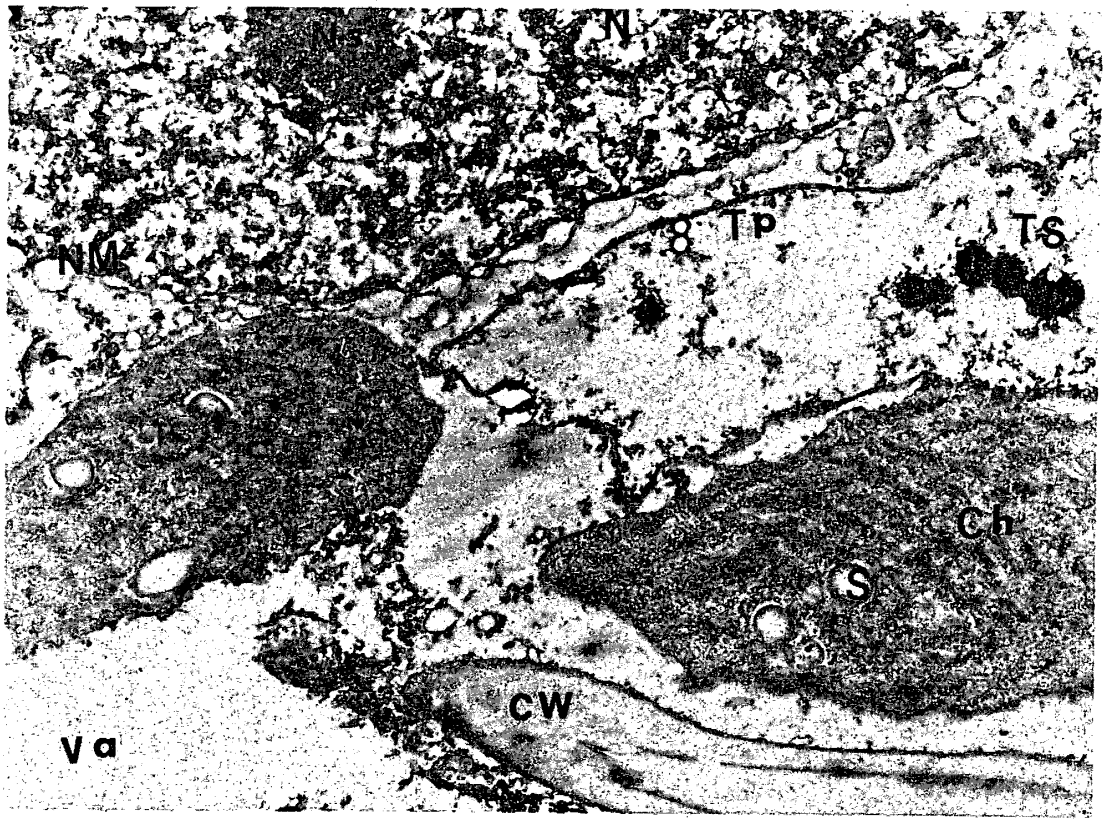

FIG. 3. The fine structure of the epidermal cell of the green part of the mosaic leaf. ×10,500

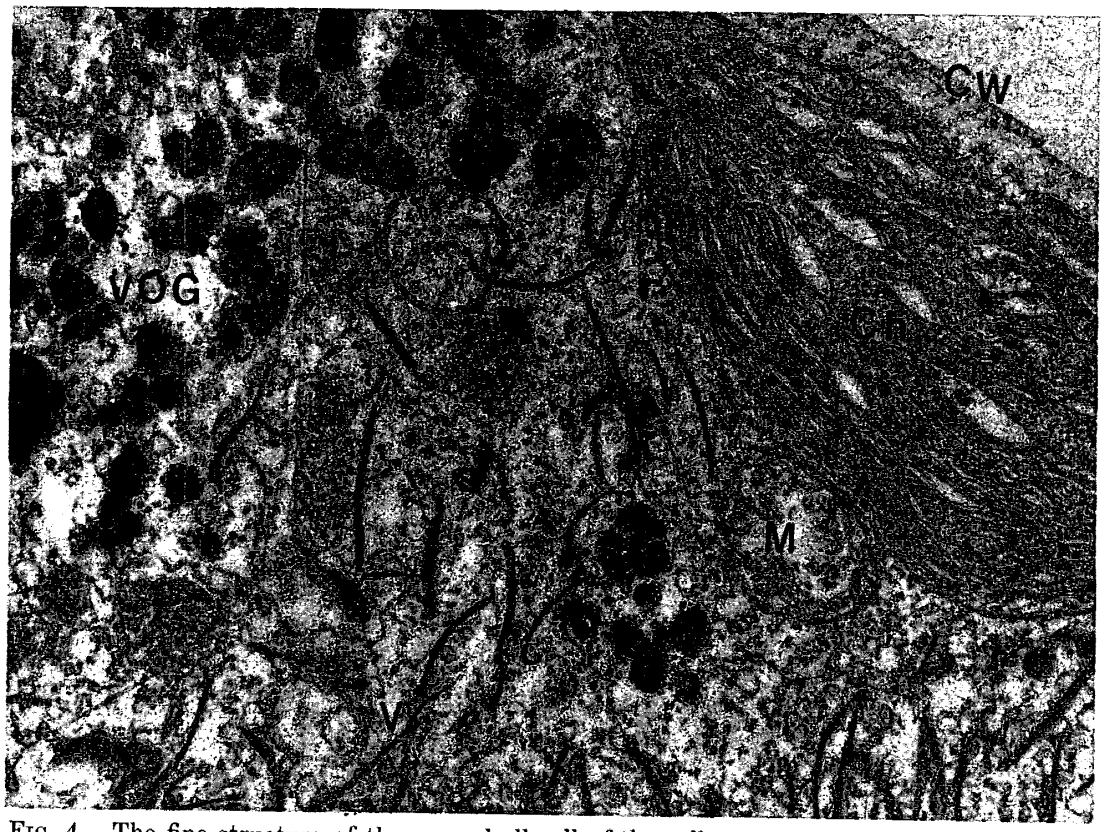

FIG. 4. The fine structure of the mesophyll cell of the yellow part of mosaic leaf. ×12,500

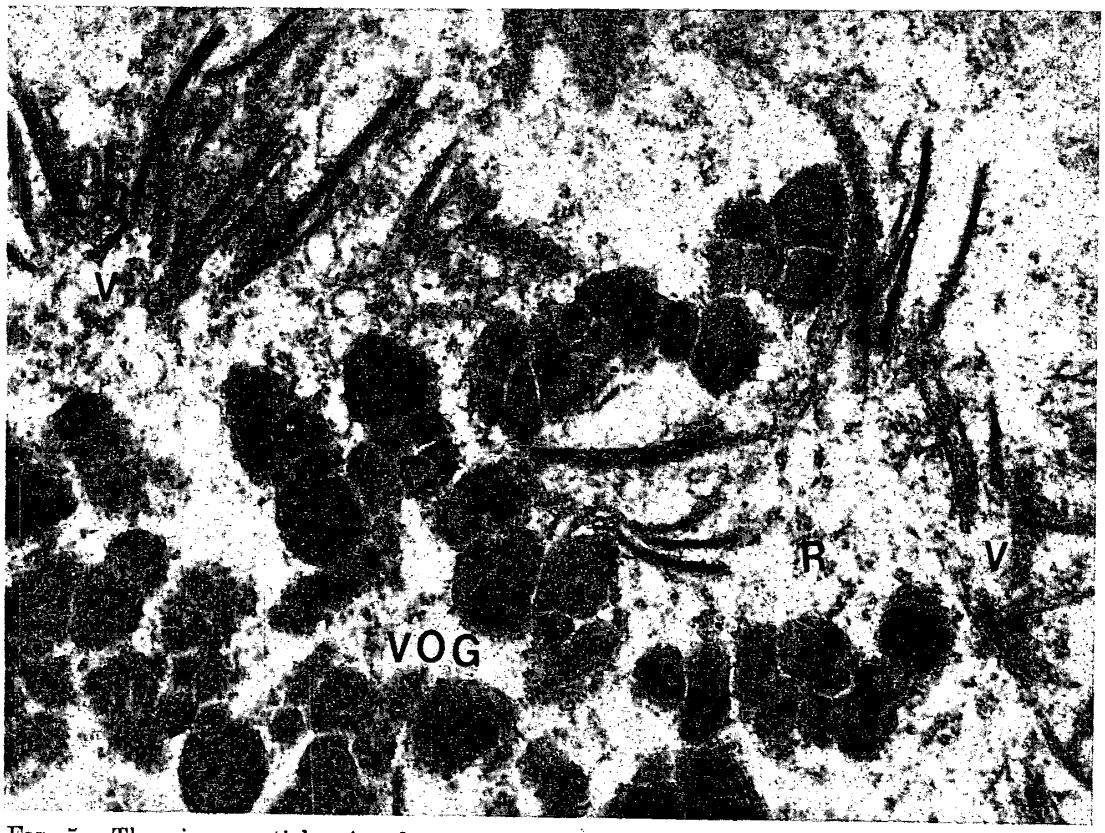

FIG. 5. The virus particles in the mesophyll cell of the yellow part of mosaic leaf. ×35,000

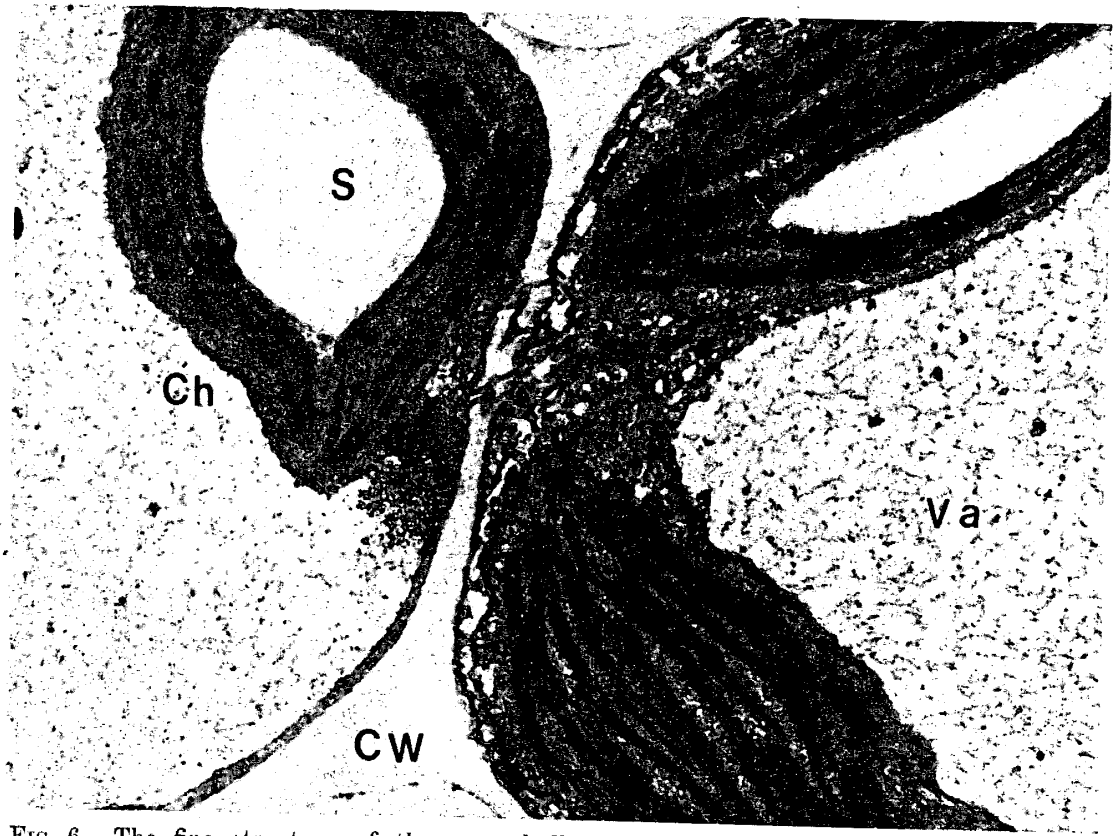

FIG. 6. The fine structure of the mesophyll cell of the green part of mosaic leaf. ×35,000

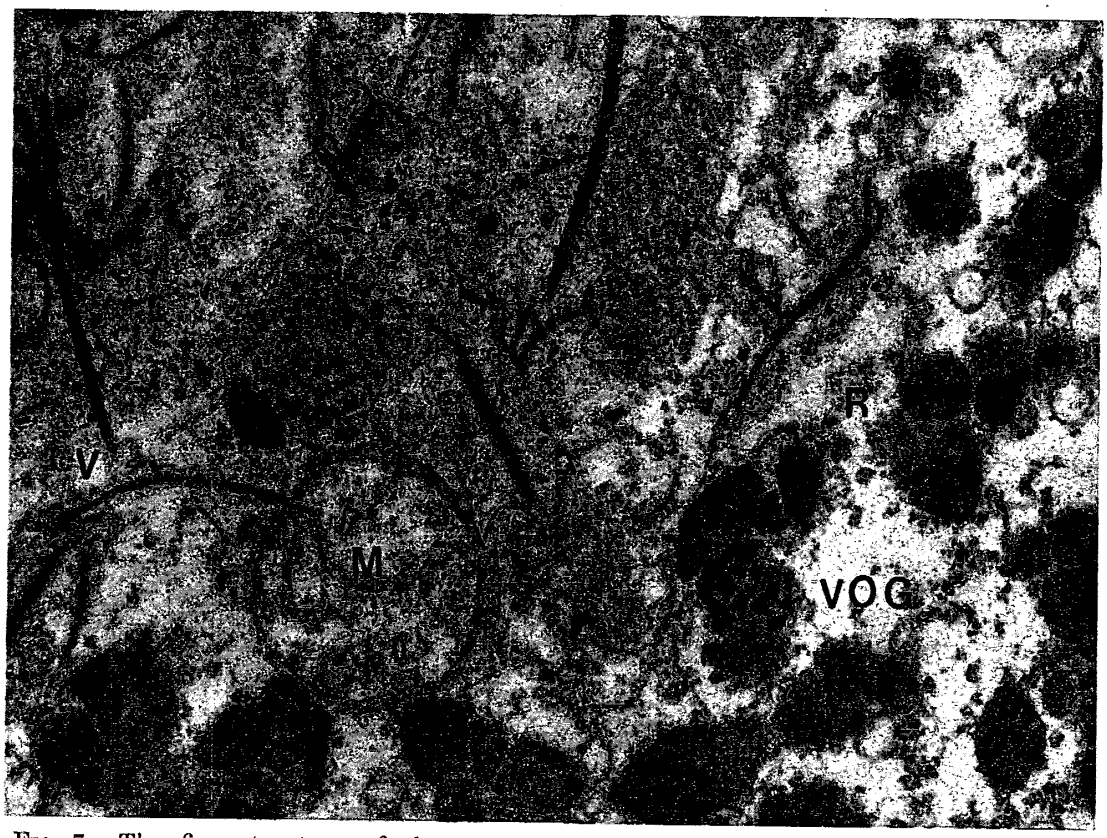

FIG. 7. The fine structure of the mesophyll cell of the green part of mosaic leaf. ×35,000

Key to Abbreviations

Ch	chloroplast	CW	cell wall	M	mitochondria	R	ribosome
S	starch grain	Tp	tonoplast	V	virus	Va	vacuole
VOG	osmiophillic grobule						

5. Virus acquisition from the yellow part and the green part of the mosaic leaf.

Either or not the acquisition rate by probing the epidermis of the yellow part is different from that of the green part in mosaic leaf, was investigated.

The aphid was allowed to acquire the virus from the epidermis of both parts separately by initial probing. After insertion, the aphids were transferred one by one to a separate healthy broad bean, and the rate of virus transmission was examined. The experiment was duplicated and fifteen plants were used for each treatment.

Results are shown in Table 4. The acquisition rate of the virus by initial probing was 66.7% from the yellow part and 16.7% from the green part. The acquisition rate from the epidermal cell of the green part was remarkably low. When the aphid was allowed to probe freely on the mosaic leaf, the acquisition rate was 53.3%.

Therefore as shown in a previous section, the low content of the virus particle

TABLE 4. *Virus acquisition from yellow and green parts of the mosaic leaf.*

Probed part \ Replication	I	II	An average transmission rate
Yellow part	11/15	9/15	66.7 %
Green part	3/15	2/15	16.7
By free probing	9/15	7/15	53.3

in the epidermal cell of the green part is presumed to be the cause of the low acquisition rate. These results indicate that in the mosaic leaf, the virus acquisition rate by aphids differes according to the part of the leaf where the aphid probes.

6. The frequency of stylet insertion into the yellow and green part of the mosaic leaf.

When the aphid was allowed to probe on a mosaic leaf, whether the insertion was on the epidermis of the yellow part or on the green part was observed. Thirty aphids were placed on each leaf and their behavior was observed. After this observation, the total area of yellow parts was measured. The ratio of total yellow parts to total leaf surface was measured as follows: each mosaic leaf was copied on paper, and then the yellow parts were cut off from the copied paper and weighed. Thirteen leaves were used for this observation.

Results are shown in Table 5. In the mosaic leaves of broad bean, the ratio of total yellow parts was 59 to 87% (average 72.1%) of the leaf surface in this

TABLE 5. *The ratio of the yellow part to total leaf surface in mosaic leaves and the frequency of stylet insertion.*

Experimental number	The ratio of the yellow part	Number of aphids penetrating into the yellow part	The frequency of stylet insertion into the yellow part
1	65.8 %	20*	66.7 %
2	84.8	25	83.3
3	62.3	19	63.3
4	60.4	20	66.7
5	74.2	22	73.3
6	75.0	24	80.0
7	60.0	20	66.7
8	76.6	23	76.7
9	79.7	24	80.0
10	87.0	26	86.7
11	78.3	21	70.0
12	73.5	22	73.3
13	59.4	18	60.0
An average	72.1	21.8	72.8

* Number of aphids which penetrated the yellow part of 30 aphids per one leaf.

experiment. This indicates that the area of the yellow part is more than half of the total leaf surface.

Sixty to 87% of the aphids inserted their stylets in the yellow part, and the others inserted in the green part. That is, most aphids inserted their stylets into the yellow part. This phenomenon does not appear to be caused by the aphid selecting in the yellow part. The reason appears to be simply that the yellow part was larger than the green part.

The virus acquisition rate from the yellow part by initial probing was 66.7% as shown in Table 4. Also, the frequency of stylet insertion into the yellow part averaged 72.8% (see Table 5). From these two results, it is calculated that when the aphids are placed on a mosaic leaf, the aphids acquire virus from the yellow part at the rate of 48.6%. On the other hand, it was indicated that the acquisition rate from the green part by the initial probing was 16.7%. When the aphids are placed on a mosaic leaf, the frequency of the stylet insertion into the green part averaged 27.2%. From these results, it is calculated that the aphid aquires virus from the green part in a rate of 4.5%. The sum of calculated value of 48.6% (acquisition rate from the yellow part) and 4.5% (acquisition rate from the green part) is 53.1%. This value indicates total virus acquisition rate from both parts on the mosaic leaf. Moreover this value coincided with the measured value of 53.3% of the acquisition rate of the virus obtained by initial probing on the mosaic leaf.

From the above results, it is concluded that in virus acqusition by probing from a mosaic leaf of broad bean infected with BYMV-O, the aphid acquires virus from the epidermis of the yellow part at a high rate.

Discussion

Bradley (3) suggested that when the stylet penetrated into a tobacco leaf infected with potato virus Y, the aphid acquired virus from the tip of the leaf more readily than from the middle or the base, and from the interveinal areas more than from the tip of the leaf. This result seems to show that the virus distribution differs according to the section of the leaf. If this fact exists, we can suppose that the difference of transmission rate by the aphid is decided by the stylet insertion site. Arnott and Smith (1) suggested that in the case of rod-shaped virus attacking a sunflower, the virus concentration in the chlorotic areas is higher than in the green areas in the mosaic pattern of the leaf. These authors' results seem to show a difference of virus concentration in the mesophyll cell, because they have not discussed the acquisition of virus by the aphid.

Our experiments indicated that the virus distribution was different between the epidermal cell and the mesophyll cell. Many virus particles were recognzied in the epidermal cell of the yellow parts of mosaic leaves, but the virus particles were very few in the epidermal cell of the green parts. Further there was a

tendency to lower virus concentration in the epidermal cell than the mesophyll cell. This tendency was recognized also in the yellow and the green parts. It is supposed that these results indicate the main reason for a high virus acquisition rate from the yellow parts by probing. Considering the probing behavior, such a differential distribution of the virus content in the mosaic pattern of the diseased leaf is an important factor in the acquisition of viruses by aphids. Our results indicated that many more aphids probed on the yellow part than the green part, but it is not clear from our experiments, whether or not the aphid selected the yellow part intentionally. Johnson (7) indicated that aphids were attracted to yellow color. The attractive effect of yellow color was recognized in the experiments presented here.

Acknowledgement

The author is grateful to Professor Dr. T. Misawa for encouragement and suggestions.

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