FEEDING BEHAVIOUR OF THE APHIDS *MYZUS PERSICAE* AND *BREVICORYNE BRASSICAE*, STUDIED WITH RADIOPHOSPHORUS

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[*Manuscript received September* 26, 1952]

Summary

The volume of material ingested and excreted by the aphids *Myzus persicae* and *Brevicoryne brassicae* was measured by feeding them on leaves or solutions containing radiophosphorus. No uptake could be measured within the first few minutes of penetration by the stylets. *Myzus* ingested approximately 0.07 mg. plant material in 1 hr. *Brevicoryne* ingested less than one-twentieth of that amount in the same period. *Myzus* ingested more from the lower than from the upper surface of the leaf. No artificial diet was found that was ingested by *Myzus* as readily as leaf tissue.

Both species reinjected a fraction of 1 per cent. of the amount ingested during the subsequent feed. No evidence was obtained for the occurrence of regurgitation from the midgut.

Periods of starvation from 30 min. to 4 hr. did not affect the amount subsequently ingested by *Myzus* during a 30-min. feeding period.

The volume of liquid mechanically transported on the stylets of *Myzus* approximated 2×10^{-7} ml.

The relation of these observations to hypotheses of the transmission of viruses by aphids is briefly discussed.

I. INTRODUCTION

It is over 30 years since aphids were first incriminated as vectors of virus diseases of plants (Allard 1917). However, in no case has the mechanism of transmission been definitely established (Bawden 1950), although several hypotheses concerning this mechanism have been advanced (see for example Doolittle and Walker 1928; Watson 1936, 1946; Smith and Lea 1946; Bradley 1952). It is clear that further data are needed concerning details of the feeding behaviour of aphids to aid evaluation of the hypotheses that have been put forward.

Hamilton (1935) has already reported results on the feeding of *Myzus persicae*, using polonium as a tracer, but advances in the knowledge of isotopes since that time have made it desirable that some of her experiments be repeated. The results of a study of some aspects of feeding and excretion by the green peach aphid *Myzus persicae* (Sulz.) and the cabbage aphid *Brevicoryne brassicae* (L.) are presented in this paper.

II. METHODS

Feeding was studied by permitting the insects to ingest radiophosphorus. This was obtained as $Na_2H^{32}PO_4$ or as $NaH_2{}^{32}PO_4$ from the British Atomic Energy Establishment through the Tracer Elements Investigations Section,

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C.S.I.R.O. The isotope was incorporated in sucrose solutions or into plant tissues by permitting the petiole of an isolated leaf of Chinese cabbage (*Brassica chinensis* L.) to stand in Knop's solution containing the isotope until a measured volume of solution had been absorbed by the leaf. Most of the equipment and

TABLE 1

INGESTION OF ^{32}P BY *M. PERSICAE* FROM LEAF OF CHINESE CABBAGE

Five insects per test, feeding time 1 hr.

Leaf No.	Position on the Leaf	Ingested ^{32}P (counts/min.)
1	Upper surface	106
	Underside	994
2	Upper surface	182
	Underside	2300

techniques were the same as described by Day and McKinnon (1951). All data were corrected for background and when necessary, for decay. The controls and measures to eliminate contamination were satisfactory for each test. The work was greatly facilitated by the use of the plastic material "Plas-B-Loon" used by Day and McKinnon (1951). From this material very thin transparent membranes (hereinafter called "plastic membranes") were made. These were easily penetrated by the stylets of the aphids, but no diffusion of phosphate solutions occurred through the membranes even when highly active (0.1 ml. of solution containing 0.5 mc./ml.) sources of ^{32}P were used.

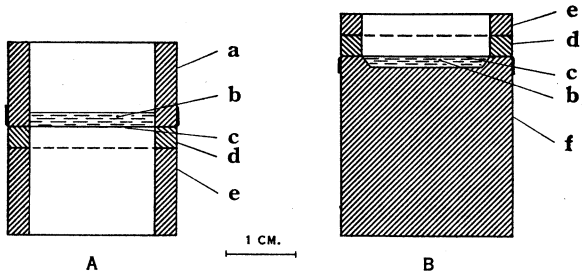

Fig. 1.—Arrangement for feeding aphids on artificial diets. *A, M. persicae*; *B, B. brassicae*. *a,* Plastic ring; *b,* feeding solution; *c,* plastic membrane; *d,* separating plastic ring; *e,* container for aphids; *f,* plastic block.

Apterae of both species of aphids starved for 30 min. were used in all experiments unless the contrary is stated.

III. Observations

(*a*) *Ingestion*

An examination was made of the amount of plant juices and of sucrose solutions ingested by *Myzus* and *Brevicoryne*. First, an experiment was performed with *Myzus* to determine whether this species ingests more when feeding from the upper or lower surface of the leaf. The data (Table 1) show that a greater amount was ingested when the insects fed on the underside of the leaf. Two factors may be involved, one resulting from differences in ^{32}P content of different leaf tissues, the other from the preferred orientation of the insects when feeding. The second alternative was shown to be concerned during artificial feeding, for *Myzus* ingested sucrose through a plastic membrane only when it was presented in the apparatus illustrated in Figure 1*A*. This arrangement was therefore used in subsequent experiments with *Myzus*. *Brevicoryne*, however, was unable to maintain its position on the plastic membrane under these conditions, and was therefore fed on sucrose solutions as shown in Figure 1*B*.

Table 2

WEIGHT OF PLANT MATERIAL INGESTED BY *M. PERSICAE*

Feeding Time	No. Insects (*a*)	Ingested ^{32}P (counts/min.) (*b*)	^{32}P Content in 1 mg. Leaf (counts/min.) (*c*)	Wt. of Material Ingested by One Insect (mg.) $\frac{(b)}{(a)\times(c)}$	Mean Wt. of Material Ingested by One Insect (mg.)
10 Min.	10	64	$1{\cdot}9\times10^4$	0·0003	0·0002
10 Min.	10	21	$1{\cdot}9\times10^4$	0·0001	
15 Min.	10	357	$1{\cdot}9\times10^4$	0·0019	0·0027
15 Min.	10	650	$1{\cdot}9\times10^4$	0·0034	
20 Min.	10	301	$1{\cdot}9\times10^4$	0·0016	0·0023
20 Min.	10	544	$1{\cdot}9\times10^4$	0·0029	
30 Min.	5	760	$1{\cdot}9\times10^4$	0·0080	0·0078
30 Min.	5	717	$1{\cdot}9\times10^4$	0·0075	
1 Hr.	5	6520	$1{\cdot}9\times10^4$	0·0686	0·0687
1 Hr.	5	6540	$1{\cdot}9\times10^4$	0·0688	
2 Hr.	5	8860	$1{\cdot}9\times10^4$	0·0934	0·0898
2 Hr.	5	8180	$1{\cdot}9\times10^4$	0·0862	
4 Hr.	10	12380	$6{\cdot}9\times10^3$	0·1795	0·1795
6 Hr.	10	19300	$6{\cdot}9\times10^3$	0·2795	0·2795

The weight of plant material, excluding the amount excreted, ingested by *Myzus* is shown in Table 2, and the comparable data for *Brevicoryne* in Table 3. It will be observed that the rate of uptake by the green peach aphid is many times greater than the rate of uptake by the cabbage aphid. In spite of the high concentrations of isotope employed (0.1 mc. in approximately 5 g. leaf) no evidence of uptake was measurable before 5 min., although extra-

polation of data obtained after 15, 30, and 60 min. feeding indicates that, had it been linear with time, uptake should have been measurable. It is concluded that a measurable amount of ingestion does not occur during the initial

TABLE 3

WEIGHT OF PLANT MATERIAL INGESTED BY *B. BRASSICAE*

Feeding Time (hr.)	No. Insects (*a*)	Ingested ^{32}P (counts/min.) (*b*)	^{32}P Content in 1 mg. Leaf (counts/min.) (*c*)	Wt. of Material Ingested per Insect (mg.) $\frac{(b)}{(a)\times(c)}$	Mean Wt. of Material Ingested per Insect (mg.)
1	10	248	27600	0·0009	0·0027
1	30	3100	27600	0·0037	
1	30	2800	27600	0·0034	
2	10	2580	27600	0·0094	
2	30	15500	27600	0·0187	0·0166
2	30	17950	27600	0·0217	
4	10	3790	27600	0·0137	
4	30	17530	27600	0·0212	0·0275
4	30	39500	27600	0·0477	
6	5	18800	27600	0·136	
6	30	118000	27600	0·143	0·1310
6	30	94500	27600	0·114	

stages of stylet penetration. However, during punctures of short duration the behaviour of the insect suggests that it can distinguish, presumably by taste, the tissues through which its stylets are penetrating. Gustatory organs appear to be absent from the stylets, but occur in the functional mouth just anterior to the pharynx. It seems likely that sufficient solution is ingested to stimulate these organs.

TABLE 4

VOLUME OF 10 PER CENT. SUCROSE SOLUTION INGESTED BY *M. PERSICAE*

Feeding Time	No. Insects (*a*)	Ingested ^{32}P (counts/min.) (*b*)	^{32}P Content in 1 cu. mm. Sucrose (counts/min.) (*c*)	Volume of Sucrose Ingested per Insect (cu. mm.) $\frac{(b)}{(a)\times(c)}$
45 Min.	40	303	3860	0·002
16 Hr.	20	920	2890	0·016

The volume of 10 per cent. sucrose solution ingested by *Myzus*, excluding the amount excreted, is shown in Table 4, and the comparable data for *Brevi-*

coryne in Table 5. *Brevicoryne* ingested sucrose almost as readily as it ingested plant material, but *Myzus* ingested sucrose very poorly. This is partly accounted for by a reluctance by *Myzus* to penetrate the plastic membrane, but other factors are also involved. Attempts to increase the acceptability of the artificial diet by the addition of several sugars, proteins, protein hydrolysate, asparagine, or mixtures of several amino acids were unsuccessful. Similarly, an experiment to determine whether *Myzus* showed pH preferences was made by feeding the

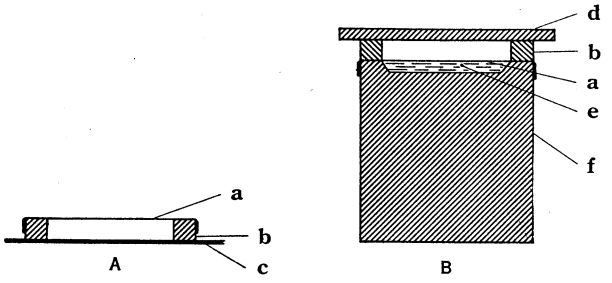

Fig. 2.—Arrangement for studying excretion by aphids. *A*, *M. persicae*; *B*, *B. brassicae*. *a*, Plastic membrane; *b*, plastic ring; *c*, leaf on which aphids were fed; *d*, glass cover; *e*, feeding solution free of radioactivity; *f*, plastic block.

insects on artificial diets, buffered by dilute phosphate buffers of pH 5.3-8.0. The results showed low uptake from all solutions but no difference between them. It was concluded that, until a more acceptable artificial diet was available for *Myzus*, the experiments using artificial media for the species were of doubtful value.

TABLE 5

VOLUME OF 10 PER CENT. SUCROSE SOLUTION INGESTED BY *B. BRASSICAE*

Feeding Time (hr.)	No. Insects (a)	Ingested ^{32}P (counts/min.) (b)	^{32}P Content in 1 cu. mm. Sucrose (counts/min.) (c)	Volume of Sucrose Ingested per Insect (cu. mm.) $\frac{(b)}{(a)\times(c)}$
1	10	273	11800	0·002
2	10	1370	11800	0·012
3	10	130	11800	0·001
4	10	2970	11800	0·025
24	5	1480	11800	0·025

(*b*) *Excretion*

Excretion of isotope was measured by estimating the activity remaining in small containers after the removal of the insects. Excretion thus includes material originating from the cornicles as well as that of alimentary origin, but

in these short feeding periods could not have included any reinjected material (see following section). This procedure may be expected to give low values for *Brevicoryne,* for some of the excreta of this insect normally remain on the

TABLE 6

EXCRETION OF ^{32}P BY *M. PERSICAE* DURING FEEDING ON CHINESE CABBAGE LEAF

In each test 20 insects exposed on leaf containing ^{32}P for 3 hr.

^{32}P Ingested by Insects (counts/min.)	Time of Exposure to Radioactive Leaf (hr.)	^{32}P Content on Leaf (counts/min.)	^{32}P Content in Container (counts/min.)	^{32}P Excreted (% of amount ingested)
8940	½	7	232	2·7
7960	½	8	51	0·7
8120	½	0	168	2·1
9140	1	0	491	5·4
9940	1	0	379	3·8
5630	2	58	467	9·3
6290	2	149	844	17·4

insect. The arrangement of apparatus used to obviate contamination in this experiment is illustrated in Figure 2. The results of experiments to determine the percentage of ingested material excreted are given in Tables 6 and 7 for *Myzus* and *Brevicoryne* respectively. The variability of the results, especially where no excretion was recorded, indicates the discontinuous nature of the process.

TABLE 7

EXCRETION OF ^{32}P BY *B. BRASSICAE* DURING FEEDING ON 10 PER CENT. SUCROSE SOLUTION

Thirty insects used in each test

Time of Exposure to ^{32}P Leaf (hr.)	Ingested ^{32}P (counts/min.)	Time of Exposure to Sucrose (hr.)	Excreted ^{32}P (counts/min.)	Excreted ^{32}P (% of amount ingested)
1	3100	1	0	0
1	2800	1	189	6·8
2	15400	2	0	0
2	17800	2	90	0·5
4	17300	2	27	0·2
4	39000	2	646	1·7
6	112700	17	5300	4·7
6	89900	17	4020	4·5

Broadbent (1951) has reported that *Myzus* does not excrete on the leaf upon which it is feeding. This is frequently so, but it will be noted in Table 6

that sometimes substantial amounts of excreted phosphorus were detected on the leaf upon which the insects were feeding.

(*c*) *Reinjection of Ingested Radiophosphorus*

Using the data obtained on ingestion and excretion a series of experiments was designed to determine whether reinjection of ingested ^{32}P occurred. Precautions were taken to ensure against contamination from excreta and other sources. The liquid (*e*) in Figure 2*B* was removed quantitatively and its activity measured. The data in Tables 8 and 9 demonstrate that ejection of ingested ^{32}P by both species does occur, but that the amount is smaller than the 6.9 per cent. of ingested material reported by Hamilton (1935).

TABLE 8

EJECTION OF ^{32}P BY *M. PERSICAE* DURING FEEDING

No. Insects	Time of Exposure to ^{32}P Leaf (hr.)	Ingested ^{32}P (counts/min.)	Time of Exposure to Sucrose (hr.)	Excreted ^{32}P (counts/min.)	Excreted ^{32}P (% of amount ingested)
40	17	46600	24	66	0·14
40	17	46600	24	70	0·15
30	17	35000	24	71	0·20
30	17	35000	24	0	0·0
30	17	35000	24	51	0·15
30	23	97000	22*	11	0·01
30	23	59000	22*	8	0·01
		Mean percentage ejection for insects fed on sucrose solution			0·13

* Insects were fed through plastic membrane on non-radioactive leaf.

There are three routes by which this ejected ^{32}P could have reached the second feeding medium. Either the isotope was taken into the midgut, absorbed into the haemocoele, and reinjected with the saliva; or it was regurgitated directly from the midgut; or it reached the feeding medium by contaminating the stylets. The absorption of phosphate occurs very readily; an attempt was made to determine by radio-autographs whether the isotope was present in the *Myzus* salivary glands at various times after feeding. At all times studied, from 15 min. to 24 hr., the isotope was concentrated in the midgut and developing embryos, but it was also present throughout the body and in the salivary glands. In view of the long feeding times (17-24 hr.) required before reinjected ^{32}P could be detected, it seems more likely that it was reinjected in the saliva than by the other routes.

(d) Effects of Duration of Starvation on Quantity of Material Ingested

It has been confirmed by several authors following the observations of Watson (1938) that aphids transmit certain viruses more efficiently after a brief period of starvation. It has generally been assumed that the insects ingest

TABLE 9

EJECTION OF ^{32}P BY *B. BRASSICAE* DURING FEEDING

No. Insects	Time of Exposure to ^{32}P Leaf (hr.)	Ingested ^{32}P (counts/min.)	Time of Exposure to Sucrose Free of ^{32}P (hr.)	Excreted ^{32}P (counts/min.)	Excreted ^{32}P (% of amount ingested)
30	6	112700	17	637	0·57
30	6	89900	17	369	0·41
				Mean	0·49

more efficiently following starvation. It was, therefore, of interest to determine whether the quantity ingested differed following periods of starvation previously known to influence the efficiency of virus transmission. Groups of 20 *Myzus* were starved for periods from 30 min. to 4 hr. and were then permitted to feed

TABLE 10

EFFECT OF STARVATION ON INTAKE OF FOOD BY *M. PERSICAE*

Twenty insects fed on radioactive leaf of Chinese cabbage for ½ hr.

Experiment No.	Time of Starvation (hr.)	Intake of ^{32}P (counts/min.)
1	No starvation	358
	½	272
	1	315
	2	294
	4	985
2	No starvation	628
	½	825
	1	301
	2	782
	2	700
	4	668
	4	856

for 30 min. on a leaf of Chinese cabbage containing ^{32}P. The amount ingested in two such experiments is shown in Table 10. Although variable, the data suggest that starved aphids ingest approximately the same amount as do unstarved aphids.

(*e*) *Amount of Material Carried Mechanically on Feeding Stylets*

Certain workers on the mechanism of transmission of non-persistent viruses have suggested that the pathogens are carried mechanically on the mouth-parts. It was therefore of interest to determine the quantity of material that could be so carried. Stylets were dissected from *M. persicae* alatae and apterae. Approximately half the length of the stylets was immediately immersed for 10 sec. in droplets of water containing a known amount of isotope. The volume of liquid remaining on the stylets could then be determined (Table 11). Occasionally surface tension prevented the stylets from penetrating or caused the solution to contaminate the stylets over their entire length. Such stylets were discarded.

TABLE 11
VOLUME OF ^{32}P SOLUTION CARRIED MECHANICALLY ON FEEDING STYLET OF *M. PERSICAE*

Freshly Dissected Stylet Immersed in ^{32}P Solution*		Stylet Dissected and Dried in Air 30 Min., Then Immersed in ^{32}P Solution†		Dissected and Dried-off Stylet Immersed in ^{32}P Solution+Wetting Agent†	
^{32}P Carried on Stylet (counts/min.)	Volume Carried on Stylet (ml.)	^{32}P Carried on Stylet (counts/min.)	Volume Carried on Stylet (ml.)	^{32}P Carried on Stylet (counts/min.)	Volume Carried on Stylet (ml.)
14	$4{\cdot}0\times10^{-8}$	3	—	0	—
35	$1{\cdot}0\times10^{-7}$	47	$1{\cdot}8\times10^{-7}$	2	—
4	—	20	$7{\cdot}7\times10^{-8}$	4	—
29	$8{\cdot}3\times10^{-8}$	88	$3{\cdot}4\times10^{-7}$	16	$6{\cdot}2\times10^{-8}$
224	$6{\cdot}4\times10^{-7}$	81	$3{\cdot}1\times10^{-7}$	2	—

* 1.0×10^{-6} ml. ^{32}P solution = 350 counts/min.
† 1.0×10^{-6} ml. ^{32}P solution = 260 counts/min.

Some stylets were permitted to dry out for approximately 30 min. in air before immersing them. This did not alter the volume of liquid picked up. Nor was there any difference between the results with alatae and apterae. The incorporation of a small amount of wetting agent ("Santomerse D," Monsanto Chemical Co.) in the water permitted the stylets to penetrate the droplet readily, but reduced the amount picked up almost to the limit of volume measurable. This limit approximated 4.0×10^{-8} ml. with the isotope available. It was observed that the stylets separate and the mandibles curve away from the maxillae in a solution containing wetting agent, whereas they remain fasciculated when introduced into water alone.

IV. DISCUSSION

The results presented may be compared with those obtained by Hamilton (1935) on *Myzus persicae* and by Day and McKinnon (1951) on the leafhopper

Orosius argentatus. Plant-feeding Hemiptera generally ingest large volumes relative to body weight. Under the most favourable conditions *Orosius* ingested about 15 per cent. of its weight per hour. Hamilton's (1935) data show that the mean weight of a single *Myzus* approximates 0.54 mg. The *Myzus* used in the present study were smaller and weighed approximately 0.2 mg. This species therefore ingests roughly 35 per cent. of its weight in 1 hr. This figure is greatly in excess of that reported by Hamilton; but this is partly accounted for by the observation that *Myzus* ingests so much less of a sugar solution than it does when feeding upon a leaf.

Whereas Hamilton's data on ingestion are too low, her results on reinjection are higher than those reported above. This is undoubtedly due to the fact that some excreted isotope was absorbed by the plant. Broadbent (1951) has shown that the excreta of *Myzus* does not ordinarily lodge on the surface on which the insect is feeding, but the data reported here prove that occasionally substantial amounts of excreta may appear on the leaf.

The amount excreted by the aphids is considerably less than that excreted by *Orosius.*

Certain of the data obtained have a bearing on hypotheses relating to the mechanism whereby aphids transmit phytopathogenic viruses. Thus non-persistent viruses may be transmitted within a 2-min. feeding period. Our data indicate that no ingestion occurs in so short a period after penetration by the stylets. Short periods of starvation increase the food uptake of *Orosius* but do not appear to affect *Myzus.* It is possible that the 30-min. feeding period necessary to record amounts of ingested material sufficient for accurate measurement may vitiate the effects of starvation, but it seems likely that the effect of starvation on the ability of aphids to transmit non-persistent viruses may be due to factors other than the amount ingested.

The reliability of the data on the quantity of material reinjected by *Myzus* would be improved if that species fed better on the artificial medium. The results for *Brevicoryne* are higher and more reliable, but the volume reinjected is still very small. A low percentage of ^{32}P in salivary glands was also reported by Hahn, Haas, and Wilcox (1950) for the mosquito *Aedes aegypti* and by Flemion, Weed, and Miller (1951) for the bug *Lygus oblineatus.* Reinjection by this bug took place through salivary secretions (Flemion, Miller, and Weed 1952). As with *Myzus* there was no evidence for regurgitation from the midgut.

It is apparent that the amount of virus transmitted by an aphid to a susceptible host is extremely small, whether it be transported through the saliva or by contamination of the mouth-parts. Infection by mechanical inoculation is rarely successful even for the most infectious viruses at dilutions of cell sap greater than about 10^5. The ability of the aphid to place the virus in a manner in which it can infect the host is therefore far greater than can be effected by any of the methods of mechanical inoculation so far devised.

V. Acknowledgments

Grateful acknowledgment is made to Dr. T. H. Oddie of the Tracer Elements Investigations Section, C.S.I.R.O., for his assistance with the supply and measurement of the isotope, and to Mr. L. A. Marshall for preparing the figures.

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